PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

UNIVERSITY OF ABOU-BEKR BELKAID - TLEMCEN

DISSERTATION

Submitted to:

FACULTY OF SCIENCE - DEPARTMENT OF CHEMISTRY

For the award of the degree of:

MASTER IN CHEMISTRY

Materials chemistry

By:

Mr HOUBAD Ahmed Nadir
Mr BEKHTI Mahmoud

On the subject of

Biopolymeric based-materials: Preparation, Characterization and applications

Presented publicly on 3 June 2024 in Tlemcen before a jury composed of:

Mr BOURAS Brahim	Professor	University of Tlemcen	President
Mr HOCINE Tayeb	Lecturer A	University of Tlemcen	Supervisor
Mr ZIANI CHERIF Houcine	Professor	University of Tlemcen	Examiner

Academic year: 2023 ~ 2024

Dedication

This work is dedicated to all those who supported and guided me through the journey of completing my final project at university.

To my parents, your unwavering love, support, and encouragement have been the foundation of my success. Your belief in me has given me the strength to pursue my dreams and the courage to overcome challenges. Thank you for your sacrifices and for always being there for me.

To my family, My Grandfather and my Grandmother May Allah have mercy on her soul and my uncles, your constant support and understanding have been invaluable. Your words of wisdom and encouragement have been my source of motivation. I am grateful for your presence in my life.

To my friends, Islam, Mohammed, Youcef, Oussama and Abdel Ali, your companionship, support, and laughter have made this journey enjoyable and memorable. Thank you for being there through the highs and lows, and for your unwavering belief in my abilities. Your friendship has been a source of strength and comfort.

To my colleague and friend who I shared this work with Mahmoud and also Noussaiba for all the help she provided.

To the ChemClub Family, your resources and community have provided me with countless learning opportunities and invaluable experiences. Being a part of this scientific club has significantly enriched my knowledge and skills. I am deeply grateful for the support and the environment that fostered my growth.

This project is a testament to the collective effort and support of each one of you. I am deeply grateful for your contributions and for being a part of this journey with me.

HOUBAD Ahmed Nadir

I would like to extend my sincere gratitude and appreciation to the people without whom this work would not have been possible.

First to my beloved parents for being there for me since day one, for their infinite support and encouragement, all their sacrifices and hard work to see me succeed.

A debt which I can never repay. For that I will always be more than grateful.

To my little sister for her constant support.

To all my family members.

And to my late Grandfather and Grandmother may Allah have mercy on them.

To my childhood friends Seif eddine, Kamal, Houssam, Abbderahmen.

To my colleague and friend whom I shared this work with Nadir.

To all my fellow classmates with whom I have shared my college experience .

And finally to all the people who supported me and prayed for me.

BEKHTI MAHMOUD

Acknowledgments

First and foremost, we give thanks to the Almighty God for blessing us with the strength, determination, fortitude, and health to complete this meager task, no matter how difficult it may have been.

Lab of Organic Electrolytes and Polyelectrolyte's Applications (LAEPO) at ABOU-BAKR-BELKAID TLEMCEN University is the primary focus of this dissertation.

To begin, we'd like to express our gratitude to Professor B. BOURAS, director of LAEPO, for inviting us to his lab and for agreeing to serve as the head of jury.

We express our gratitude to Pr. H. ZIANI CHERIF from the University of TLEMCEN for his interest in the thesis and for agreeing to be our examiner.

We extend our heartfelt gratitude to our esteemed supervisor, Mr. T. HOCINE, Lecturer A at the University of TLEMCEN. His initial suggestion of this topic set the foundation for our work, and his unwavering support has been invaluable. We deeply appreciate his constant availability, insightful advice, wise guidance, boundless patience, and tireless efforts. His contributions have been instrumental in the success of this project.

We dedicate this work to our esteemed professors and the staff of LRM who have guided and supported us throughout our Master's course. Your collective wisdom, patience, and dedication have been instrumental in shaping our academic and personal growth. We are deeply grateful for the invaluable knowledge and insights you have imparted to us.
To our entire teaching team, each one of you has left an indelible mark on our journey. We cherish the time and effort you have invested in our education. This work stands as a testament to your unwavering commitment and profound impact on our lives.

And a special thank you to Professor K.I. BENABADJI for his commitment to teaching us throughout our master's program, as well as for providing us with all the knowledge we needed and his guidance while we worked.

We are grateful to Mrs. S. BOUAYAD AGHA, the Laboratory (LAEPO) engineer, for all of her assistance, courtesy, and availability.

We would like to extend our sincere thanks to S.A. BENABDELLAH the LRM engineer at the university of TLEMCEN. his expertise and support have been invaluable to our project. We deeply appreciate his assistance and dedication.

Mr. A. BELAID, Mrs. C. KAHOUADJI and Mr. O. BOURAS, PhD researchers at the University of TLEMCEN were invaluable resources, and we are very grateful to them.

We wanted to extend our sincerest thanks to S. BENYEKHLEF for his invaluable guidance and support throughout our work. Your dedication and expertise played a crucial role in helping us completing our work. his time and mentorship are deeply appreciated.

We also address our sincere thanks to PhD researcher K. BOUZOUINA for guidance and insights have been instrumental, and we are profoundly grateful for the time and effort you have invested in helping us completing our work.

We would like to express our profound gratitude to the Faculty of Sciences' SEM operator.

Dear classmates and colleagues, Just a quick note to say a heartfelt thank you for your support and friendship. who have made our study a rewarding and enjoyable trip.

List of content

CHAPTER III RESULTS AND DISCUSSION

PART I. EXTRACTED CHITIN AND CHITOSAN RESULTS

PART II. STUDY OF CHITOSAN FILMS

PART III. APPLICATION OF CHITOSAN FILMS

List of figures

CHAPTER I LITERATURE REVIEW

CHAPTER II MATERIALS AND METHODS

CHAPTER III RESULTS AND DISCUSSION

List of tables

CHAPTER I LITERATURE REVIEW

CHAPTER III RESULTS AND DISCUSSION

List of abbreviations

DD: Degree of deacetylation.

ECh: Extracted chitin from shrimp shells.

Cs: Chitosan

ECs: Extracted from shrimp shells.

CCs: Commercial chitosan.

PEG or P: Polyethylene glycol.

Gly or G: Glycerol.

XRD: X-ray diffraction.

FTIR: Fourier transform infrared.

^{1}H-NMR: Nuclear magnetic resonance.

TGA : Thermogravimetric analyses

DMA: Dynamic mechanical analysis.

SEM: Scanning Electron Microscopy.

CrI: The degree of crystallinity.

w/v: Weight/Volume.

v/v: Volume/Volume.

η: Viscosity.

η_{sp}: Specific viscosity.

η_{red}: Reduced viscosity.

INTRODUCTION

Chitin-based materials are one of the most promising abundant polysaccharide biopolymers for developing constructs with advanced functionality due to their unique features; especially in biomedical research and technology. They have extensive applications in pharmaceuticals and biology. Seafood companies produce a large volume of crustacean shells that contains chitin up to around 30% of its volume; a big waste source with high costs of pollution. These worthy wastes can be transformed into additional high grade, low-volume by-products.

On commercial scale, chitin/chitosan is typically removed chemically from shrimp shells. The following article describes the current chemical procedures for chitin recovery and synthesis of its most frequent derivative, the deacetylated form named chitosan, from shrimp shell wastes and squid pen. The main extraction steps are addressed individually and are highlighted [1].

Chitosan is an acid soluble amino-polysaccharide with great potential due to its poly-cationic features which distinguish it from other natural polysaccharides and polymers (usually anionic). The macromolecular chains of chitin and chitosan are characterized by their molecular weight and their degree of acetylation (DD) [2].

Pure chitosan film is frail and water-soluble, restricting its practical utility in food packaging. In this work, combining glycerol and polyethylene glycol could concurrently increase the flexibility of chitosan film. Through the use of X-ray diffraction (XRD), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR), Thermogravimetric analyses (TGA), Dynamic mechanical analysis (DMA).

The purpose of this study is as follow, chitin and chitosan are extracted, thus it is required to discover a high DD ratio for the use of chitosan as film for antibacterial activity [1].
The current work is organized into three chapters:

- The first chapter is formed of four sections: -The first comprises a generalization on chitin, its chemical structure, physicochemical properties, its source, also the extraction of chitin from the nature and the degree of deacetylation.
- The second one provides a generalization about chitosan, its chemical structure, physicochemical properties, source and degree of deacetylation and application of chitosan in different disciplines
- The Third part dedicated to a brief overview of Edible Packaging [3].

• The fourth part is about different methods of determination the degree of deacetylation using various analytical techniques [4].

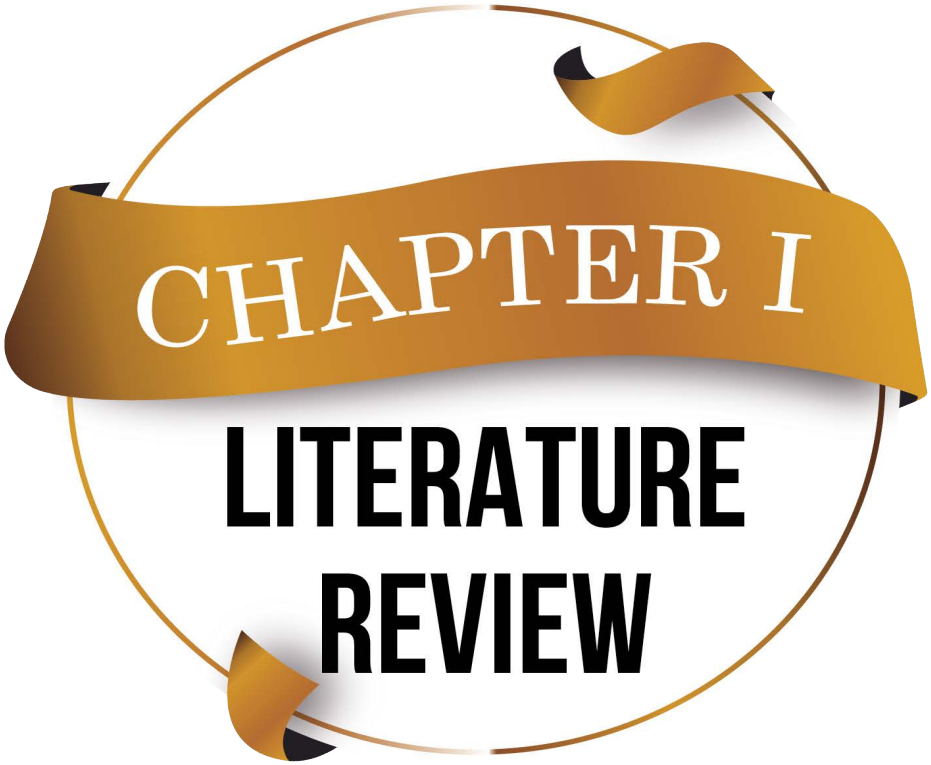
The second chapter depicts the products employed in our experiment, the operating mode of the extraction of chitin and chitosan as well as the numerous methods of characterization [5].

The third chapter offers a presentation of the different results and discussions on:

- Synthesis outcomes (yield and structure, solubility test).
- Characterization of chitin and chitosan by infrared (IR) and X-ray diffraction (XRD) [6].
- Characterization of chitosan films by X-ray diffraction (XRD), Scanning electron microscopy (SEM) ,Thermogravimetric analyses (TGA), Dynamic mechanical analysis (DMA) [7].
- Calculation of DD using potentiometry and conductimetry titration and infrared (IR).
- Determination of the molecular weight by viscosimetry [8].
- Antibacterial activity of the Films using the Mueller Hinton (MH) agar diffusion method [9].

REFERENCES

1. Pakizeh, M., A. Moradi, and T.J.E.P.J. Ghassemi, *Chemical extraction and modification of chitin and chitosan from shrimp shells.* 2021. **159**: p. 110709.
2. Crini, G., et al., *Chitine et chitosane: du biopolymère à l'application*. 2009: Presses Univ. Franche-Comté.
3. Kumar, N.J.N. and F. Science, *Polysaccharide-based component and their relevance in edible film/coating: A review.* 2019. **49**(5): p. 793-823.
4. Czechowska-Biskup, R., et al., *Determination of degree of deacetylation of chitosan-comparision of methods.* 2012(17): p. 5-20.
5. Abdou, E.S., K.S. Nagy, and M.Z.J.B.t. Elsabee, *Extraction and characterization of chitin and chitosan from local sources.* 2008. **99**(5): p. 1359-1367.
6. Rodriguez-Veiga, I., et al., *Exploring Saduria entomon (Crustacea Isopoda) as a new source for chitin and chitosan isolation.* 2022. **23**(24): p. 16125.
7. Li, C., et al., *Preparation and plasticizing mechanism of deep eutectic solvent/lignin plasticized chitosan films.* 2023. **240**: p. 124473.
8. Czechowska-Biskup, R., et al., *Procedure for determination of the molecular weight of chitosan by viscometry.* 2018(23): p. 45-54.
9. Jiang, L., et al., *Evaluation of diffusion and dilution methods to determine the antimicrobial activity of water-soluble chitosan derivatives.* 2013. **114**(4): p. 956-963.


CHAPTER I
LITERATURE
REVIEW

I. Biodegradable polymers

A polymer consists of monomers, which can be either identical or different single units. Polymers' degree of polymerization refers to the quantity of monomer units comprising a macromolecule. Biodegradability refers to a material's susceptibility to decomposition by microbial activity. This attack's structure is gradually simplified and ultimately transformed into carbon dioxide, water, methane, and new biomass. Biodegradable polymers can be produced in two distinct methods based on their source and method of synthesis: from the petrochemical industry or from renewable resources.[1]

I.1. Natural polymers or biological polymers

The largest family is made up of polysaccharides (carbohydrates) such as starch (potato, corn, wheat, etc.), cellulose (plant cell walls), lignin (wood) or chitosan (crustacean chitin). Another family consists of proteins derived from oilseeds (rapeseed, sunflower, soy), protein crops (peas, faba beans), or animal tissues (collagen, gelatin, casein) [1].

I.1.1. Categorization of Natural Origin Biopolymers

Packaging that is edible or biodegradable is composed of renewable macromolecules called biopolymers, which include polysaccharides (celluloses and derivatives, starches and derivatives, chitin and chitosan, etc.), proteins (gelatin, gluten, etc.), lipid compounds (waxes, fatty acids and derivatives, etc.), and polyesters (poly(3-hydroxybutyrate) (PHB) or poly(3-hydroxyvalerate) (PHV), poly(lactic acid) (PLA), etc.) [2].

I.1.2. Biopolymers from agro-resources

Natural macromolecules that are renewable and decomposable are called biopolymers, or agro-materials. They come from a variety of agro-composites, including lipids and fatty acids and hydrocolloids (proteins and polysaccharides). The majority of them can be utilized alone, in combination with other polymers, or in their natural or plasticized state. Their function is as biofilms [3].

II. Edible Packaging

II.1. Definition of edible film

Food products have long been enhanced in appearance and preserved through the application of edible films and coatings. The most popular ones include:

- coating fruit with natural waxes, which has been done in China since the 12th century

- coating chocolate on candies or pastries
- coating meat with fats; and wrapping a variety of foods in a lipoprotein film made from soy milk (yuba, fukuk) to enhance their presentation and prolong their shelf life.

A film, coating, or thin protective layer with active or selective (transfer) qualities is known as edible packaging. It is consumed in its entirety as it is an essential component of the meal . By functioning as a barrier against gases, moisture, and fats, it shields the product from the elements and enhances its quality by minimizing the loss of aroma and maintaining its flavor, texture, and appearance [4].

A coating that helps preserve product quality even after opening and increases the shelf life of fresh fruit without resulting in anaerobiosis is ideal . Two primary categories of packaging exist:

1. Structures that are not dependent on food: films, sachets, wrappers (sausage skins), trays (for meat or fruit)[5].

2. Items that are placed directly on food, like sugar coatings for almonds A "chocolate bar" has chocolate surrounding the cookie, "candy bars" have paraffin wax surrounding the cookie, and sugared almonds have sugar surrounding the almond. Kerosene wax encircling some fruits [5].

Table I.1. *Advantages and disadvantages of edible coatings.*

Materials edible	**Fruits treated**	**Advantages**	**Disadvantages**
Waxes and oils	Lemon Pear Good texture. Banana Tomato	-Excellent barrier to moisture and gas exchange. -Good texture. -Extend shelf life.	Sometimes cause anaerobic respiration.
Polysaccharides (Cellulose, starch, alginate, pectin, gum arabic, carrageenan and chitosan)	Lemon Apple Pear Banana Mango Strawberry	-Selective permeability to gases (CO2 andO2) without causing anaerobia. -Extend shelf life. -Slow ripening. -Good texture.	Minimal impermeability to water vapor due to their hydrophilic nature.
Proteins (keratin, corn zein, wheat gluten)	Gluten (corn zein)	-Excellent barrier to gas exchange. -Corn zein is the only one impermeable to water vapour.	Waterproofing to water vapour.

III. Chitin and Chitosan

III.1. Chitin

Chitin, which is made up of N-acetyl D-glucosamine units (2-acetamido-2-deoxy-D-glucosamine) connected by β(1–4) bridges, is the most extensively distributed biopolymer in nature after cellulose . Chitin, which is thought to be the acetamide derivative of cellulose (Figure 1), is distinguished from other polysaccharides by up to 7% of nitrogen present in its polymeric chain [6].

The crystalline structure of the chitin molecule forms a network of well-organized fibers in its natural state. It is possible to arrange chains differently. These chains all run along the same axis, forming a helix that can take on three different polymorphic forms [6].

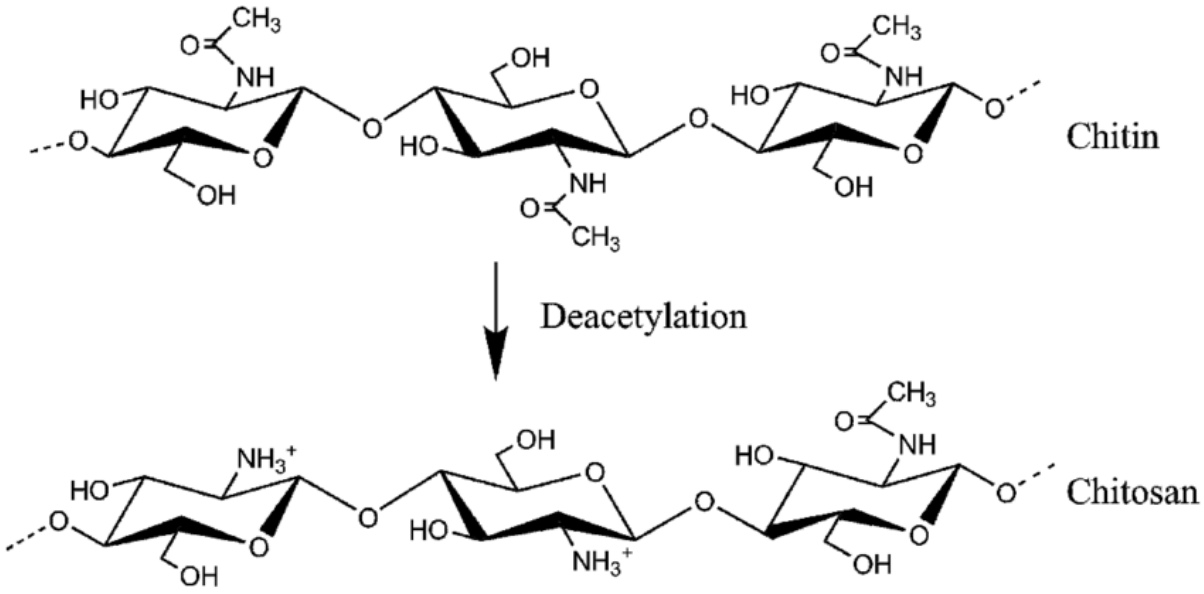


Figure I.1. *Chemical structure of chitosan and chitin.*

III.2. Chitosan

It wasn't until 1954 that chitosan was found in nature; it was found in the phycomycete yeast Blakes leeanus [7]. It is found exclusively in the cell walls of specific fungi, primarily Zygomycetes (Davies and Bartnicki, 1984), as well as in a few insects, including termite queens' abdominal walls [8].

Chitosan is a linear polysaccharide composed of β (1–4) bridges connecting D-glucosamine units (2-amino-2-deoxy-D-glucose). As a result, the chemical structures of chitosan and cellulose are similar; the only distinction is that the amine function replaces the hydroxyl group

at position C-2 (Figure I.3). Lastly, deacetylated chitin with a nitrogen content greater than 7% is what chitosan is.

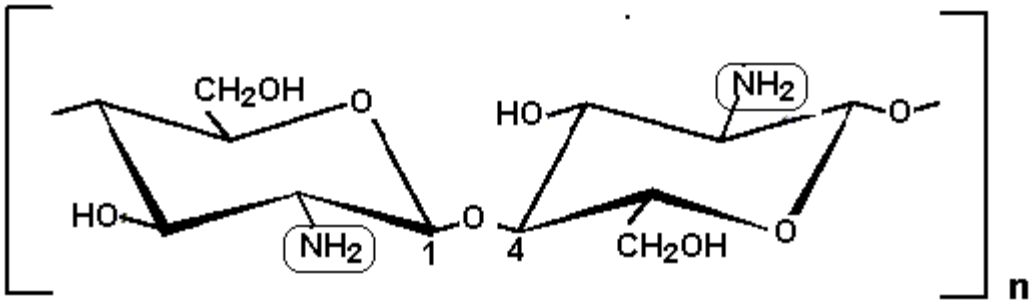


Figure I.2. *Chemical structure of chitosan.*

III.3. Physico-chemical properties of chitin and chitosan

III.3.1. Degree of deacetylation (D.D)

Chitin is now the primary source of chitosan due to its rarity in nature. Most chitosan available in the market are produced by heating chitin to an alkaline deacetylation temperature [9].

Acetyl groups (-$COCH_3$) are removed during deacetylation in order to release amine groups (-NH_2). Because of this, the term "chitosan" is used to refer to a family of copolymers with different fractions of acetylated units that are differentiated from one another by the degree of deacetylation, or the percentage of amine groups in the polymer chain [10].

Chitosan's degree of deacetylation ranges from 56% to 99%, with an average of 80%; the amount varies depending on the living species and method of preparation [11].

However, based on the value of this parameter, a precise distinction between chitin and chitosan has never been made. When the D.D. is more than 50%, some researchers refer to the substance as chitosan [12]. When the D.D. is more than 20%, some people prefer to refer to it as chitosan. This discrepancy can occasionally be attributed to chitosan's and chitin's solubility in diluted acid solutions. Ultimately, when the nitrogen content surpasses 7% by weight, the term "chitosan" is appropriated [13].

The most significant physico-chemical factor affecting chitosan's performance in its varied applications is the degree of deacetylation. This parameter's value is frequently set based on its intended application; for instance, chitosan needs to be deacetylated in the medical field [14].

A number of methods, including UV spectrophotometry, I-R spectroscopy, NMR spectroscopy, HPLC, potentiometric titration, thermal analysis, and others, have been developed to ascertain the D.D. value [15].

The most effective method for quickly characterizing D.D. is infrared spectroscopy. IR spectroscopy, since it's user-friendly and enables us to work with solid materials.

III.3.2. Molecular weight

For DD values in the 70% range, the macromolecular weight of chitin can reach 8×10^5 to 106 g/moles.

Using the relation of MARK HOWINK(A),(Assaad E. Montréal; 2006), the viscosity of the chitin solution can be used to determine this important character.

$$[\eta] = K \times M_v^{\alpha} \qquad I$$

With :

$[\eta]$: Intrinsic viscosity.

K and a: Constants depending on the nature of solutions and temperature.

M_v: Viscosimetric molar weight.

III.3.3. Solubility

Chitin has a stiff fibrous structure and is a white, hard, inelastic solid in its natural state. This indicates that it is insoluble in the majority of common solvents, meaning that there will be very few uses for this kind of molecule [16].

Nonetheless, it dissolves in a few hazardous and corrosive solvents, including methylene chloride, chloroacetic acid, and chlorinated alcohols [17]. Additionally, it dissolves in fluorinated solvents like hexafluoro-2-propanol and hexafluoroacetone. The solubility of chitin in the following two mixtures was investigated by [18]:

- Dimethylacetamide/lithium chloride (DMAc/LiCl)
- N-methyl-2-pyrrolidone/lithium chloride (NMP/LiCl)

They pointed out that more than 5% of lithium chloride is needed for chitin to dissolve completely. -[19] compared the effectiveness of these two solvent systems with dozens of others, including mixtures of two chloroethanol and nitric acid, two chloroethanol and sulfuric acid, two chloroethanol and phosphoric acid, and two chloroethanol and sulfuric acid.

Despite being extremely toxic, they came to the conclusion that DMAc/LiCl and NMP/LiCl had the best solubilization performance.

In water, concentrated acids, bases, and organic solvents, chitosan in free amine form is insoluble. As a reference, HCl (1%) and C_2H_5COOH (1%), at pH ≈4, are utilized. On the other hand, it is soluble in diluted acids such as hydrochloric acid, formic acid, acetic acid, oxalic acid, and lactic acid at pH < 6.5 [20]. According to [21], phosphoric acid, sulfuric acid, citric acid, and sebacic acid are poor solvents for chitosan.

The protonation of amine groups in chitosan is responsible for its solubility:

$$-NH_2 + H_2O \rightleftharpoons -NH_3^+ + OH^-$$

$$-NH_3^+ + H_2O \rightleftharpoons -NH_2 + H_3O^+$$

The degree of deacetylation also affects chitosan solubility; total solubilization is thought to be possible when the D.D. is between 80% and 90% .

III.3.4. Determination of the degree of deacetylation of chitosan

Extracted chitin and chitosan from various sources such as crab and shrimp, analyzed the products, and determined the degree of deacetylation using various analytical techniques.

- **Potentiometric** titration Alpha, beta chitin, and chitosan solutions in a mixture of water and hydrochloric acid were titrated with a basic solution (NaOH). The pH values recorded after each addition of HCl resulted in a curve with two inflection points. Calculating the DD values involves determining the volumes added at these two points [22].
- **Nuclear Magnetic Resonance (NMR) spectroscopy ^{1}HNMR** is used to detect protons and determine the proportion of each type of hydrogen in a structure for analysis. Chitosan obtained from pink shrimp shells using 40% NaOH was analyzed using 1HNMR and the findings are shown in Figure I.4. The most important peaks on this study are those of acetamide and amino groups (situated on 2.06 and 4.9 ppm respectively) which refer to the amount of acetylated and deacetylated monomers in chitosan and the DD is calculated using the number of H in each peak [23].

$$DD\% = \frac{numberofHon\ 4.9}{numberofHon\ 4.9 + (numberofHon\ 2.06)/3)} \qquad II$$

III.4. Extraction of chitin

Chitin is never found pure in the natural world. It usually takes the form of microfibers with diameters ranging from 2.5 to 2.8 nm, embedded in a protein matrix, and is frequently linked to proteins, lipids, calcium, and pigments [24]. These microfibers can grow to a diameter of d25 nm in the cuticles of crustaceans. Such configurations allow chitin to spiralize (microfibrous). Only after certain substances have been eliminated is this feasible: the proteins that hold the microfibers in place and calcium carbonate [25].

Based on chitin's percentage in each of the three primary sources listed below [26], one can select the appropriate extraction technique:

Chitin makes up 1.4% of an insect's weight, which explains why they aren't exploited.

- The proportion of chitin in microorganisms can exceed 20% of their dry cell weight. But the only application for this source is in experimentation. basis for experimentation.

-Lastly, crustaceans, the primary source of chitin, have the highest yields. origin of chitin.

III.4. Chemical extraction

There are two crucial steps involved in the chemical extraction process:

III.4.1. Deproteinization

An alkaline solution is applied to the shell powder at this stage, usually using NaOH to get rid of proteins [27]

III.4.2. Demineralization

Using acidic treatment with nitric acid and sulfuric acid, carbonate calcium is separated from the product obtained by the deproteinization process. However, it is advised to use chlorohydric acid at this point [27].

$$CaCO_3 + 2HCl \longrightarrow CaCl_2 + CO_2 + H_2O$$

III.4.3. Decolorization

The chitin that is left over after demineralization and deproteinization is frequently a solid that is pink in color. This indicates that the pigments, primarily carotenoids, are resistant to the first two treatments. In order to achieve a more pure white chitin, it is first depigmented using acetone and then bleached in a NaOCl solution (0.315% v/v) for five minutes with a solid/liquid ratio of 1/10 (weight/volume) [28]. This process is followed by drying the chitin at room

temperature. Depending on the final product specification, this step is optional. Furthermore, bleaching is not very desirable because it significantly lowers the final product's viscosity [11].

III.5. Production of chitosan [18]

The following figure illustrates the different steps involved in the chemical synthesis of chitosan:

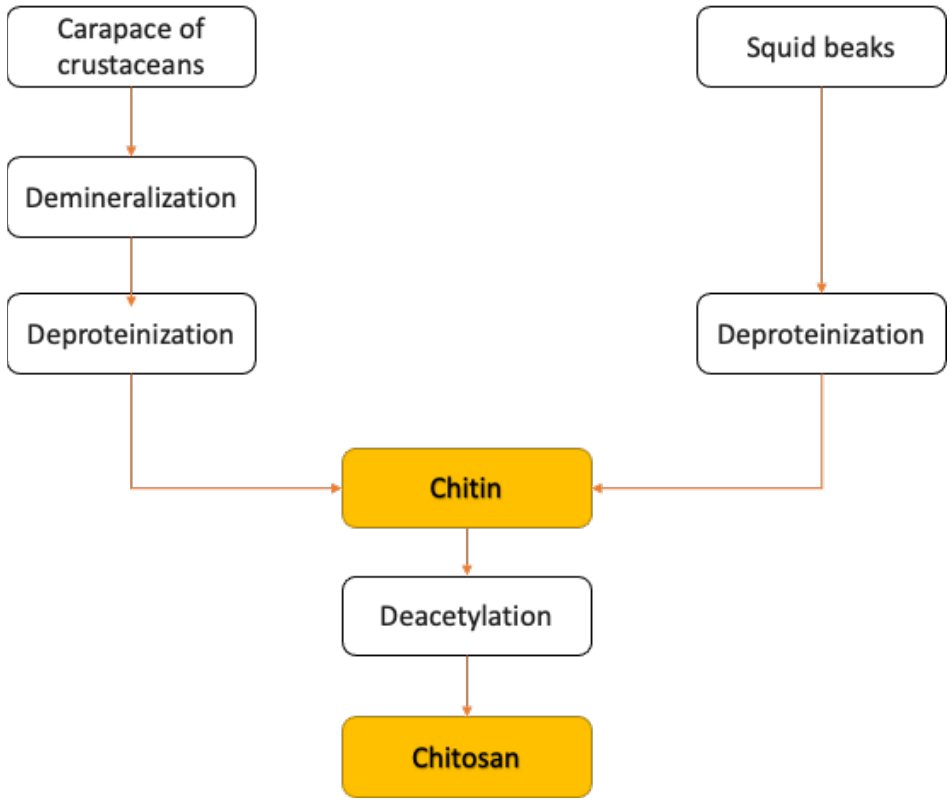


***Figure I.3**. Conventional chitosan production method.*

III.6. Sources and chemical extraction of chitosan

Typically, a process called deacetylation is used to partially convert acetamide groups into amino groups in order to extract chitosan from chitin. An alkali treatment at a high temperature was required for this procedure.

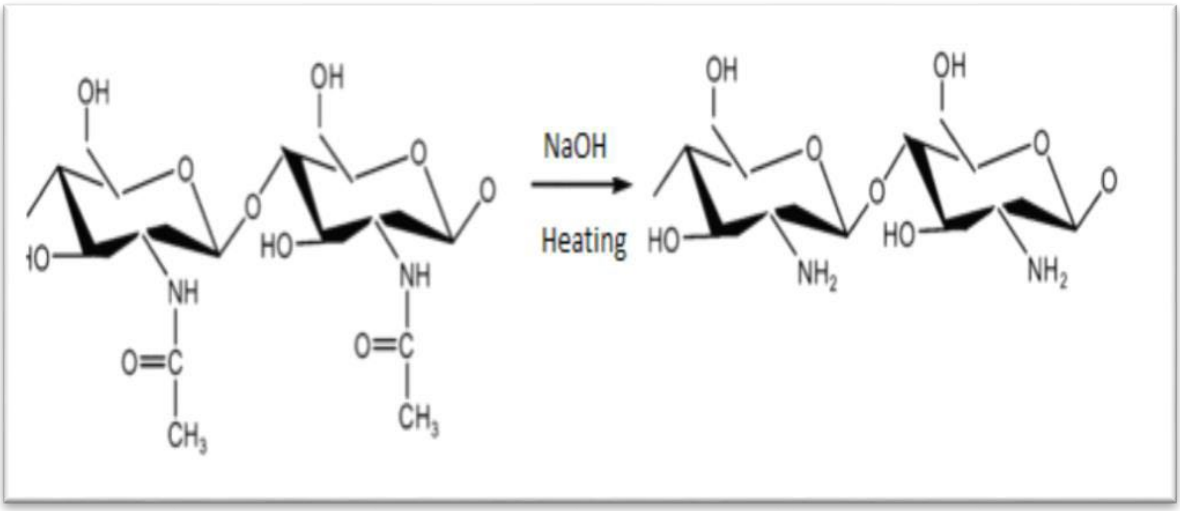


Figure I.4. *Deacetylation reaction**[29].***

This table offers a thorough summary of the sources of chitin. From well-known crab shells, such as those included in the table :

Table I.2. *Sources of Chitin and their percentage.*

The source	% of chitin
Brown shrimp shells	22.53
Pink shrimp shells	23.92
Squid pens	49
Crab shells	16.78

III.7.Major applications of chitin and chitosan in the different fields

This table explores the remarkable adaptability of chitosan and chitin. Find out the many uses for these biopolymers in a variety of industries, from water purification to medical wound healing miracles to environmentally friendly solutions and more are included in the table :

Table I.3. *Applications of chitin and chitosan.*

Field of Application	**Applications**
Biomedical and Pharmaceutical applications	Antioxidant Antimicrobial agent Drug delivery Gene therapy Wound management
Health care products Food Industry	Cosmetics formulations Packaging edible coatings antioxidant agent Flocculation/Clarification and deacidification of fruits and beverages
Agriculture	Antimicrobial activities against various plant pathogens. Fruit preservative. Increase in the auxin concentration and urea release in the soil
Industrial application	Functional materials Paper manufacture
Construction industry	wood adhesive, fungicide, wood quality enhancer, and preservative
Waste treatment	Flocculating, and negative charge

Refrences

1. Chesterman, J., et al., *Biodegradable polymers.* 2020: p. 317-342.
2. Aggarwal, J., et al., *The realm of biopolymers and their usage: an overview.* 2020. **8**(2): p. 1005-1016.
3. Sandhya, P., M. Sreekala, and S. Thomas, *Biopolymer-based blend nanocomposites*, in *Handbook of Biopolymers*. 2022, Springer. p. 1-28.
4. Kumar, N.J.N. and F. Science, *Polysaccharide-based component and their relevance in edible film/coating: A review.* 2019. **49**(5): p. 793-823.
5. Umaraw, P., A.K.J.C.r.i.f.s. Verma, and nutrition, *Comprehensive review on application of edible film on meat and meat products: An eco-friendly approach.* 2017. **57**(6): p. 1270-1279.
6. Moussian, B.J.T.c.-c.o., *Chitin: Structure, chemistry and biology.* 2019: p. 5-18.
7. Terbojevich, M. and A.J.C.h. Cosani, *Molecular weight determination of chitin and chitosan.* 1997: p. 87-101.
8. Seng, J.-M.J.B., *Chitine, chitosane et dérivés: de nouvelles perspectives pour l'industrie.* 1988. **71**: p. 40-44.
9. Fernández, M. and P.F.J.F.c. Fox, *Fractionation of cheese nitrogen using chitosan.* 1997. **58**(4): p. 319-322.
10. Roberts, G.A. and G.A.J.C.c. Roberts, *Structure of chitin and chitosan.* 1992: p. 1-53.
11. Fernandez-Kim, S.-O., *Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols*. 2004: Louisiana State University and Agricultural & Mechanical College.
12. Esquenet, C., *Propriétés structurales et dynamiques des solutions de polyélectrolytes rigides et semi-rigides et de polysaccharides associatifs*. 2003, Université Joseph-Fourier-Grenoble I.
13. Gagné, N., *Production of chitin and chitosan from crustacean waste and their use as a food processing aid.* 1993.
14. Baxter, A., et al., *Improved method for ir determination of the degree of N-acetylation of chitosan.* 1992. **14**(3): p. 166-169.
15. Lavertu, M., et al., *A validated 1H NMR method for the determination of the degree of deacetylation of chitosan.* 2003. **32**(6): p. 1149-1158.
16. Zargar, V., M. Asghari, and A.J.C.r. Dashti, *A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications.* 2015. **2**(3): p. 204-226.
17. Muzzarelli, C., et al., *Susceptibility of dibutyryl chitin and regenerated chitin fibres to deacylation and depolymerization by lipases.* 2004. **56**(2): p. 137-146.
18. Uragami, T., Y. Naito, and M.J.P.B. Sugihara, *Studies on synthesis and permeability of special polymer membranes: 39. Permeation characteristics and structure of polymer blend membranes from poly (vinylidene fluoride) and poly (ethylene glycol).* 1981. **4**: p. 617-622.
19. Austin, P., et al., *Chitin: New facets of research.* 1981. **212**(4496): p. 749-753.
20. Sashiwa, H. and Y.J.C.P. Shigemasa, *Chemical modification of chitin and chitosan 2: preparation and water soluble property of N-acylated or N-alkylated partially deacetylated chitins.* 1999. **39**(2): p. 127-138.
21. Kurita, K.J.P.i.p.s., *Controlled functionalization of the polysaccharide chitin.* 2001. **26**(9): p. 1921-1971.

22. Pérez-Álvarez, L., L. Ruiz-Rubio, and J.L.J.J.o.C.E. Vilas-Vilela, *Determining the deacetylation degree of chitosan: opportunities to learn instrumental techniques.* 2018. **95**(6): p. 1022-1028.
23. Sánchez-Machado, D.I., et al., *Measurement of the degree of deacetylation in chitosan films by FTIR, 1H NMR and UV spectrophotometry.* 2024. **12**: p. 102583.
24. Watkins, D., et al., *Hyperhomocysteinemia due to methionine synthase deficiency, cblG: structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L.* 2002. **71**(1): p. 143-153.
25. Kumar, M.N.R.J.R. and f. polymers, *A review of chitin and chitosan applications.* 2000. **46**(1): p. 1-27.
26. Santini, F., et al., *Communication: Introduction of a Nitrogen Heterocycle into Sulphated Chitosan Oligomers.* 1999.
27. Al-Manhel, A.J., A.R.S. Al-Hilphy, and A.K.J.J.o.t.S.S.o.A.S. Niamah, *Extraction of chitosan, characterisation and its use for water purification.* 2018. **17**(2): p. 186-190.
28. Pakizeh, M., A. Moradi, and T.J.E.P.J. Ghassemi, *Chemical extraction and modification of chitin and chitosan from shrimp shells.* 2021. **159**: p. 110709.
29. Islam, S., et al., *Chitin and chitosan: structure, properties and applications in biomedical engineering.* 2017. **25**: p. 854-866.

CHAPTER II

MATERIALS AND METHODS

This chapter presents the materials and products employed to achieve the study's goal, together with characterization analyses, definitions, and principles of operation.

Part I. The equipment

I.1. Raw materials

Chitin Derived from shrimp shells and squid pen.

I.2. Reagents

We have utilized the following products:

-Shrimp shells and squid pen

-Distilled water

-Sodium hydroxide (NaOH)

-Hydrogen chloride (HCl)

-Acetic acid

-Ethanol

-Commercial Chitosan

-Hydrochloric Acid

-Polyethylene Glycol 3000 [PEG]

-Glycerol

I.3. Materials

-Beakers of different capacities (10, 50, 250, 500 mL).

-Erlenmeyer flask.

.-Heating plates.

-Vials (25, 50, 100, 250 mL).

-Petri dishes.

-Digital balance.

-Wash bottle.

-Pipettes (50, 25, 5, 1 mL).

-Burettes.

-Magnetic stirrer.

-Spatulas.

-Bec Bunsen

-Whatman Discs.

-Reflux

-Oven

-Analytical balance

-Ultrasound

Part II. Experimental Methods

II.1. Chemical Extraction of chitin and chitosan

The experiment utilized 100% natural raw materials, specifically shrimp shells sourced from GHAZAOUET seaside in western Algeria. These shells were processed and chitin and chitosan were extracted in the laboratory of Abou-Bakr Belkaid University, Faculty of Chemistry, following a specific procedure:

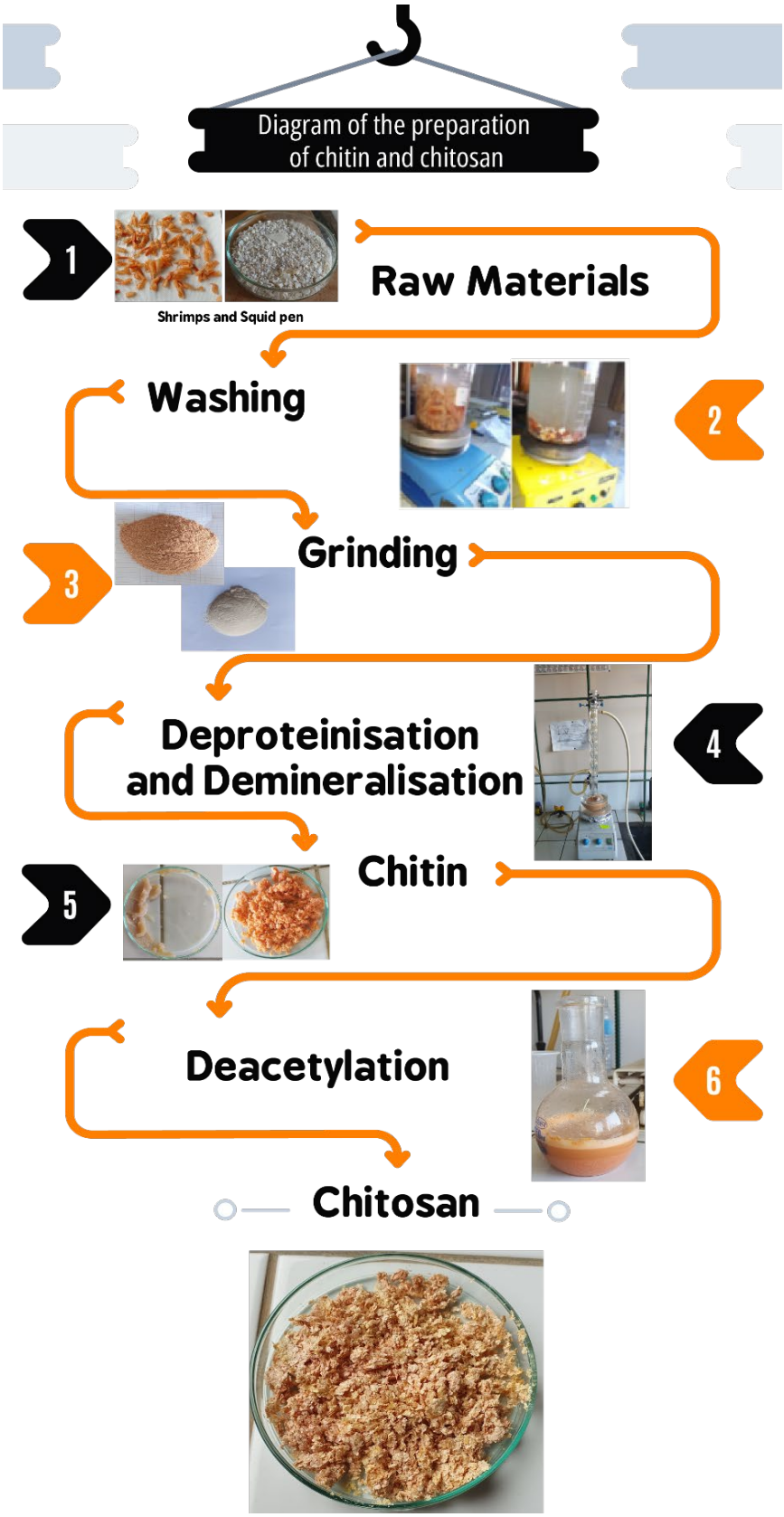


Figure II.1. *Diagram of the preparation of chitin and chitosan.*

II.1.1. Shell purification

Place 40g of shrimp and squid pen in a 1000 mL beaker, cover with 650 mL of distilled water, and shake for 1 hour. Filter and measure the acidity (pH). The next stage is to crush the material thoroughly into a slurry to make future work easier after it has dried for 24 hours at 60°C [1].

II.1.2. Deproteinization

Proteins can be removed by dissolving 8 g of NaOH in a 100 mL flask of distilled water in triplicate, In a 100 mL round-bottom flask add 20g of shrimp (ratio 1/5 w/v) followed by adding 100 mL of NaOH solution. Reflux the mixture for 3 hours and 30 minutes, filter (neutralize), and dry in an oven for 24 hours at 60°C [1].

II.1.3. Demineralization

This step consists in removing the calcium carbonate (CaCO3)

$$2\ HCl_{(aq)} + CaCO_{3(s)} \rightarrow CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)}$$

Since the ratio is 1/15w/v, prepare a solution 500 of mL HCl (2N). Add 18.15g of shrimp to a 250 mL ground flask, add 272.25 mL of HCL solution, bring to reflux for 2 hours at 60°C, filter (adjust pH until neutralization), and dry in the oven for 24 hours [1].

II.1.4. Deacetylation

It is found when a portion of chitin's acetyl group is removed using sodium hydroxide (NaOH).

$$R-NH-CO-CH_{3(s)} + NaOH_{(aq)} \rightarrow R-NH_{2\ (s)} + CH_3COONa_{\ (aq)}$$

Make a 60% NaOH solution (30g NaOH 50 mL H2O). We have 5g grams of shrimp extracted, so we put it in an Erlenmeyer with 50 mL of NaOH solution, reflux for 3 hours (at 120°C.), filter, wash (neutralization), and repeat this process twice more before drying the goods [1].

II.2. Conductimetry/potentiometry solution preparation (determination)

2.88 g of shrimp chitosan is obtained after deacetylation. In a 50 mL flask, combine 20 mg of chitosan and 25 mL of HCl (0.1 M). Adjust with distilled water, shake to dissolve the product, and titrate with 0.1 M NaOH [2].

II.3. Preparation of solutions to determine viscosity and molecular weight

In order to determine the macromolecular weight of chitosan. 10 samples were prepared from both commercial chitosan and the extracted chitosan by two means, first one chitosan on its own and the second one chitosan with salt NaCL. A 2.5g/L chitosan solution was prepared by

dissolving 25mg of chitosan in 25 mL of acetic acid (1%) for both types of chitosan, then from this solution 4 others were prepared by dilution (2-1,5-1-0.75)g/L.

First the solvent used acetic acid was put throw the Ubbelohde U-tube viscometer to record it flow time (t_0) followed by each sample in order to record it flow time (t) (this procedure was repeated twice). And the values of (t and t_0) were used to calculate the specific viscosity which was then used to calculate the reduced viscosity [3].

II.4. Preparation of the films

Films were fabricated using the extracted chitosan and commercial chitosan samples. A (2%) weight/volume (w/v) solution of chitosan was prepared in a (1%) volume/volume (v/v) solution of aqueous acetic acid in all case, by dissolving 0.5g of chitosan in a 25mL acetic acid solution (1%) followed by adding the desired plasticizer (PEG or GLY) with different percentages (20% and 40%) then mixed with magnetic stirring until a homogeneous solution is obtained. The solution obtained was degassed using an ultrasound bath (20 minutes, at room temperature) and cast on a glass Petri dish 7 cm in diameter, and placed in an oven at 60°C for 24h.

The dried films were removed from the glass dish and set aside for further analyses. 10 films were obtained in total, 5 with commercial chitosan and 5 with the extracted chitosan.

II.5. Preparation of antibacterial activity for the films

The antibacterial activity of chitosan is assessed on the reference strain Escherichia coli ATCC 25922 using the Mueller Hinton (MH) agar diffusion method, according to the protocol described by Jiang and colleagues in 2013. In order to accomplish this, a Mueller-Hinton agar plate is coated with an inoculum containing 108 colony-forming units per milliliter (0.5 McFarland) in three different orientations. 6 mm diameter sterile Whatman paper discs, each containing 20µL of the solution being tested, are placed on the surface of the MH agar that has been previously seeded, using sterile forceps. The plate is incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition are measured using a caliper [5].

Part III. Characterization methods

III.1. Spectroscopy using infrared (IR)

Figure II.2. *Infrared Spectroscopy (CARY 600 SERIES).*

III.1.1. Fundamental

It is a method of analysis for gases, solids, and liquids. It is possible to determine the existence of certain covalent bonds and, consequently, distinctive groups inside a molecule using infrared spectroscopy. The molecule's internal vibration, which modifies bond normal angles or interatomic distances, is correlated with the absorption of these radiations.

III.1.2. Tools

FTIR CARY 600 SERIES infrared equipment was utilized for DD computation and characterization. Using a model IR spectrophotometer (4100 Yasco, Japan) in the range from 400 to 4000 cm -1, the produced chitosan samples were characterized in KBr pellets, and the DD was calculated using the following formula :

$$DD\% = \left[1 - \left(\frac{A1665}{A3450} \times \frac{1}{1.33}\right)\right] \times 100 \qquad 1$$

III.2. XRD, or X-ray diffraction

Figure II.3. *X-ray diffraction apparatus.*

III.2.1. Fundamental

This method seeks to characterize the material's structure, measure the mesh parameters, determine the size and orientation statistics of crystallites, and is mostly suitable for crystalline materials (solid or powder, mono-crystalline or polycrystalline). Bragg's law is followed for the diffraction with:

The angle between the diffracting planes and the incident beam is represented by

$$\eta \lambda = 2d \sin \theta \qquad 2$$

θ: The angle between the incident beam and the diffracting planes.

λ: The wavelength of the incident beam.

d: the reticular distance between the diffracting planes.

III.2.2. Tools

We employed a DRX device of type "RIGAKU ULTIMA-IV" operating at 20°C under 40 kV and 30 mA, with copper CBO radiation Kα (=1.54 Å).

S. Govindan and his team operate within these constraints:

Using a Rich Siefert 3000 XRD apparatus with Cu Kα1 radiation (λ = 1.5406 Å), the produced samples' structure was examined, and a HITACHI-SU 6600 FESEM was used to examine the surface morphology of the CS-Ag nanocomposites. Using a Shimadzu FTIR infrared affinity spectrometer, group confirmation was assessed.

The measured diffraction patterns ranged from 2° to 80° (2 at). 5°/min scanning speed to describe the crystalline substance. We compared and characterized this device with commercial chitosan.

III.3. The pH (potentiometer)

Figure II.4. *Potentiometer device OHAUS STARTER 2C (LAEPO)*

III.3.1. Fundamental

A pH meter is a scientific tool that quantifies the activity of hydrogen ions in aqueous solutions and uses the resultant pH value to indicate how acidic or alkaline the solution is. The potential difference between a pH electrode and a reference electrode is measured by a pH meter.

III.3.2. Tools

We measured and determined the value of DD in our experiment using the pH scale.

III.4 Measurement of conductivity

Figure II.5. *Conductivity meter device CDM210 (LAEPO).*

III.4.1. Fundamental

In the electrolyte, two parallel, flat stainless-steel electrodes of area S are placed face-to-face at a distance L from one another.

We observe that L is the rms current (in A) of the current passing through the solution when a sinusoidal AC voltage of rms value U (in V) is applied between these electrodes.

III.4.2. Tools

In our investigation, we calculated DD and made measurements using a conductivity meter**.**

III.5. Determination of the macromolecular weight by viscosity

Figure II.6. U-tube viscometer *(LAEPO)*.

III.5.1. Definition

A U-tube viscometer is a tool used to measure the viscosity of a liquid, especially Newtonian fluids where viscosity is constant with flow rate. It is often referred to as a glass capillary viscometer or an Ostwald viscometer (after Wilhelm Ostwald).

III.5.2. Fundamental

Viscosity is measured with a U-tube viscometer by measuring the flow of a liquid through a small tube. Slower flow is caused by higher viscosity, and Poiseuille's Law uses the instrument's design to relate viscosity to flow time.

III.5.3. Tools

Viscometry or viscosity technique was one of the first methods used for estimating the MW of polymers. In this method, the viscosity of polymer solution is measured, and the simplest method employed is capillary viscometry. This method involves recording both the flow time of the polymer solution (t) and the flow time of the pure solvent (t0). The ratio of the polymer solution flow time (t) to the flow time of pure solvent (t0) is equal to the ratio of their viscosities ($\eta/\eta 0$) only if they have the same densities .

The Ubbelohde capillary viscometer is used to determine the polymer's solution viscosity. The method entails injecting the polymer solution into the reservoir of the viscometer, and then aspirating to the top bulb. Afterward, the admittance of the air causes the polymer solution to flow down in the capillary by gravity. To obtain the average MW by this technique, the process should be performed for pure polymer solution and solvents accordingly. The pressure drop (ΔP) fluctuates based on the viscosity (η) of the solutions due to the fact that the polymer solution that flows through the capillary follows Poiseuille's rule for laminar flow .Chitosan molecular weight is determined from the intrinsic viscosity η by applying the Mark–Houwink equation [3].

$$[\eta] = K.M_v^{\alpha} \quad (3)$$

where:

• K and α are constants that depend on the polymer-solvent system at a given temperature

• Mv is the molecular weight in dalton (Da);

• η is the intrinsic viscosity.

K and α are the Mark–Howink coefficients whose values are respectively

And the specific viscosity was calculated with this equation [4].

$$\eta sp = \frac{t - to}{to} \quad (4)$$

where

Ƞsp = specific viscosity (sec)

t = time required for samples to flow (sec)

to = time required for solvent to flow (sec)

III.6. Thermogravimetric analysis (TGA)

Figure II.7. SDT Q 600 TGA *(LAEPO).*

III.6.1. Definition

A thermogravimetric analyzer (TGA) is a scientific equipment used to quantify the variations in mass of a sample in relation to temperature or time. These measurements can yield valuable data regarding a material's thermal stability, composition, decomposition kinetics, moisture content, and other relevant factors.

III.6.2. Fundamental

The fundamental premise of a TGA machine is rather straightforward: it tracks the alteration in mass of a sample while maintaining precise control over temperature and atmospheric conditions.

III.6.3. Tools

The thermal stabilities of the composite films were characterized using a synchronous thermal analyzer (SDT Q 600 TGA, TA Instruments).

For this aim, the temperature was increased from 30 to 800 ◦C at a rate of 10 ◦C/min under a nitrogen environment at a ventilation rate of 20 mL/min.

III.7. Dynamic mechanical analysis (DMA)

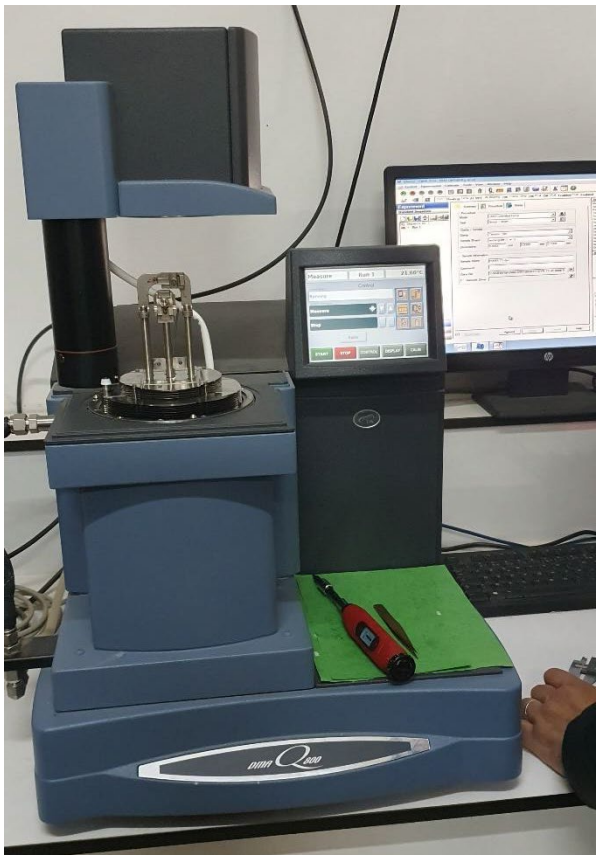


Figure II.8. DMA Q 800 *(LRM).*

III.7.1. Definition

Dynamic mechanical analysis (DMA) is a technique used to examine the mechanical characteristics of materials, particularly polymers, as a function of temperature, time, and frequency. It is a powerful instrument that may provide information about a material's stiffness, elasticity, damping, and transitions.

III.7.2. Fundamental

(DMA) focus on understanding how a material reacts under oscillating stress or strain, notably its viscoelastic qualities.

III.7.3. Tools

We measured the mechanical characteristics using DMA.

III.8. Scanning Electron Microscopy (SEM)

Figure II.9. Scanning Electron Microscopy (SEM) TM-1000.

III.8.1. Definition

Scanning electron microscopy (SEM) is a microscopy technique that employs a concentrated electron beam to examine the surface of a material. The electrons interact with atoms in the sample, generating diverse signals that include information regarding the surface topography and composition of the sample.

III.8.2. Fundamental

SEM utilizes electrons instead of light to examine the surface of a sample. The resulting signals reveal minute features such as texture and composition. This enables the visualization of fine details beyond the resolution capabilities of conventional light microscopes.

III.8.3. Tools

We employed scanning electron microscopy (SEM) to get highly detailed images of the surfaces of CCs and Ecs Films, allowing us to observe features as small as a few nanometers.

REFERENCES

1. Pakizeh, M., A. Moradi, and T.J.E.P.J. Ghassemi, *Chemical extraction and modification of chitin and chitosan from shrimp shells.* 2021. **159**: p. 110709.
2. Pérez-Álvarez, L., L. Ruiz-Rubio, and J.L.J.J.o.C.E. Vilas-Vilela, *Determining the deacetylation degree of chitosan: opportunities to learn instrumental techniques.* 2018. **95**(6): p. 1022-1028.
3. Derraz, M., et al., *Extraction and physicochemical characterization of an environmentally friendly biopolymer: chitosan for composite matrix application.* 2023. **7**(6): p. 260.
4. Marianti, A., Y. Anggraito, and W. Christijanti. *Effective gamma irradiation dose on viscosity and molecular weight reduction of chitosan*. in *Journal of Physics: Conference Series*. 2020. IOP Publishing.
5. Jiang, L., et al., *Evaluation of diffusion and dilution methods to determine the antimicrobial activity of water-soluble chitosan derivatives.* 2013. **114**(4): p. 956-963.
6. HIMED, L., S. MERNIZ, and M.J.A.J.N.P. BARKAT, *Evaluation des activités antioxydante et antibactérienne de l'huile essentielle de Citrus limon (variété Lisbon) extraite par hydrodistillation.* 2016. **4**(1): p. 252-260.

CHAPTER III

RESULTS AND DISCUSSION

PART I. Extracted chitin and chitosan results

After being recovered from sea garbage, shrimp shells and squid pen were washed for two hours using distilled water, dried, and then treated with NaOH and HCl to remove chitin. The resultant product was deacetylated using a concentrated solution of 60% NaOH; in order to get a high degree of DD.

I.1. Overview of extraction

The following table lists the masses of the products, both obtained and used:

Table III.1. *The results of the extraction of chitin and chitosan from squid pen and shrimp shells.*

	Shrimp shells		Squid pen	
Process	**The mass used (g)**	**The mass obtained (g)**	**The mass used (g)**	**The mass obtained (g)**
Purification Time: 1 Hour Temperature: RT	50g	40.80g	120g	120g
Deproteinization Time: 4 Hours Temperature: 60°C	20g	13.89g	20g	18.15g
Demineralization Time: 4 Hours Temperature: 60°C	13.89g	5.04g	18.15g	0.56g
Deacetylation Time: 4 Hour Temperature: 120°C	5.04g	2.88g	-	-

The outcomes of the chitin method's extraction from marine waste demonstrate that every action is followed by a mass loss, indicating that the product's content needs to be removed.

Because of the high calcium carbonate content in these earliest stages of demineralization, there was a significant mass loss, particularly for squid pen shells. The outcomes were comparable to those of the priceless works.

The percentages of mass loss in Squid pen and shrimp for the two crucial steps in the chitin synthesis process are shown in the table below, along with a brief comparison with the literature [1].

Table III.2*. The percentage of proteins and minerals in squid pen and shrimp shells used.*

	Shrimp		Squid pen	
	Experimental	Literature	Experimental	Literature
% proteins	30.55	34.02	9.25	10-30
% minerals	63.71	42.26	96.30	60-80

We essentially have no remaining squid pen due to deproteinization and demineralization, which indicates that the supply we have is mineral-rich.

I.2. Solubility test

The primary distinction between chitosan's original form and its acetylated version is that the former is a polymer with good solubility in acid solutions within the pH=4 range [2].The degree of deacetylation determines how soluble the product is; the higher the DD value, the more soluble the product is in an acidic medium. This means that a deacetylated product (one with a high DD value) will have many NH_2 groups, which can readily be protonated in an acidic medium to become soluble NH_3^+.

The results of a solubility test for chitosan using several solvents are displayed in the following table:

Table III.3. *Solubility test*

	Ethanol	Water	Acetone	Chloroform	Hydrochloric acid	Acetic acid
Solubility test	-	-	-	-	+	+

With: – means insoluble and + means soluble.

The findings demonstrate that synthesized chitosan is not soluble in any of the solvents tested, with the exception of acetic acid, where the product was found to be somewhat soluble, which may provide insight into the degree of deacetylation (less than 50%).

PART II. Study of chitosan Films

The figure showcases chitosan films produced as a result of our work. The following images provide a visual study of their essential properties such as their morphology.

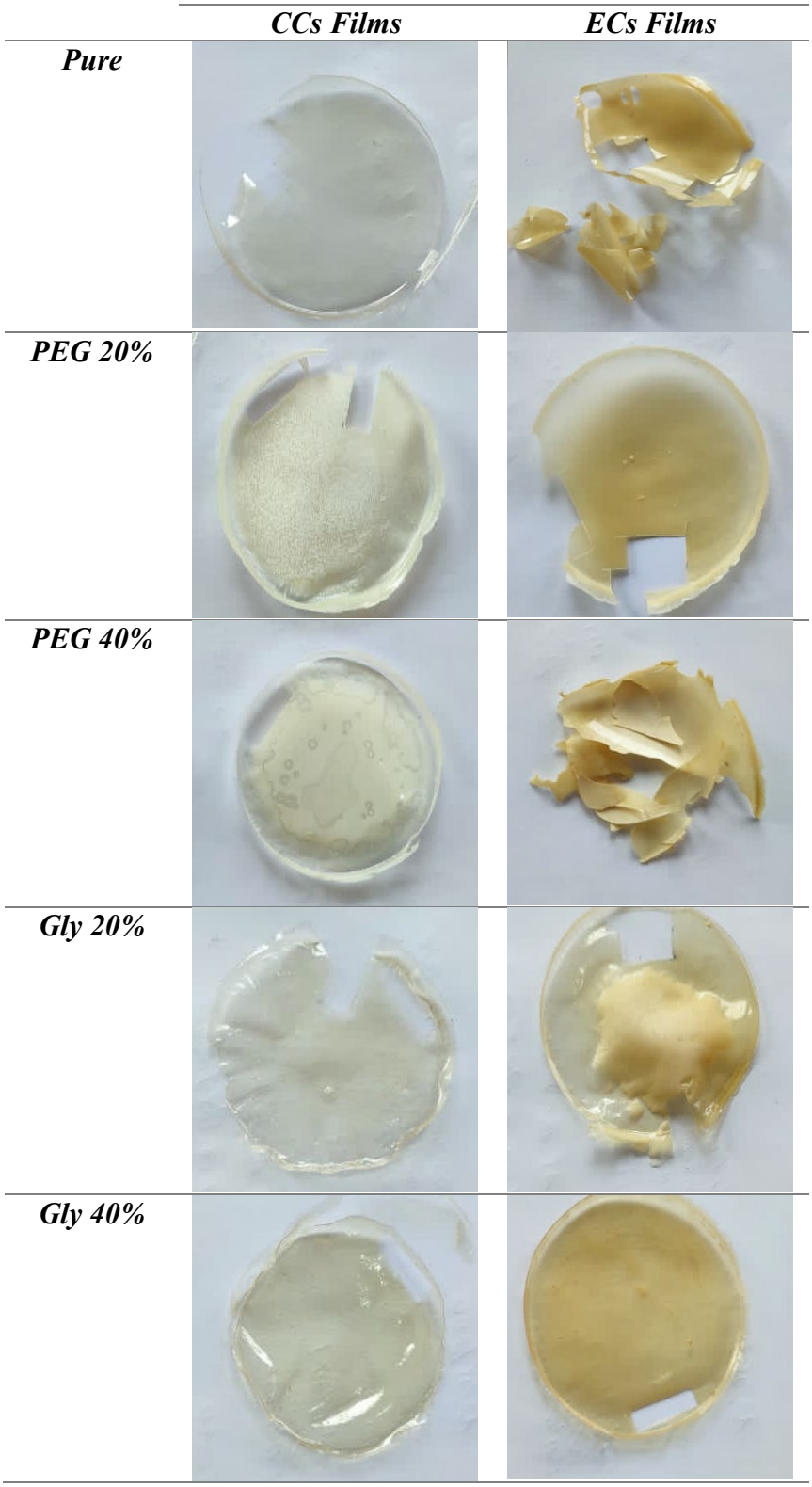


***Figure III.1**. Pictures of the produced films ECs/CCs*

II.1. Chitosan and chitin characterization

The rate of deacetylation, the presence of functional groups in the final product, and the crystallinity of the extracted material can all be tested analytically to assess how effective the extraction process was.

II.2. FTIR spectroscopy characterization of the produced products

The goal of FTIR analysis on our products is to identify their characteristic bands and compare them with those found in existing literature. The study's findings are displayed in figure III.2:

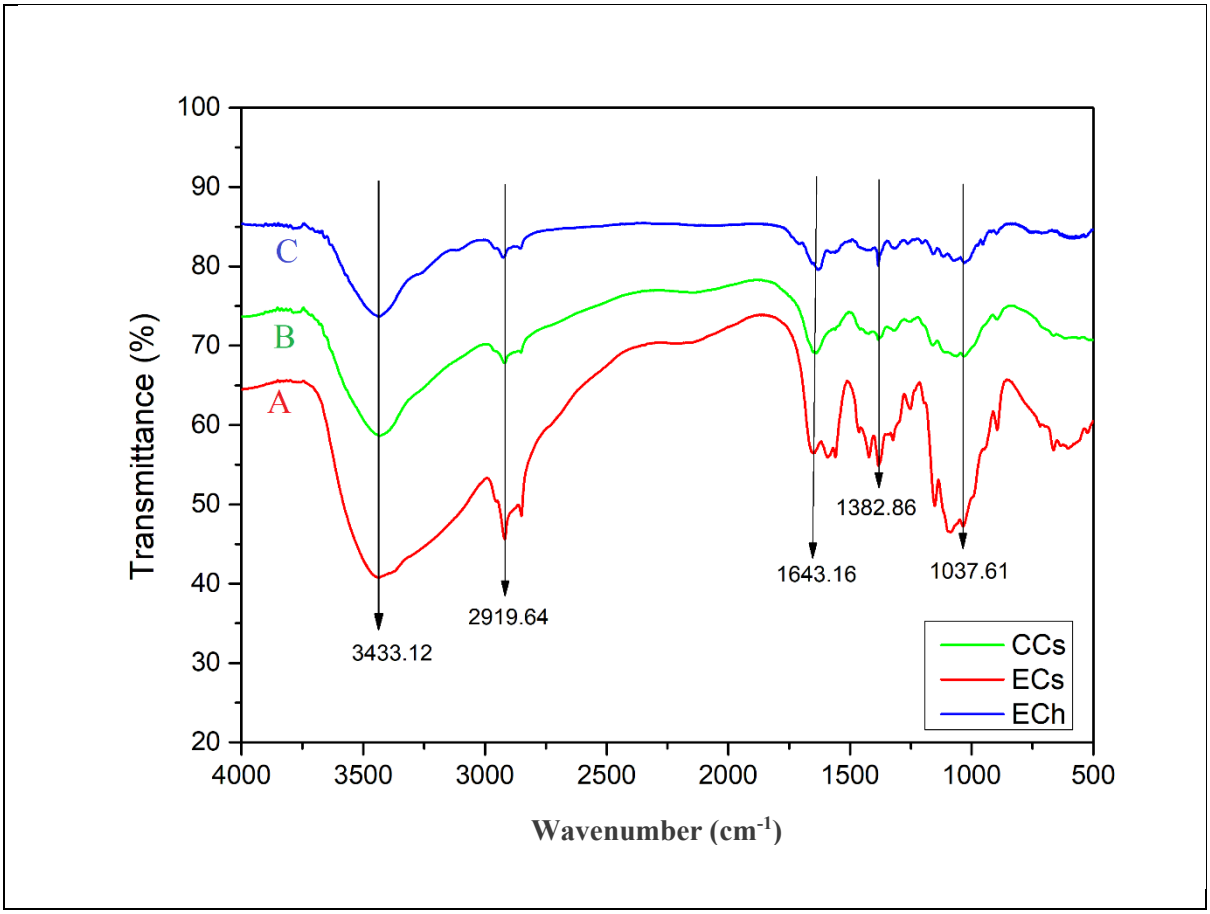


Figure III.2. *FTIR spectrum of ECs[A],CCs[B], ECh[C].*

Functional groups and vibrational modes can be seen in the FTIR analysis of chitin powder, which was carried out at wavenumbers between 400 and 4000 cm^{-1}.

The adsorption of the C-O-C group, which is the international standard for chitin production, at a wavenumber of 1073 cm^{-1} with a vibrational mode resembling a glucosamine ring, indicates the characteristic of chitin creation (Table III.1). Another common chitin IR absorption is the C-N group. With a stretching vibration mode, we obtained the C-N functional group at wavenumber 1318.57 cm^{-1}.

Table III.4. *FTIR characterization of chitin*

Functional Group	Wavenumber (cm^{-1})	
	Literature [3]	Research
-OH (stretch)	3448	3442
C – H (stretch)	2891	2919
C = O (stretch)	1680 – 1640	1650
N – H (bend)	1560 – 1530	1556
CH_3 (stretch)	1419	1382
C – O – C (glucosamine ring)	1072	1073
C-N (stretch, -$NHCOCH_3$ -)	1319	1318
N – H (bend)	750 – 650	756

Once chitin was obtained, the acetyl group was removed by deacetylation, which turned the chitin into chitosan. According to these findings, 10.3 g of chitosan were synthesized from 60 g of shrimp shell powder (Table III.1). Put another way, from the first period of shrimp shell powder, up to 17% of chitosan was created. The yield will decrease as more material is eliminated during the operation. To determine the effectiveness of chitosan and synthetic chitin, organoleptic tests were conducted. We came to the conclusion that physical changes including shape, color, and smell occur during every synthesis phase. Following the effective formation of chitin during the synthesis process.

The FTIR graph of the chitosan characterization results, which was produced using the same analytical technique as chitin, also reveals the distinctive functional groups of chitosan. Apart from the functional groups, the vibrations that take place and the chitosan's DD% were also determined. The research results' wavenumber pattern graph aligns with the pattern found in the literature [4].

Table.III.5. *FTIR characterization of chitosan*

Functional Group	Wavenumber (cm^{-1})	
	Literature [4]	Research
-C-O-C- (glucosamine ring)	1038.46	1037.61
N-H (bend, primary amine)	1642.72	1643.15
-CH_3 and –CH_2 (bend)	1427.33 - 1383.74	1382.86 - 1420.89
-C=O (amide)	1642.72	1643.15
-C-H (stretch)	2920.60	2919.64
-OH (stretch)	3433.70	3433.12

II.3. X-Ray Diffraction

XRD examination elucidates a material's crystallographic fingerprint. By studying the diffraction pattern of X-rays interacting with the material, the crystalline structure of chitosan depends extremely on its deacetylation process as well as on its amorphous chitin form.

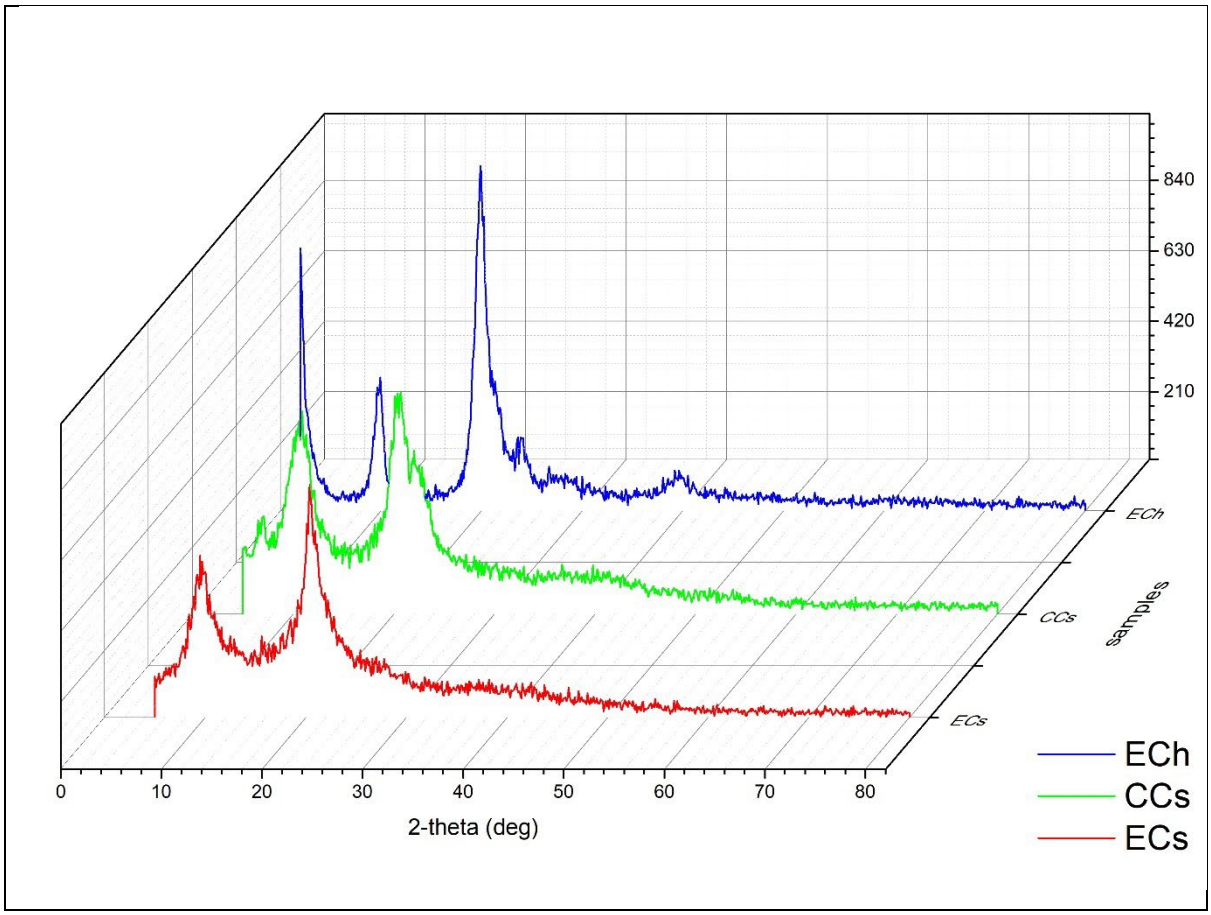


Figure III.3. *XRD diffraction pattern of the chitosan*

The XRD pattern of ECh shows two diffraction peaks occurring at (9.88° and 19.87°), and at 11° and 20.75° in CCs. For ECs, two peaks at 9.54 and 20.46° are shown in Fig. 4. The following equation is used to calculate the chitosan crystallinity index (CrI) [5]:

$$CrI = \frac{K \times I_C}{(I_c + I_a)} \qquad 1$$

where CrI, I_c, and I_a represent the degree of crystallinity, diffraction intensity in the crystalline region, and diffraction intensity in the amorphous region, respectively. K is a modulus having a value of 1.1 ~ 0.8. The crystallinity index values of chitosan obtained from the ECh, CCs, and ECs were 73%, 65%, and 68% respectively, Whereas in other studies, the CrI value of chitosan isolated from other insects, including beetles, cuttlefish, shrimp, and silkworms (B. mori), ranged from 36 to 95% [5] [6].

II.4. Conductimetric titration

The purpose of the conductimetric titration is to ascertain the degree of conversion of chitin to chitosan in the resulting product. The variations in the conductivity of the chitosan solutions (chitosan+ HCl) due to the addition of NaOH at different levels allow for the creation of the following curves:

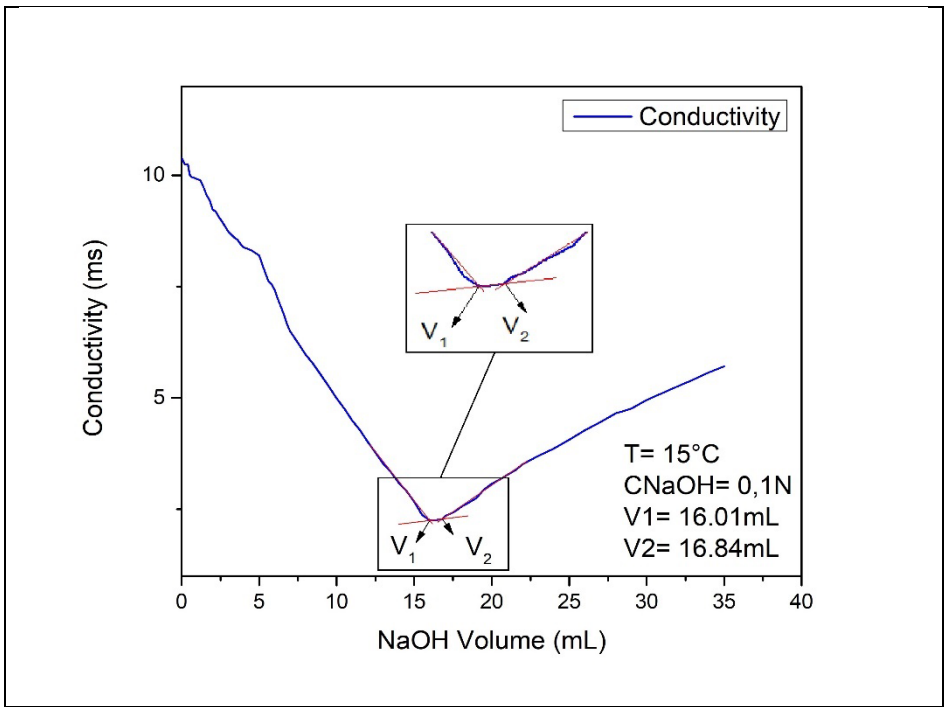


Figure III.4. *Results of conductivity changes for chitosan extracted from shrimp shells.*

The conductivity-volume graphs are separated into three distinct sections.

- The initial portion signifies a reduction in conductivity caused by a decrease in the quantity of H+ ions in the reaction medium as a result of their neutralization by OH^- ions.
- The second section demonstrates a stabilization of the conductivity in relation to the volume of base injected. This stabilization occurs because the NH_3^+ ions are converted into NH_2 ions.
- The final segment signifies a rapid surge in conductivity, resulting from the complete neutralization of ammonium ions and the abundance of OH^- ions.

The curves exhibit two sites of inflection, denoted as V_1 and V_2, respectively:

- V_1: Tells us how much sodium hydroxide is needed to counteract the hydronium ions from the hydrochloric acid that didn't help protonate the NH_2 groups in chitosan.
- V_2: Shows how much NaOH is required to protonate the NH_3^+ groups in chitosan. The volume of NaOH required to change NH_3^+ ions into NH_2 is represented by the difference between the two volumes, V_1 and V_2.

II.5. Potentiometric titration

The equivalent volumes of the neutralization of amino groups and H^+ ions are calculated for the DD values using a pH-metric dosage of chitosan solutions. The concept is the same as that of conductimetric titration. The study's findings are displayed in the curves below:

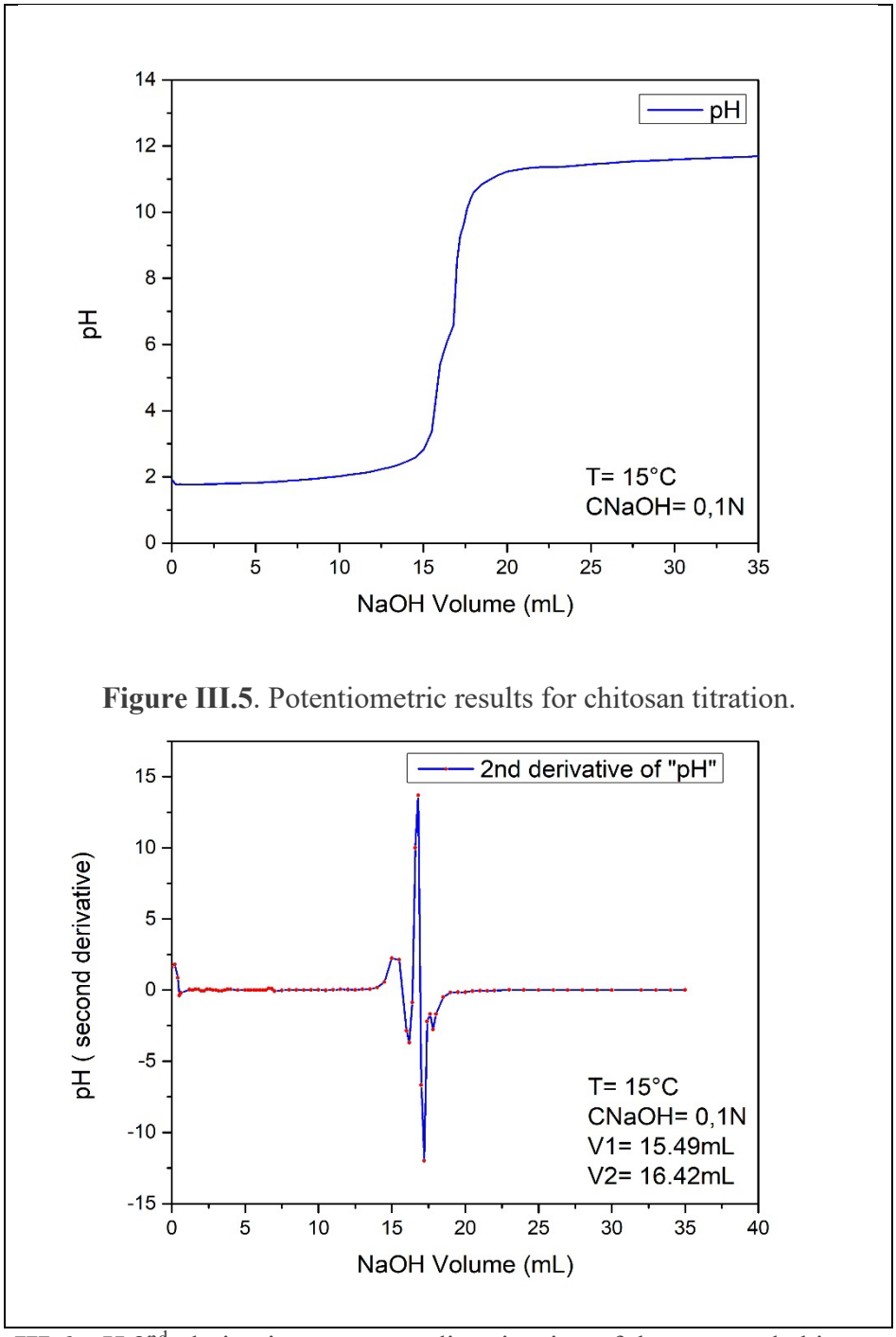


Figure III.5. Potentiometric results for chitosan titration.

Figure III.6. pH 2nd derivative corresponding titration of the prepared chitosan solution

II.6. Thermogravimetric analyses (TGA)

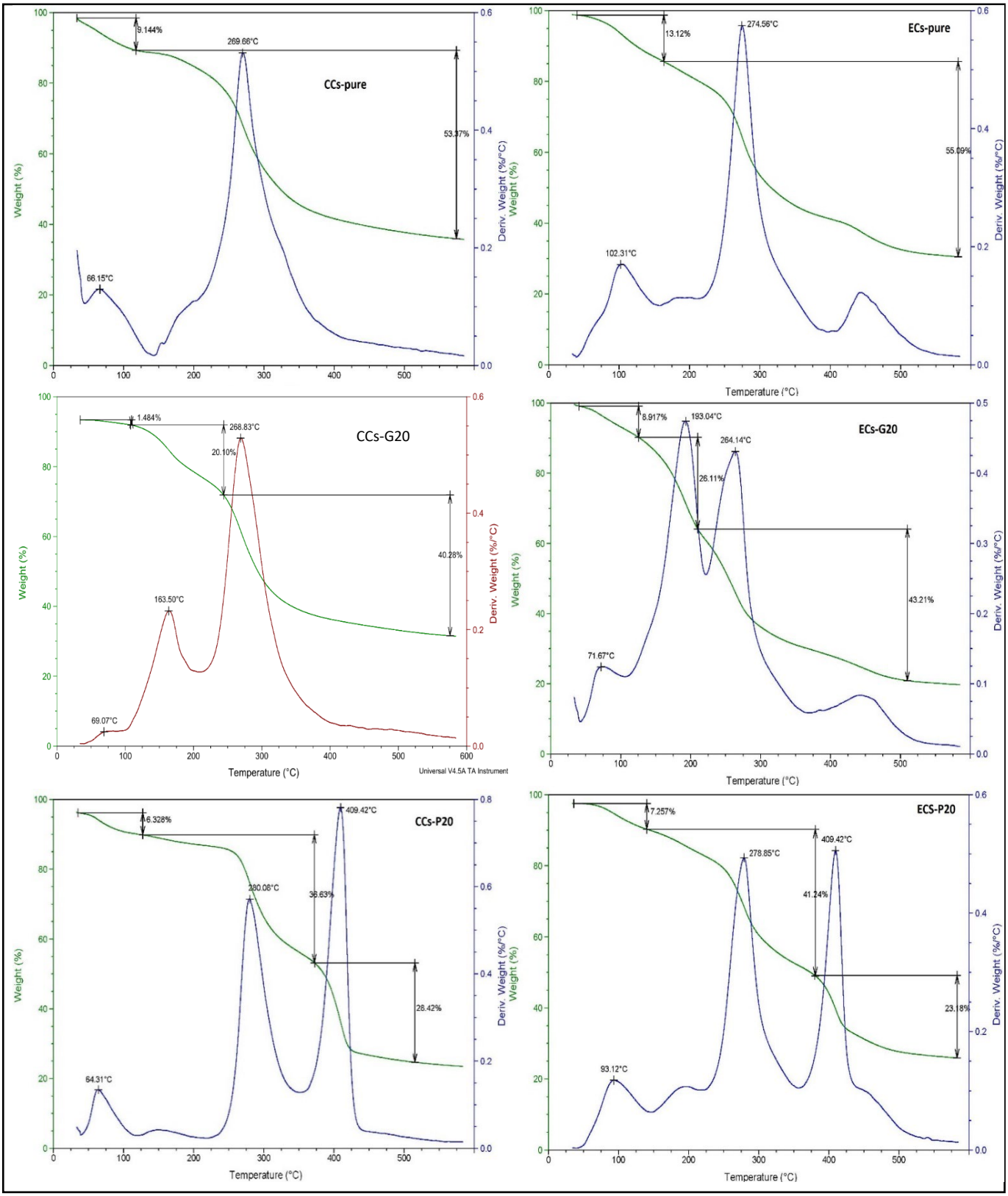


Figure III.7. *ATG Thermograms of Chitosan Films.*

TGA was used to investigate the effects of plasticizers PEG and glycerol on the thermal stability of the CS films, as shown in Figure III.4.

Three stages of thermal degradation of the CS main film were identified based on the TGA traces.

All samples lost weight during the first stage as a result of the temperature rising and the water in the film and the water adsorbed on the film surface gradually evaporating. between 70 and 100°C for both CCs and ECs (Pure/P20/P40) samples, the second stage of breakdown involves the breakage and destruction of the CS ring units between 230 and 500 °C. (that is, the glycosidic links in the main chains). On the other hand, the glycerol-containing samples (G20/G40) are related to the glycerol plasticizer's degradation. According to ECs-G20, the greatest mass loss connected to the CS component was roughly 43.21wt%. For the samples containing glycerol (G20/G40), the third step, which occurs between 500 and 600◦C , entails breakage and destruction of the CS chains. However, the weight loss associated with the PEG plasticizer's degradation was observed in the CCs and ECs (Pure/P20/P40) samples. All the results are shown in Table III.6

Table III.6. Extracted Chitosan Weight loss and Temperature of degradation.

Commercial Chitosan Films						
Samples	**Cs-Pure**		**Cs-P20**		**Cs-G20**	
	Weight %	**Temperature C**	**Weight %**	**Temperature C**	**Weight %**	**Temperature C**
1st stage	9.14	66.15	6.32	64.31	1.48	69.07
2nd stage	53.37	296.66	36.63	280.08	20.10	163.50
3rd stage	-	-	28.42	409.42	40.28	268.33
Extracted Chitosan Films						
Samples	**ECs-Pure**		**ECs-P20**		**ECs-G20**	
	Weight %	**Temperature C**	**Weight %**	**Temperature C**	**Weight %**	**Temperature C**
1st stage	13.12	102.31	7.25	93.12	8.91	71.67
2nd stage	55.09	274.56	42.24	278.85	26.11	193.04
3rd stage	-	-	23.18	409.42	43.21	264.14

II.7. Dynamic mechanical analysis (DMA)

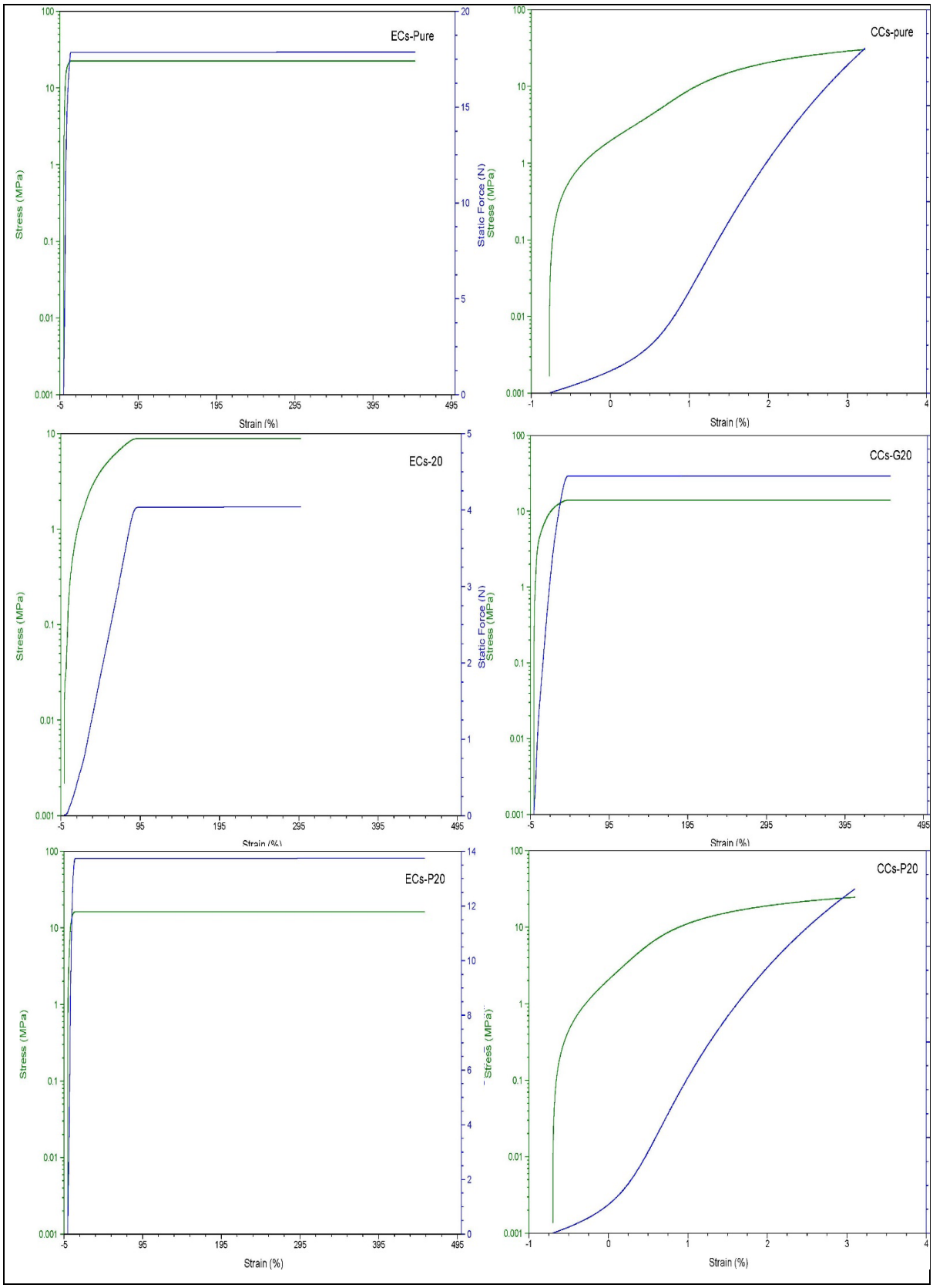


Figure III.8. *Mechanical properties of Chitosan films.*

Despite having favorable film-forming capabilities, CS is delicate and has inadequate flexibility. Plasticizers are commonly used to enhance the flexibility of CS and decrease the rigid interactions amongst CS. [7].

Table III.7. *Mechanical properties of Chitosan samples.*

	Commercial Chitosan Films				
Samples	**Cs-Pure**	**Cs-P20**	**Cs-P40**	**Cs-G20**	**Cs-G40**
Change in length (mm)	0,21	0.15	1.1	2.04	3.02
Strain (%)	3.22	3,09	361,5	452.2	421
Static Force (N)	18	18	17.4	12.51	5.11
Tensile Strength (MPa)	30.36	24.67	16.83	13.98	4,75
Breaking statue	-	-	+	+	+
	Extracted Chitosan Films				
Samples	**ECs-Pure**	**ECs-P20**	**ECs-P40**	**ECs-G20**	**ECs-G40**
Change in length (mm)	0.43	0.5	0.78	5.02	2.45
Strain (%)	447.3	452.2	16.61	296.1	618.2
Static Force (N)	17.87	13.75	18	4.01	4.65
Tensile Strength (MPa)	22,46	16.19	17.71	8.89	7.35
Breaking statue	+	+	-	+	+

Figure III.5 shows the stress and elongations at break for the all ECs/CCs films, indicating that adding a plasticizer increases the flexibility of the CS structure effectively. The highest elongations at break for the ECs-G40 is 618.2 %. In addition, upon increasing the amount of added plasticizer PEG/Glycerol, the tensile strength and elongation at break from G20 to G40 for both of ECs and CCs has been decreased. For ECs-P20 and ECs-P40 we notice a increasing of the tensile strength and elongation at break, on the other hand the rest (CCs-P20 and CCs-P40) their tensile strength and elongation at break is reduced. For CCs-pure, the maximum tensile strength of 30.01 MPa was achieved without adding any plasticizer, while the maximum elongation at break of 618.2 %was achieved upon adding 40% of Glycerol to extracted chitosan. Because of its small molecule size, glycerol interacts with the structure of chitosan nicely. The chitosan chains become less rigid and more mobile as a result of this interaction, which also weakens the hydrogen bonds that hold them together. [8] . However, excess glycerol or polyethene glycol can also destroy the hydrogen bonds between the CS chains, reducing the tensile strength of the film. [9]. Figure III.9 shows the tensile strength of the Cs samples and the added plasticizers.

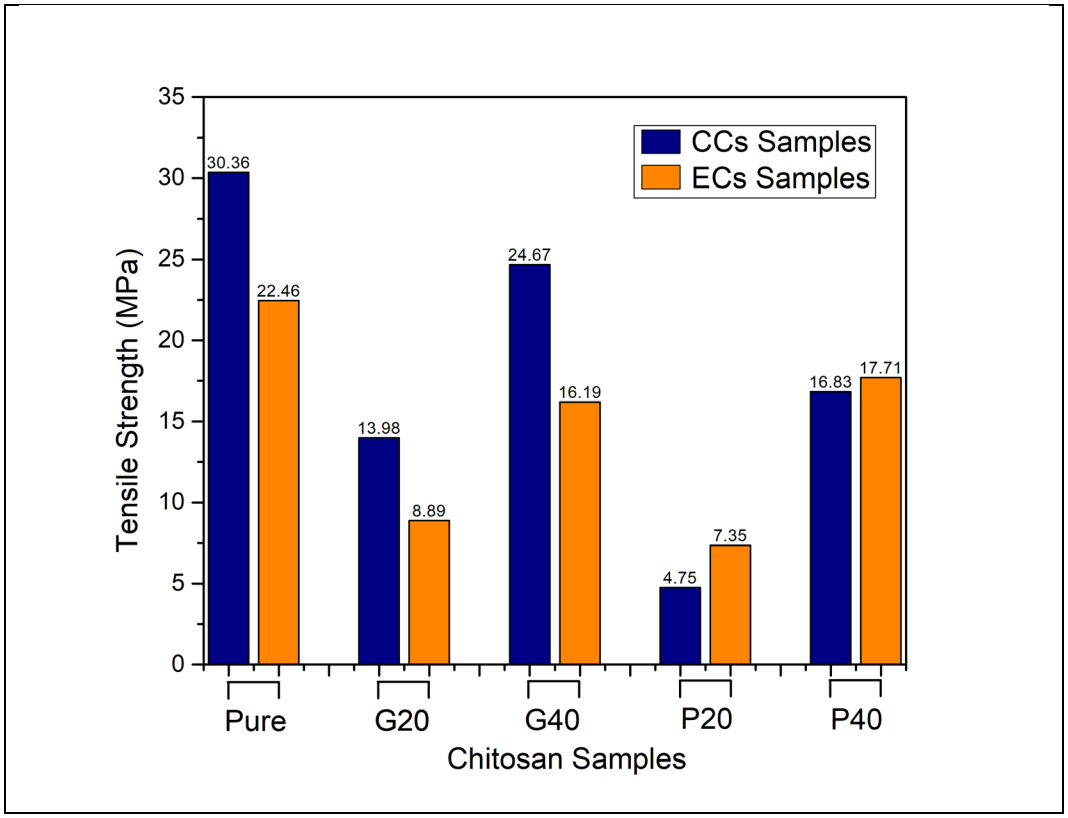


Figure III.9. *Tensile Strength of CCs and ECs films.*

II.8. X-Ray Diffraction of CCs and ECs Films

X-ray diffraction (XRD) was used to assess the impact of adding PEG and Glycerol on the crystallinity of the films that were produced. Figure III.10 demonstrates that there were no more characteristic absorption peaks observed in the CCs film when the amount of plasticizers was increased. However, the characteristic absorption peak of 2θ=9◦ initially decreased in intensity, indicating reduced film crystallinity. Upon further increasing the concentration of PEG or Gly, the film exhibited a subsequent increase in the characteristic absorption peaks, followed by a subsequent decrease. These results can be attributed to the fact that in the presence of a small amount of PEG or Gly, the ordered arrangement of the molecular chains was disturbed, and the crystallinity was reduced. XRD patterns of the CCs-Pure, CCs-G20, CCs-G40, CCs-P20, and CCs-P40 films can be observed in Figure III.10. These patterns reveal the presence of distinct absorption peaks for the CCs-P and CCs-G films. Upon increasing the PEG and Gly content in both films, the characteristic absorption peaks at 2θ values of 9◦ disappeared, and the intensities of the other peaks also weakened significantly; these observations indicate the presence of interactions between PEG/Gly and CS in the crystal zone, which disrupted the molecular chain arrangement of CS, reducing the crystallinity. Moreover, a higher proportion of PEG/Gly leads to a reduction in film crystallinity, particularly when the PEG/Gly percentage exceeds 20%. These findings suggest that the presence of PEG/Gly

disturbs the molecular arrangement of chitosan in the CCs-P and CCs-G films. While it forms partial hydrogen bonds with Cs, it decreases the overall crystallinity of the film [10].

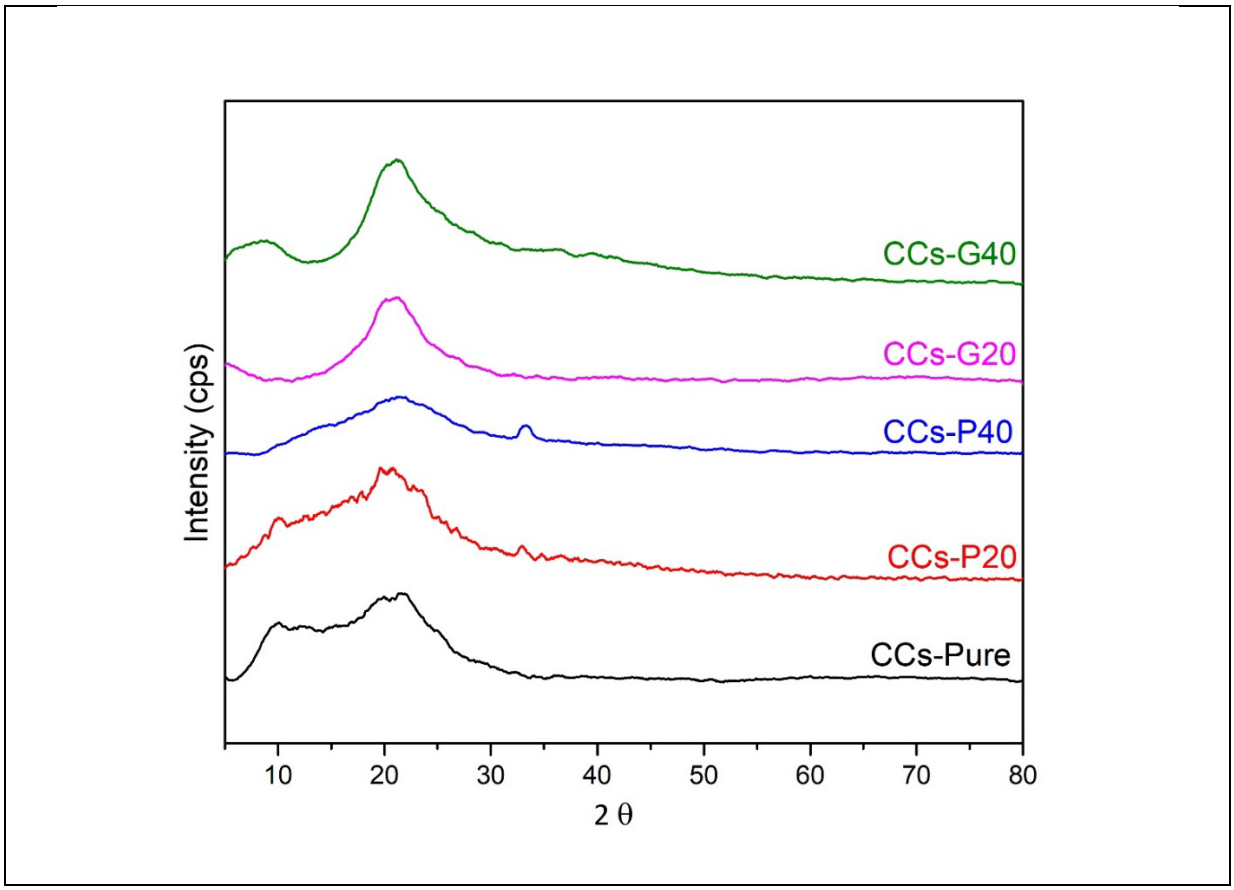


Figure III.10. *XRD spectra of all CCs samples.*

II.9. Determination of the macromolecular weight by viscosity

In order to determine the macromolecular weight of chitosan we must first find the intrinsic viscosity. 8 samples were prepared from both commercial chitosan and our own product in two different ways: first one chitosan on its own and the second one chitosan with salt NaCL.

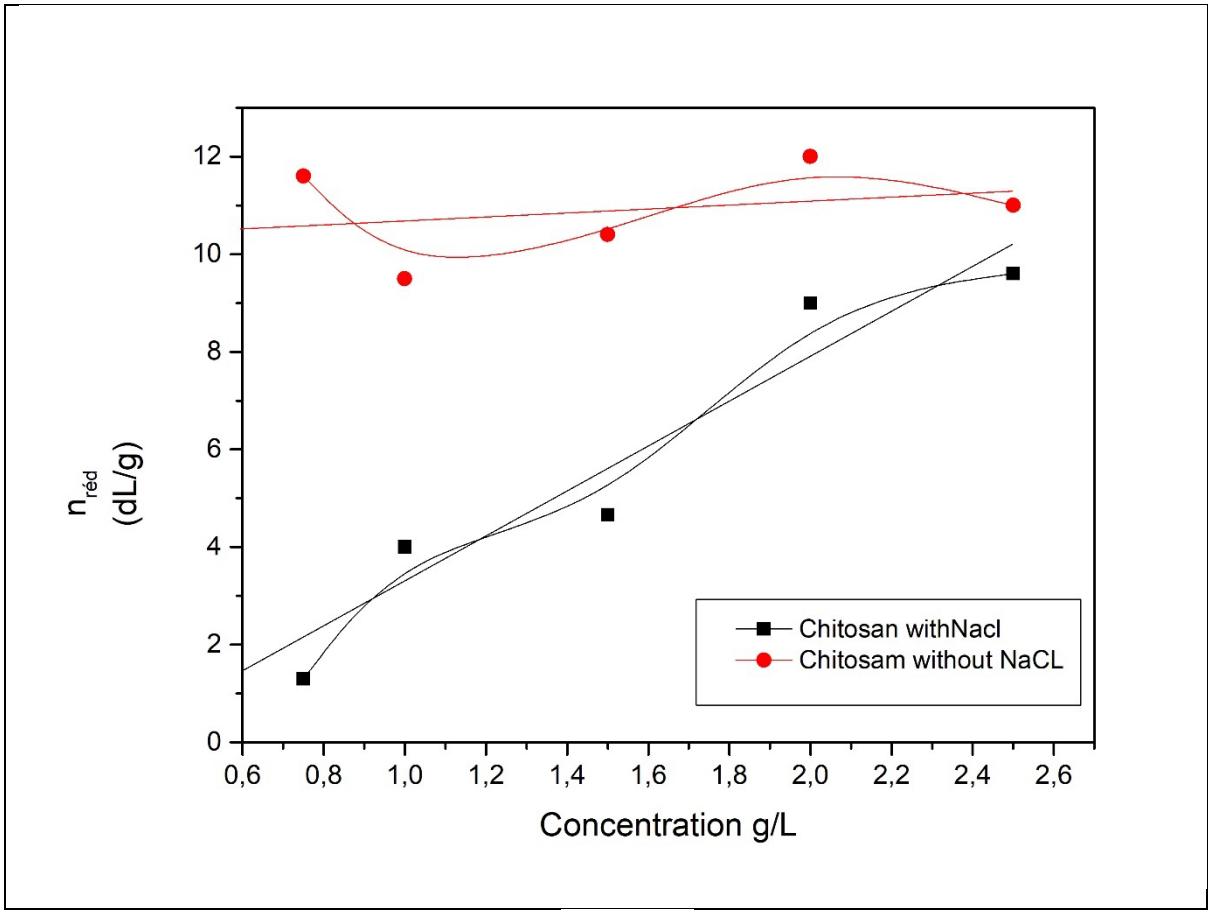


Figure III.11. *Reduced viscosity of ECs in* Acetic acid *solution with and without NaCl 0.1N at temperature 23*°C

II.1.2.1. *Viscosimetric study in the absence of salt (NaCl)*

The ECs under study exhibited polyelectrolyte behaviour. The production of the polymer results in the formation of anionic charged sites due to partial hydrolysis. In the low concentration zone, the hydrophobic interactions between the macromolecular chains are preferred, resulting in an elevated reduction in viscosity. Consequently, there is a reduction in viscosity, indicating the relaxation of macromolecular chains.

II.1.2.2. *Viscosimetric study in the presence of salt (NaCl)*

The addition of salt generates a considerable reduction in reduced viscosities compared with those obtained in the absence of salt. This phenomenon was noticed in the case studied. We remark that the anti-polyelectrolyte behaviour does not exist in our case. The polymers demonstrate polyelectrolyte behaviour. The addition of salt creates chain charges which results in reduced viscosity [11]

Macromolecular viscosimetric masses are calculated by the Mark Houwink relation using the parameters k and α.

K and α are the Mark–Howink coefficients whose values are respectively $1.81.10^{-3}$ $mL \cdot g^{-1}$ and 0.93 in the situation where acetic acid is taken as solvent [12].

Table III.8. Results of a viscometric study of chitosan solutions.

	Intrinsic viscosity dL/g	**Macromolecular weight.10^{-3} g/mol**
In the absence of NaCl	10.50	478
In the presence of (NaCl 0.1N)	1.47	192

II.10. Scanning Electron Microscopy (SEM)

The CCs and ECs was plasticized with glycerol and PEG at 20%, resulting in the formation of small particles dispersed within the matrix. This may be attributed to poor homogenization during the preparation of the plasticized chitosan solutions. However, this is not consistent with the results obtained from the mechanical properties tests, which indicate that glycerol and PEG are effective plasticizers for both of CCs and ECs. Therefore, there should be a strong complexation. Because glycerol and PEG include hydroxyl groups, they can interact with the functional groups of chitosan (-OH and -NH2).

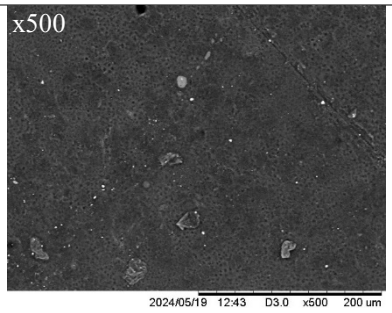
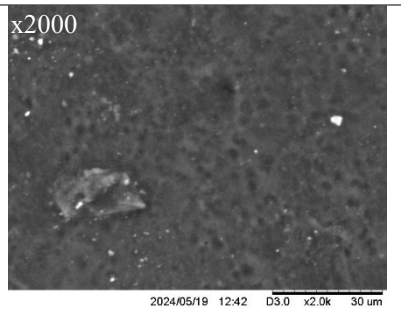
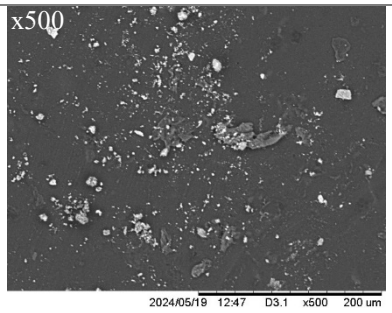
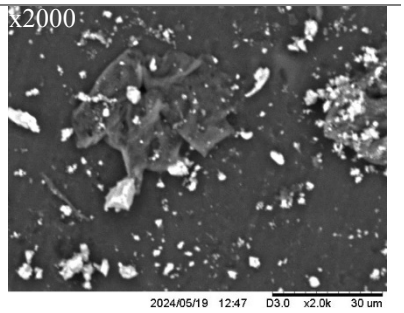
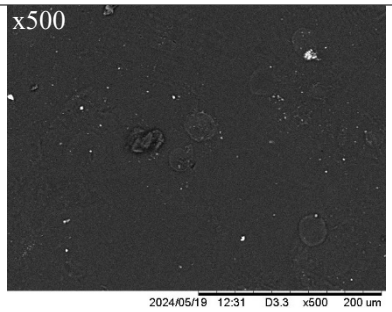
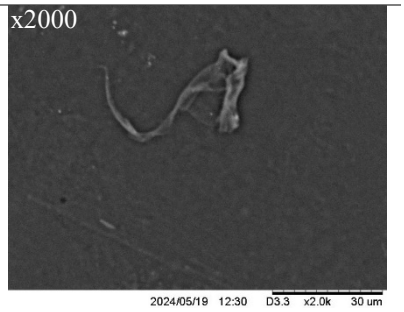
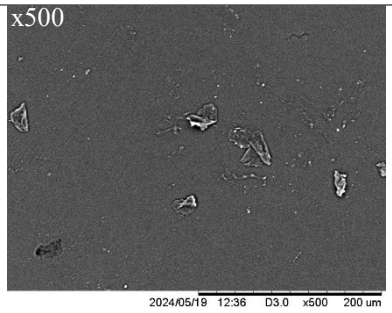
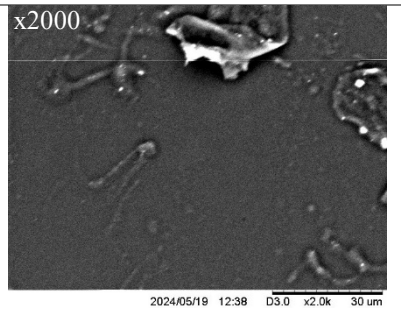
ECs-P20		
ECs-G20		
CCs-P20		
CCs-G20		

Figure III.12. Scanning electron microscopy images of the prepared films.

II.11. Determination of DD values

The characterization techniques employed in this work have the goal of determining the level of chitosan deacetylation:

Table III.9. Degree of deacetylation.

Determination Methods	DD%
Conductimetric analysis	71.74%
Potentiometric	78.97 %
FTIR	79 %

II.11.1. FTIR spectroscopy and DD

The following expression can be used to determine the deacetylation degree values by infrared analysis [13].

$$\%DD = \left[1 - \left(\frac{A1643}{A3433} \times \frac{1}{1.33}\right)\right] \times 100 \qquad 2$$

A: The absorbance at 1643, corresponds to NH absorbance.

A: The absorbance at 3433, corresponds to the absorbance of OH groups.

1.33: The theoretical constant, or the ratio of A_{1643}/A_{3433}, for completely acetylated chitosan.

A point to be remembered:

The transmittance values as a function of wave number are provided by the previously obtained results; however, in order to use the previously stated relation, one must first apply the relation between absorbance and transmittance:

A=-log(T).

As an example of a calculation, we use the previous formula to determine the DD of chitosan extracted from shrimp [4].

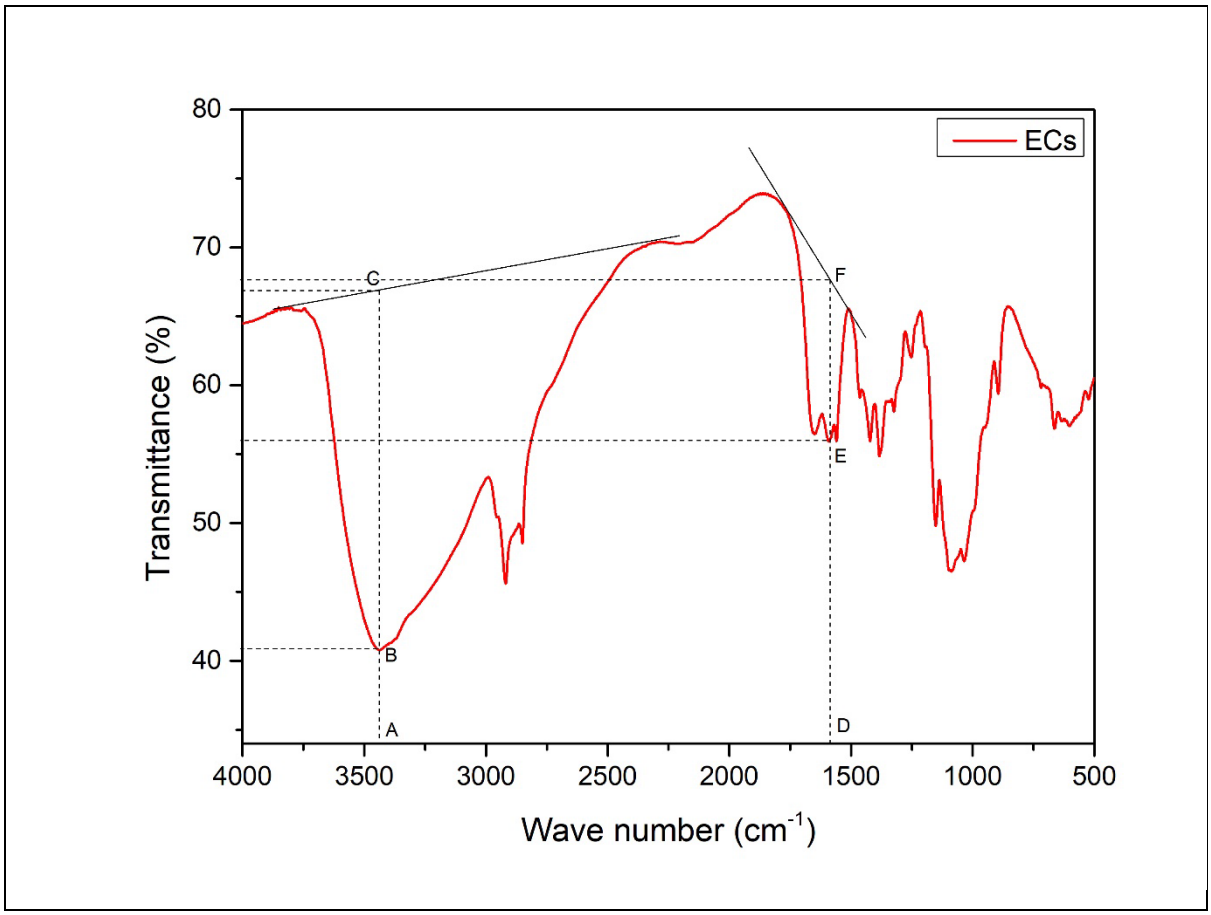


Figure III.13. *Determination of deacetylation degree of chitosan from shrimp shells.*

$$\boldsymbol{A_{1643}} = \frac{\log DF}{\log DE} = \log\frac{69.55 - 34}{56{,}46 - 34} = \log 1.58 = \mathbf{0.19}$$

$$A_{3433} = \frac{\log AC}{\log AB} = \log \frac{66.77 - 34}{40.91 - 34} = \log 4.74 = \mathbf{0.67}$$

$$\%\mathbf{DD} = \left[1 - \left(\frac{0.19}{0.67} \times \frac{1}{1.33}\right)\right] \times 100$$

%DD = 79 %

II.11.2. Conductimetric analysis

We were able to determine the volume required to protonate the deacetylated monomers using the conductimetric assay; we will use this volume to determine the DD value of each synthesized sample using the following relation [13].

$$\%\mathrm{DD} = \left[203 \times \frac{(\mathrm{V2} - \mathrm{V1}) \times \mathrm{N}}{\mathrm{m} + 42 \times (\mathrm{V2} - \mathrm{V1}) \times \mathrm{N}}\right] \times 100 \qquad 3$$

With:

V1: The NaOH volume needed to neutralize the excess HCl.

V2: The amount of NaOH needed to neutralize -NH3+.

m: The chitosan mass (in milligrams) used.

N: The titrant solution's normality (mol/L).

203: The acetylated sample's macromolecular mass.

42: The distinction between the acetylated and deacetylated chitosan's macromolecular masses.

- Here is an example of a calculation with Conductimetric analysis:

$$\%\mathbf{DD} = \left[203 \times \frac{(16.84 - 16.01) \times 0.1}{20 + 42 \times (16.84 - 16.01) \times 0.1}\right] \times 100$$

%DD = 71.74%

II.11.3. Potentiometric analysis

- Here is an example of a calculation with pH secondary derivative curve corresponding to the titration of the prepared chitosan solution:

$$\%\mathbf{DD} = \left[203 \times \frac{(16.42 - 15.49) \times 0.1}{20 + 42 \times (16.42 - 15.49) \times 0.1}\right] \times 100$$

%DD = 78.97 %

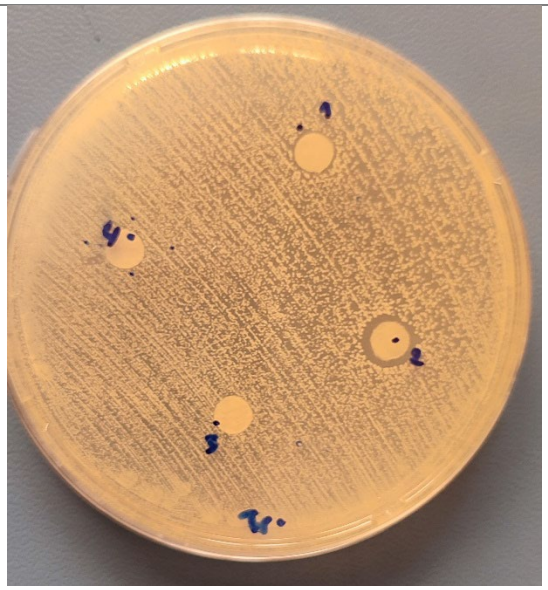
PART III. Application of chitosan Films

III.1. Antibacterial activity of the Films

Chitosan films, derived from natural substances, are attracting interest due to their antimicrobial properties. These films are both biodegradable and non-toxic to human cells. Additionally, they have inherent qualities that can either kill or hinder the growth of dangerous bacteria. Due to these characteristics, they are well-suited for use in both medical and food preservation contexts.

III.1.1. Antibacterial Function

Inhibition zone test



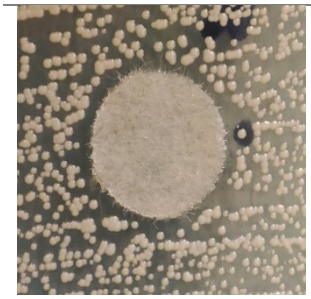
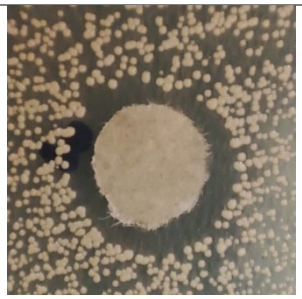
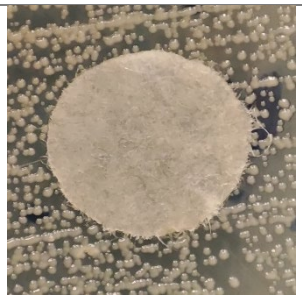
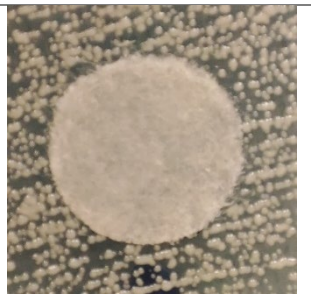
1-CCs	*2-ECs*	*3-PEG Plasticizer*	*4-Glycerol Plasticizer*
			
Diameter (mm)			
7.71	**8.86**	**6**	**6**

Figure III.14. *Inhibition zone test for commercial and extracted chitosan against E. coli.*

III.1.2. Determination of antibacterial activity

The sensitivity of the various strains to chitosan studied is classified according to inhibition diameter according to the following criteria: Ø<8 mm: resistant bacterium (-); 8≤Ø≤14 mm (+): susceptible bacterium; 15≤Ø≤19 mm: highly susceptible bacterium (++) and Ø≥20 mm (+++): extremely susceptible bacterium [14].

The diameter of our extracted chitosan is 8.86 mm, indicating that it contains a susceptible bacterium (+). In contrast, the commercial chitosan has a diameter of 7.71 mm, indicating a negative resistant bacterium. The diameters of both PEG and Gly remained unchanged which means that they don't have any resistance against E-Coli bacteria.

in technical Insights the molecular Mechanism Cs produces its antibacterial activity by interacting with the microbial cell membrane, causing leaking of cellular contents and eventually cell death.

The zone of inhibition is the clear area around the chitosan disc where bacterial growth has been stopped. In our case, the diameter of this zone is 8.86 mm.

The bacterium's susceptibility to the antimicrobial agent is established by measuring the diameter of the zone of inhibition. Various levels correlate with varying diameters. We calculated the distance between the edges of this clear area in the Zone of Inhibition to be 8.86 mm. This indicates that the bacterium is being affected by the chitosan and is not able to grow in this area.

Susceptibility the true interpretation of our 8.86 mm measurement necessitates a comparison with a reference chart. Based on the size of the inhibition zones, these charts categorize antibiotics according to their level of effectiveness.

The variables affecting the outcomes are the efficiency of chitosan can be influenced by its molecular weight and degree of deacetylation.

REFERENCES

1. Abdou, E.S., K.S. Nagy, and M.Z.J.B.t. Elsabee, *Extraction and characterization of chitin and chitosan from local sources.* 2008. **99**(5): p. 1359-1367.
2. Tezkratt, A. and I. Bir, *Optimisation des paramètres de l'obtention du chitosane et ses applications dans le traitement des eaux de lavage de la station de potabilisation TAKSEBT*. 2018, UMMTO.
3. Stuart, B.H., *Infrared spectroscopy: fundamentals and applications*. 2004: John Wiley & Sons.
4. Sulistyani, S., H. Hasanah, and T.J.J.S.D. Wijayanti, *Synthesis and optimization of chitosan nanoparticles of shrimp shell as adsorbent of Pb2+ Ions.* 2017. **6**(2): p. 143-150.
5. Zhang, M., et al., *Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia.* 2000. **27**(1): p. 99-105.
6. Mohan, K., et al., *Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects.* 2020. **105**: p. 17-42.
7. Li, C., et al., *Preparation and plasticizing mechanism of deep eutectic solvent/lignin plasticized chitosan films.* 2023. **240**: p. 124473.
8. Hao, Y., et al., *Synthesis and evaluation of bio-based plasticizers from 5-hydroxymethyl-2-furancarboxylic acid for poly (vinyl chloride).* 2020. **59**(40): p. 18290-18297.
9. Taysun, M.B., E. Sert, and F.S.J.B.J.o.C.E. Atalay, *Synthesis, characterization and acid-catalyzed application of ternary deep eutectic solvent: effect of glycerol addition.* 2022: p. 1-9.
10. Pan, M., et al., *Physicochemical transformation of rice straw after pretreatment with a deep eutectic solvent of choline chloride/urea.* 2017. **176**: p. 307-314.
11. HOCINE, T., *Etude sur un pilote de la floculation de nouveaux floculants Acrylamide-4-Vinylpyridine pour la fixation des métaux lourds et des micropolluants*. Université de Tlemcen-Abou Bekr Belkaid.
12. Khan, T.A., K.K. Peh, and H.S.J.J.P.P.S. Ch'ng, *Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing.* 2000. **3**(3): p. 303-311.
13. Zhang, Y., et al., *Determination of the degree of deacetylation of chitosan by potentiometric titration preceded by enzymatic pretreatment.* 2011. **83**(2): p. 813-817.
14. HIMED, L., S. MERNIZ, and M.J.A.J.N.P. BARKAT, *Evaluation des activités antioxydante et antibactérienne de l'huile essentielle de Citrus limon (variété Lisbon) extraite par hydrodistillation.* 2016. **4**(1): p. 252-260.


CONCLUSION

The overall objective of our work was originally to enhance the value of marine by-products (Shrimp shells, Squid pen), with the aim of using them for the preservation of multiple application were antimicrobial properties is necessary.

Following the deproteinization and demineralization procedures for the squid pen, we observed a loss in mass. This indicates that the studied product contains a significant amount of matter that needs to be removed, with the most substantial loss occurring during the deproteinization stage. all the rest of work was centered exclusively on using shrimp shells.

The chitosan obtained through this method has a high degree of deacetylation (79%), which allowed us to obtain films and use them as biodegradable packaging.
This material is well-known for its film-forming and antimicrobial properties, but it has the disadvantage of being sensitive to moisture and brittle, which limits its applications in the packaging industry.

The aim of our study was to enhance plasticity of chitosan films by plasticizing them using two hydrophilic plasticizers: Glycerol, PEG 3000.

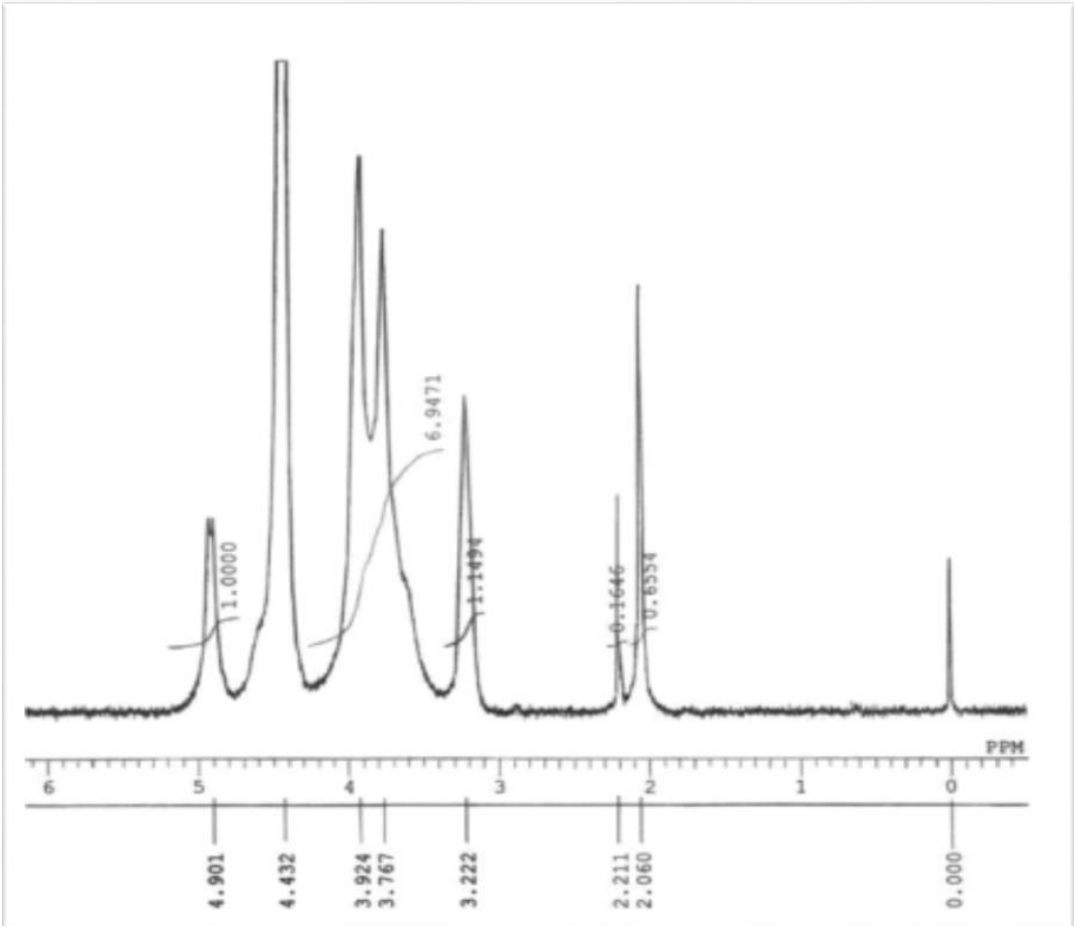
Glycerol and PEG can enhance elasticity of chitosan films up until a certain point, the amount of plasticizer injected does not cause the antiplasticization phenomenon to take shape.

After films were made, we have targeted the microbiological activity of commercial chitosan, extracted chitosan, glycerol and PEG. Since the diameter is 8.86 mm, we have observed that our extracted chitosan has very strong antibacterial activity against E-coli bacteria.

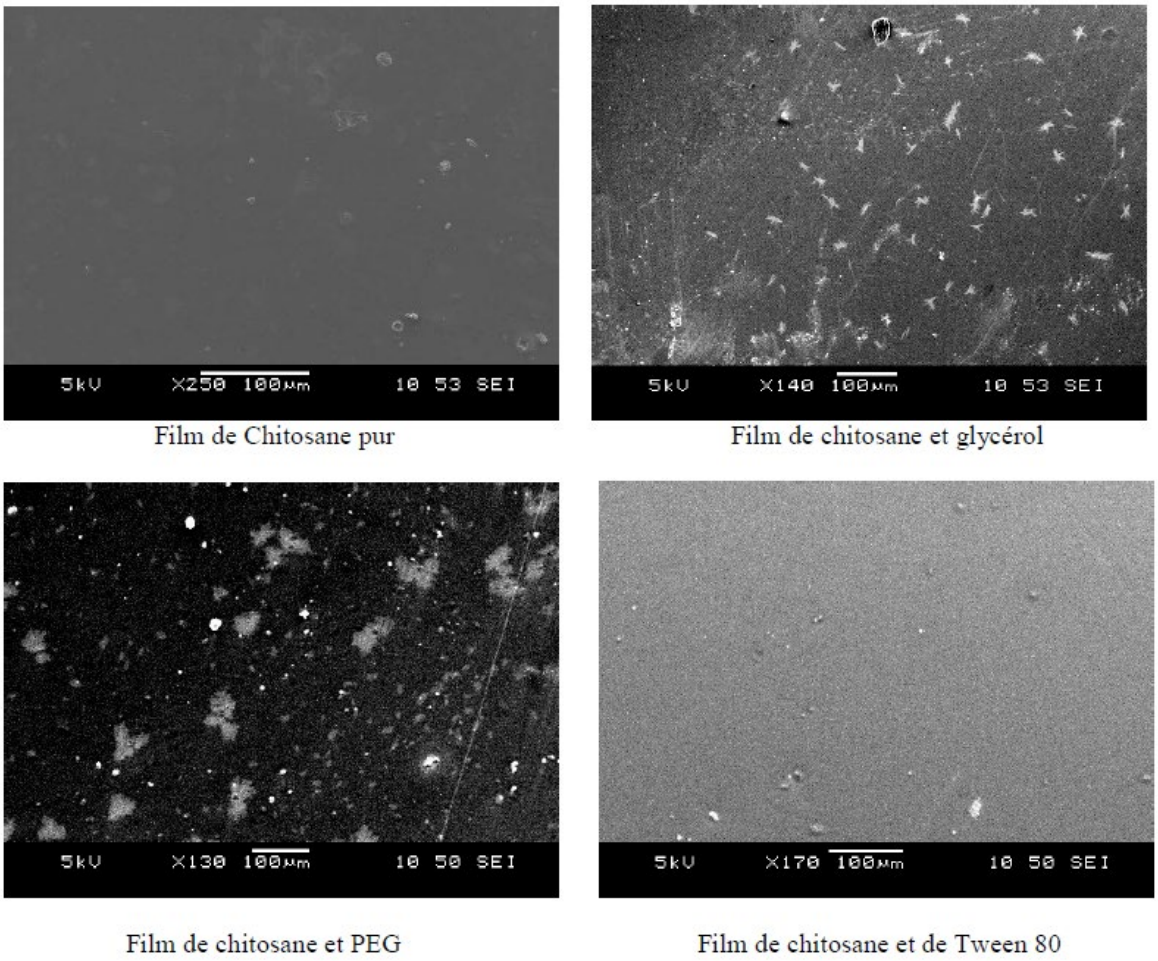
The work of this study is far from being completed and several aspects need to be further investigated:

- The water's permeability for pure and plasticized chitosan films (applied to fresh fruits and vegetables).
- Utilizing different plasticizers, such as Tween 80, PET, POM, and PEG 6000 with different ratios.
- Aim the Biomedical and Pharmaceutical applications such as Antioxidant, Drug delivery or Wound management.
- Determination of degree of deacetylation using ^{1}H-NMR analysis.
- Testing other bacteria's such as Salmonella, Vibrio cholerae and Vibrio vulnificus on the films in order to see the resistance.

ANNEXES



Annex 1. *^{1}HNMR results of chitosan extracted from pink shrimp with 40%NaOH and 1 day of deacetylation in autoclave.*



Annex 2. *SEM micrographs of chitosan membranes.*

Chitosan characteristics	Sources chitosan extracts		
	Blaps lethifera	*Pimelia fernandezlopezi*	*Musca domestica*
Yield (Y)	50.0 ± 0.3%	41.7 ± 0.5%	57.9 ± 0.2%
Moisture content (MC)	14.3 ± 0.3%	17.2 ± 0.2	7.8 ± 0.1%
Ash contents (AC)	1.5 ± 0.1%	2.0 ± 0.1	8.2 ± 0.2%
Water binding capacity (WBC)	515.1 ± 6.5%	287.0 ± 5.8	301.1 ± 4.3%
Fat binding capacity (FBC)	296.7 ± 14.5%	433.5 ± 11.3	455.1 ± 13.2%
Degree of deacetylation (DD)	87.1 ± 0.2%	88.2 ± 0.1%	84.1 ± 0.3%
Crystallinity index (CrI)	84.0 ± 0.1%	73.0 ± 0.4%	81.0 ± 0.2%

The findings are displayed as mean ± SD ($n = 3$)

Annex 3. *Characteristics of various sources of chitosan extracts.*

Alkali Concentration (Molar)	Solid to liquid ratio (g/ml)	Temperature (°C)	Duration	Remained protein in chitin (%)	Consumed NaOH/ shell weight (mole/ g)	Explanation
0.75 M NaOH	1st bath: 1:1.52nd bath: 1:1	~100	1st: 15 min 2nd: -	5.1*	0.002	2 bath
0.3 M NaOH	–	80–85	1 h	–	–	3 bath
1 M NaOH	1:15	<70	24 h	<1%	0.015	vigorous stirring
2.25 M NaOH	–	65	90 min	–	–	–
0.3 M NaOH	–	80–85	1 h	–	–	3 bath
0.75 M NaOH	1:2.5	RT	24 h	0.8%	0.002	–
0.25 & 1 M NaOH	1st stage: 1:40 2nd stage: -	40	2–6 h	~3%	–	1st: sonicate for 0, 1, and 4 h in 0.25 M NaOH 2nd: soak for 2 h in 1 M NaOH
1 M NaOH	–	105–110	–	–	–	several bath
0.5 M NaOH	1:30	90	2 h	–	0.015	–
1.0 M NaOH	1:20	70	24 h	–	–	several bath
1 M NaOH	1:20	70	–	–	0.02	–
2 M NaOH	1:4	45	4 h	~0.8%	0.008	constant stirring @ 100 rpm
2.5 M NaOH	–	65	6 h	–	–	–
0.9 M NaOH	1: 10	65	2 h	–	0.009	constant stirring
2 M NaOH	1:20	46	125 min	3.1%	0.04	–
1 M NaOH	–	1st stage: Boiling 2nd stage: cooling @ RT –	1st: 1 h 2nd: 30 min –	–	–	–
1 M NaOH	–	45	2 h	*4.8%	–	–
2 M NaOH	1:20	90	12 h	~4%	0.04	–
1 M NaOH	1:10	100	20 min	~3%	0.01	–
1 M NaOH	–	I: 80	I:2h II: 5 + 3 = 8 min	–	–	–
2.5 M NaOH	1:10	1st stage: boiling 2nd stage: -	1st: 6 h 2nd: 72 h	–	0.025	I: conventional method II: assimilated by microwave method, 5 min @ power 160 W, then 3 min @ power 350 W
0.89 M KOH	–	80	2 h	–	0.015	2nd: This stage was done after bleaching to remove the residual proteins
0.5 M NaOH	1:30	1st bath:100 2nd bath:32	1st bath: 1 h 2nd bath: 18 h	–	–	constant stirring
1.2 M NaOH	–	RT	30 min	8% (chitosan protein)	–	2 bath
0.75 M NaOH	–	RT	75 min	–	0.09	–
3 M NaOH	1:30	Boiling	1st bath: 15 min 2nd bath: 30 min	–	–	Constant stirring @ 150 rpm
0.75 M NaOH	1st bath: 1:1.5	RT	24 h	>1% (chitosan protein)	0.002	–
0.75 M NaOH	1:2.5	110	3 h	–	0.19	stirring
19 M NaOH	1:10	RT	20 h	–	0.005	–
1 M NaOH	1:5	–	10–40 min	2–6%	–	high-frequency ultrasonic bath
Deionized water	–					

Annex 3. *Deacetylation methods of α-chitin (suspension techniques, in homegenious & heterogenious state).*

Films	Force de traction (N)	Allongement à la rupture (%)
2% Chitosane	25,39	5,39
2% Chitosane + 0.1% PEG	19,29	5,24
2% Chitosane + 0.2% PEG	20,92	9,59
2% Chitosane + 0.4% PEG	21,96	8,45
2% Chitosane + 0.6% PEG	22,31	9,25
2% Chitosane + 0.1% Glycerol	25,69	6,70
2% Chitosane + 0.2% Glycerol	24,20	10,76
2% Chitosane + 0.4% Glycerol	26,81	13,65
2% Chitosane + 0.1% Tween 80	24,52	5,69
2% Chitosane + 0.2% Tween 80	23,67	5,42
2% Chitosane + 0.4% Tween 80	21,41	4,18
2% Chitosane+0,6% Tween80	17,46	1,29

Annex 3. Mechanical properties of chitosan films with different plasticizer percentages.

ملخص:

يتمثل الهدف الرئيسي من هذه الدراسة في تثمين المنتجات البحرية الثانوية (قشور الجمبري وقلم الحبار) من أجل استغلالها في مجالات مختلفة. تم تحضير الكيتوزان والظروف المثالية لنزع أسيتيل الكيتين هي كالتالي: 50 مل من 60٪ من هيدروكسيد الصوديوم بنسبة 60٪ لـ 5 جم من الكيتين لمدة 3 ساعات عند درجة حرارة 120 درجة مئوية.

ثم قمنا بعد ذلك بتصنيع أغشية الشيتوزان النقية الملدنة باستخدام الجلسرين و بولي إيثيلين جلايكول بكل من النسب و20% و 40% .

خضعت أغشية الشيتوزان لاختبارات الشد .DMA تشير النتائج إلى أن البولي إيثيلين جلايكول والبولي إيثيلين جلايكول والجلسرين يحسنان اللدونة إلى حد ما. لم تؤدي كمية الملدنات المحقونة إلى ظهور ظاهرة مقاومة اللدونة.

تشير نتائج SEM إلى وجود تعقيد فعال بين الشيتوزان والجلسرين والبولي إيثيلين جلايسرينول والبولي إيثيلين جلايسرينول.

Abstract:

The main objective of this study is to valorize marine by-products (shells) in order to exploit them in various fields. Chitosan was prepared and the ideal conditions for chitin deacetylation are as follows: 50 ml of 60% NaOH for 5g of chitin for 3 hours at a temperature of 120°C.

We then fabricated plasticized pure chitosan films using glycerol, polyethylene glycol at both 20% and 40%.

The chitosan films were subjected to DMA tensile tests. The results indicate that polyethylene glycol and glycerol significantly improve plasticity to some extent. The amount of plasticizer injected did not lead to the appearance of the anti-plasticization phenomenon.

SEM results indicate that there is an effective complexation between chitosan, glycerol and PEG.

Résumé:

Le but principal de cette étude est de mettre en valeur des sous-produits marins (carapaces) afin de les exploiter dans des différents domaines. Le chitosane a été préparé et les conditions idéales pour la désacétylation de la chitine sont les suivantes : 50 ml de NaOH à 60% pour 5g de chitine pendant 3 heures à une température de 120°C.

Ensuite, nous avons réalisé des films à base de chitosane pur et plastifiés en utilisant du Glycérol, du Polyéthylène glycol à 20% et également du 40%. Les films de chitosane ont été soumis à des tests de traction par DMA. Les résultats obtenus indiquent que le polyéthylène glycol et le glycérol ont considérablement amélioré la plasticité jusqu'à un certain point. La quantité de plastifiant injectée n'entraîne pas l'apparition du phénomène d'antiplastification.

Les résultats du MEB indiquent qu'il a une bonne complexation efficace entre le chitosane, le glycérol et le PEG.