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Research Article

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Abstract

The shells of the prawns, which is a massive by-product of seafood industry, can be used to create sustainable materials, but this remains a potential that is not fully explored. To increase the shelf life of fruits and vegetables, this study explores extraction and application of chitosan which is a biodegradable polymer derived out of prawn shells in edible coating of products. The extraction process to convert chitin to chitosan involves demineralization by use of diluted hydrochloric acid, deproteinization by use of sodium hydroxide (NaOH) and deacetylation with 50 percent NaOH. This is then followed by addition of glycerol as a plasticizer, the resulting chitosan is dissolved in 1% acetic acid and the mixture is cast in edible films at 40 °C. The chitosan films produced show excellent antifungal and antimicrobial properties with much higher efficacy at high concentrations of chitosan. These layers help to extend the shelf life of fresh foods by reducing microbial growth and loss of quality significantly. The paper highlights that edible films based on the use of chitosan are a potential, green alternative to be used in different applications such as food preservation and waste valorization.

Keywords: Chitosan, Edible coating, Demineralization, Deproteinization, Deacetylation, Shelf life

1. Introduction

Also, worldwide, 15% to 50% of agricultural products are lost post-harvest mainly because of microbiological spoilage, and even more in developing countries that do not have proper storage technologies of fruits and vegetables. The storage of fresh produce over long periods of time worsens biochemical and physiological alterations that render fresh produce vulnerable to pathogens and cause quality degradation. These losses are very high contributors to post-harvest losses. There has been a concern regarding the dependence on synthetic fungicides as a way of reducing these losses because of their adverse effects on the human health, the environment and the development of fungicide resistant pathogens. As a result, international studies have gone on the spurt to determine alternative, sustainable solutions. One of the prominent consumer trends is that of green consumption where there is focus on decreasing the synthetic additives, improving quality, security, and increasing the shelf life. Chitosan has been found as one of the promising agents in controlling the post-harvest diseases and further shelf life at preharvest and postharvest stages among the options which are being studied [1-2].

1.1 The source and structural characteristics of chitin and chitosan

The second most abundant natural-biopolymer after cellulose is chitin, a linear amino-polysaccharide that consists mainly of 2-acetamide-2-deoxy-2-deoxy-beta-D-glucopyranose 2-acetamide-2-deoxy-2-deoxy-beta-D-glucopyranose units and in part of 2-amino-2-deoxy-2-deoxy-beta-D-glucopyranose, 2-acetamide-2 It is commonly present in invertebrates, such as crustacean shells, insect cuticles, and the cell walls of fungi and yeasts, and in the wall of a few mushrooms and green algae. World production of chitin is almost as much as cellulose. Crustacean shells based on the source or species usually have 30-50% calcium carbonate, 30-40% protein, and 20-30% chitin. As an example, shrimp waste contains 31-44 % of minerals, 10-38 percent protein, 24-46 percent chitin. Different sources have breakages of chitin, ash, and protein percentages as shown in Table 1. Nowadays, chitin is a well-known versatile and useful biomaterial whose potential is great in a lot of spheres, and it becomes an even more important polymer [3-4]. Chitosan is a natural polymer made of sugar chains that consist of glucose amine and N -acetyl glucose amines, which are connected using glycosidic bonds. Its amino groups are positively charged, enabling it to react with negatively charged cell surfaces and polymeric materials. Its solubility is one of its most important qualities that are essential in numerous applications. Crustaceans like crabs and shrimp are the common sources of chitosan that are

produced using a modern process. This is done by acid treatment and alkaline treatment to remove proteins and calcium carbonate, then purification and decolorization to remove pigments to get pure and uncontaminated preparation. The chemical structure of chitosan is unique; thus it has a variety of properties, which predisposes it to wear in many important industrial applications in many sectors [5-6].

TABLE 1. Composition of Chitin, Ash, and Protein Across Various Sources [7]

Source of information	**Protein %**	**Ash %**	**Chitin %**
Shrimp shells (Penaeus durarum)	34.03	42.27	23.75
Shrimp shells	32.78	32.48	36.66
Crabs Shells	16.70	66.60	16.72
Squid gladius	36.55	02.58	31.22
Mussel shells	09.98	23.28	23.28
Insect cuticles (cicada sloughs	39.80	11.70	36.61

1.2 Solubility of Chitin and Chitosan

A significant intra- and intermolecular hydrogen bonding system gives chitin insolubility in most of both organic and inorganic solvents. Chitosan is the main derivative of chitin, which is made of units of N-acetyl-D-glucosamine and D-glucosamine. Chitosan is a resultant product of chitin deacetylation in an alkaline solution. The polysaccharide chain has functional groups such as amino and hydroxyl group which enable chitosan to form molecular polymers and structural changes. The free amino functional group is present in each unit of D-glucosamine, and it can bear a positive charge, which characterizes such important characteristics as solubility and antimicrobial activity. These amino groups are also good chelating agents which bind to metal ions and bind dye anions electrostatically [8-9].

Degree of deacetylation (DD) and molecular weight are important parameters determining the properties of chitosan such as crystallinity, hydrophilicity, cell response, and solubility. Chitin on the other hand cannot dissolve in regular solvents such as water, organic, and normal acid or alkali. It is soluble in high-concentration inorganic acids, i.e. HCl, H 2 SO 4, H 3 PO 4, and some other special solvents, i.e. N, N-dimethylacetamide (DMA) trichloroacetic acid, and lithium thiocyanate. These solvents however are usually toxic, corrosive, hazardous or non-degradable. In contrast to chitin, chitosan is soluble in an aqueous solution with weak acid like acetic acid,

citric acid, formic acid and lactic acid at pH lower than that of its pKa (around 6.5). It is also soluble in certain organic solvents, e.g., dimethyl sulfoxide and p-toluene sulfonic acid [10]. Both chitin and chitosan are soluble in several factors such as acetylation degree, molecular weight, pH of the solution, ionic strength, and temperature. It is important to note that the degree of acetylation of the N-acetyl amino groups of chitin has a greater effect on the solubility of chitin compared to the molecular weight. These parameters have a great influence on their biological and physicochemical properties [11-12].

Chitosan was made, with different characteristics, purity and quality to fit its intended uses. It is regarded as an intermediate product as opposed to a finished product to be consumed directly by the consumer. Chitosan has many uses in many industries due to the versatile characteristics it possesses. Chitosan is also used in agriculture as a growth controller in plants, a foliar fungicide, an antimicrobial agent and time release delivery of fertilizers, which helps in modifying the soil [13]. Chitosan has found use in a range of skincare and haircare items in cosmetics, including shampoos, body washes, and skin defense agents. It has also been tested to be used as a toothpaste additive to reduce erosion of enamel. Chitosan also sells as a diet supplement, especially in weight loss products. Nevertheless, it is not clear how it gets to inhibit fat digestion. In poor anion exchange, chitosan reduce the dietary cholesterol uptake and elevates bile acid secretion. Chitosan is typically used to treat obesity at 1,000 mg/kg of body weight (which is about 14.3 mg/kg body weight in the case of a 70 kg adult) per day [14-15].

The high usage of plastic packaging films made of petroleum has posed great ecological problems. To deal with these problems, the ongoing use of plastics should be banned and phased off to other more sustainable materials in order to save the environment. This movement of thinking has triggered the quest to the use of biodegradable, environmentally friendly packaging materials and processes, which also conserve natural resources reduction of pollution. A solution is provided through biodegradable materials which are sourced through agricultural feedstocks, the chitosan of sea food processing wastes, or seafood waste [16]. These materials can be decomposed into non-toxic products like CO_2 and H_2 O hence they are environmentally friendly and harmless. Also, diffusion barriers to protect food can be based on biodegradable packaging films and coatings. The coatings applied to fruits and vegetables by placing a thin layer of an

external substance over the natural skin of the produce is a protective coating to the skin and prolongs the shelf life of a piece of produce preventing spoilage [17].

There is an increase in the number of products produced in the market that are highly perishable thus the need to use effective packaging solutions to ensure the products have their maximum shelf life. Edible films (thin sheets of edible substances) can be applied to food or used as a self-supporting layer placed over the surface of the food, and they are eaten with the food. Chitosan is also the most utilized substance in the manufacture of such edible films since it maintains the fruits longer without any change in their chemical properties. This holds particular significance, because most of the chemicals applied to the old packaging are toxic and non-degradable. Once again, edible films have been identified as one of the best means of preserving quality of foods [18]. There are potential applications in these films in diverse fields since they can be applied on food surfaces, isolate various compounds, used as casings, bags, or wraps. Also, they aid in ensuring food quality as they serve as obstacles to oxygen, moisture, oil, and taste as well as including advantageous characteristics like antimicrobial and antioxidant functions. Application of the films can be done using such methods as sprinkling, dipping or immersion [19].

The shelf life and quality of post-harvest vegetables and fruits are usually very diminished during the storage period because of their vulnerability to spoilage. These produce items can have a thin layer of chitosan applied to the surface to stop the growth of indigenous or inoculated bacteria, yeasts, and molds. The concentration of the antimicrobials, the type of microorganism, and the nature of the chitosan employed may affect the antibacterial and antifungal activity of the coating based on chitosan [20].

2. Materials And Methods

2.1 Materials

This research aims at extracting chitosan of shrimp shell waste and uses it to prepare edible films to preserve fruits. The review of the relevant research papers leads to the extraction process, which follows the three main steps of demineralization, deproteinization, and deacetylation.

Purification is done by some alkali and acid precipitation process. The edible films are then made and their quality tested to check their applicability in preserving fruits.

2.2 Shrimp Shell Waste Preparation in the extraction of Chitosan.

The waste of the shells of shrimp is gathered in fish markets and seafood restaurants. The shells are well washed by using tap water to clear the impurities. They are dried in a 100 0 C oven after cleaning. The dried shells are then blended with a blender machine and reduced into fine particles. Sieving is done to attain a particle size of about 1 mm. Lastly; the ground powder of shell is placed in flasks and is awaited to proceed with the next activities in the chitosan extraction procedure [21].

2.3 Extraction of Chitosan

The chitosan recovery of shell waste follows three basic processes, which are demineralization, deproteinization, and deacetylation. All these processes are essential in separation and conversion of the chitin found in shell waste to chitosan.

2.4 Demineralization step

This stage is where the shell of the shrimp is demineralized using 4% of HCl solution stirred. This can also be done using alternative acids like acetic acid or sulfuric acid. The treatment continues until the emission of CO_2 gas is stopped. This solution is then filtered through a cloth and washed using distilled water to eliminate the traces of acidic solution until neutral pH is attained. Lastly, the sample is made dry in an electric oven within a duration of 24 hours [22].

2.5 Deproteinization step

In the given step, a demineralized shrimp shell powder undergoes 1M NaOH treatment under stirring at 150 rpm and 30 O C. The procedure is repeated until the solution turns colorless meaning that there are no proteins. The sample is then well washed to get a neutral pH and dried in an oven for 24 hours. After this process what is obtained is the final product which is chitin [23].

2.6 Deacetylation of chitin to chitosan

During this step, the chitin, obtained in the deproteinization step, receives 50% NaOH solution at 120 C and 15 psi counter of 1 hour. The sample is then dried in distilled water until it attains a neutral pH after which it is dried in an electric oven over 24 hours producing chitosan as a final product as shown in Figure 1 [24].



Figure 1. Preparation for Chitosan

2.7 Characterization of prepared Chitosan

Once chitosan was obtained using a shrimp shell waste, its characterization was realized using Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR), and X-Ray diffraction (XRD) methods and the following instruments as displayed in Table 2 were used.

TABLE 2. Characterization techniques & Instrument Model [25]

S.No	Characterization Technique	Instrument Model
Structural and vibrational properties		
1	XRD :X-ray diffraction:	X-ray diffractometer with CuK_{α} radiation (λ=0.154 nm)
2	FTIR: Fourier transform infrared spectroscopy	Thermo Nicolet IR-200 spectrophotometer
Morphological studies		

3	SEM: Scanning Electron Microscope	The Carl ZEISS-EVO MA15 FESEM System
Chemical state analysis		
4	EDS: Energy Dispersive Spectrometer	The Bruker QUANTAX 200 system

2.8 Preparation of Chitosan coating solution

The chitosan (0.5, 1.0 and 1.5 %) chitosan solutions used in coating were combined with the 1 % solution of CH 3 COOH. The mixtures were mixed at 50 ° C in 2 hours to a homogenous solution. Course filter paper was then used to filter the solution. Then 1.5 mL of glycerol ($C_3H_8O_3$) a plasticizer was added to every 1.0 g of chitosan. The solution was warmed up by stirring the mixture for 30 minutes to fully incorporate the solution. The solution of chitosan coating was now made ready to use.

2.9 Post-harvest treatment

Mangoes were picked when fully matured between May and June and sent into the laboratory. A check on all the selected samples was done to keep the sample uniform in size and free of fungal infection or mechanical damage. The mangoes were sprayed with tap water, dried in room temperature and then dipped in the chitosan solution of coating solution for 15 minutes. The mangoes were then stripped, they were dried at room temperature, weighed and kept over a period of 7 days [26-27]. In the production of chitosan, the shell waste of shrimps was initially gathered, washed and dried. Hydrochloric acid was used to perform the demineralization step and sodium hydroxide (NaOH) was used to perform deproteinization. The last procedure, which was deacetylation, was carried out by applying 50% NaOH to transform chitin into chitosan. Chitosan edible films were prepared subsequently by combining chitosan with glycerol and acetic acid.

3 Results and Discussion

3.1 Scanning Electron Microscopy (SEM) Analysis

The SEM images show that the powder of chitosan is evenly dispersed and without aggregation and this indicates a successful extraction of chitosan. Figures 2 and Figure 3 reveal the surface

morphology of the chitin and chitosan powders and the particles are well dispersed and evenly distributed. Scanning under the microscope at different magnifications showed that the chitosan samples had a coarse and thick surface texture.

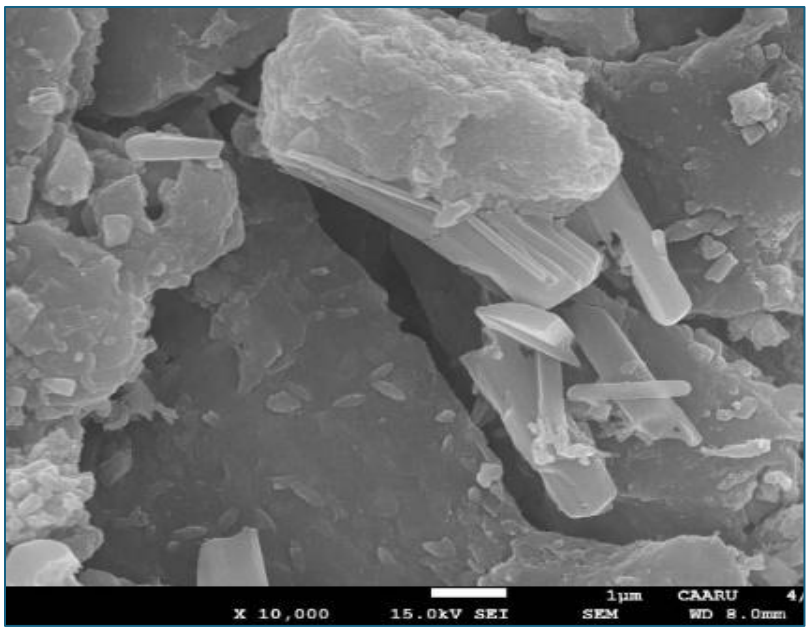


Figure 2. SEM Analysis of Chitin Surface Morphology

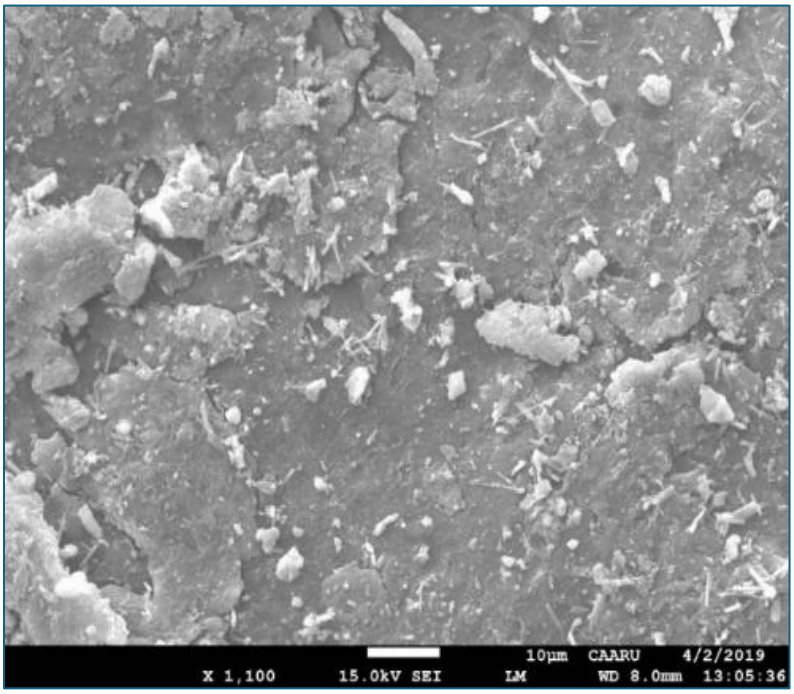


Figure 3. SEM Analysis of Chitosan Surface Morphology

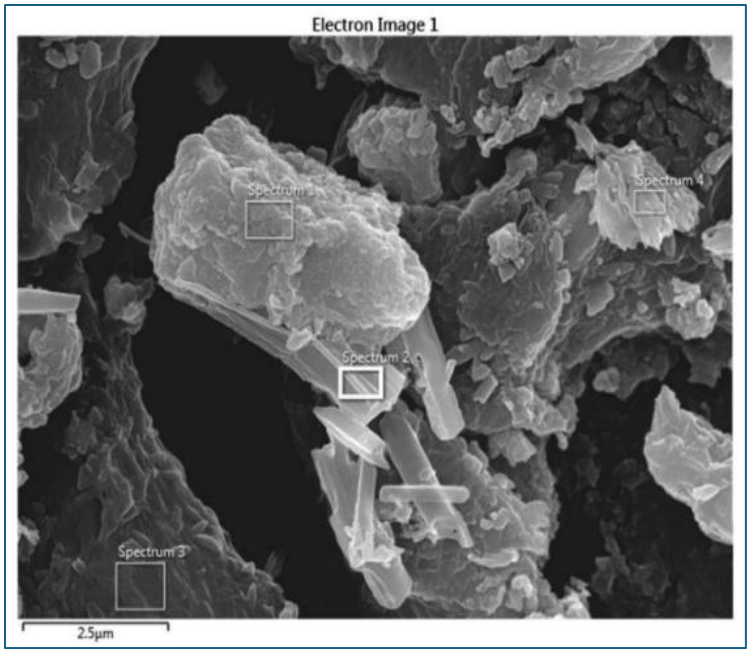
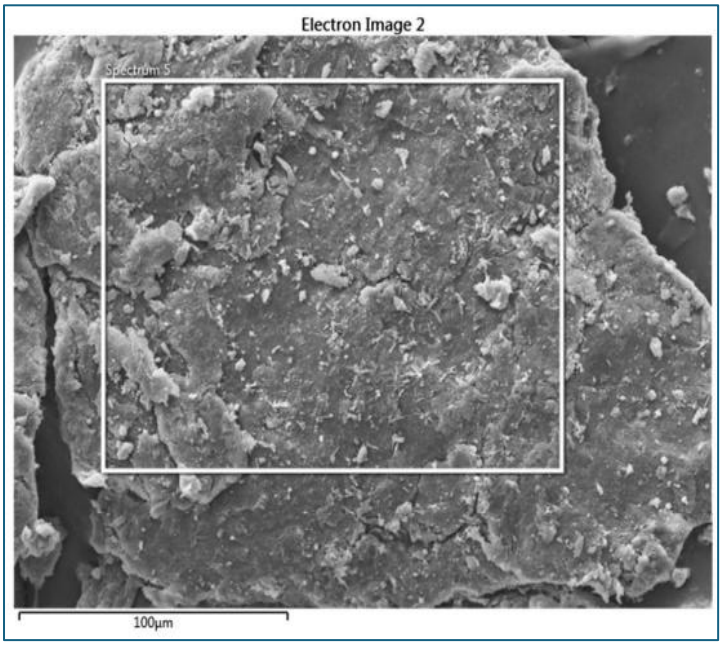


Figure 4. SEM-EDS Characterization of Chitin



F**igure 5.** SEM-EDS Characterization of Chitosan

Figure 4 and Figure 5 show the analysis of chitin and chitosan quantity using SEM-EDS. Figures 5 and 6 display spectra of energy-dispersive X-ray spectroscopy (EDS), performed using a SEM.

The chemical makeup of chitin is: 42.8% carbon (C), 40.8% oxygen (O2), 8.6% sodium (Na), 2.2% calcium (Ca), 0.9% magnesium (Mg), and 0.5% chlorine (Cl) which is consistent with available literature [28]. To avoid the accumulation of the static charges during imaging, all samples were gold-coated before EDX analysis. The chitosan elemental composition was set in motion to be 43.30% C, 36.60% O2, 16.10% Na, 2.50% Ca, 0.90% Mg, 0.60% aluminum (Al) as presented at Figure 6 and Figure 7.

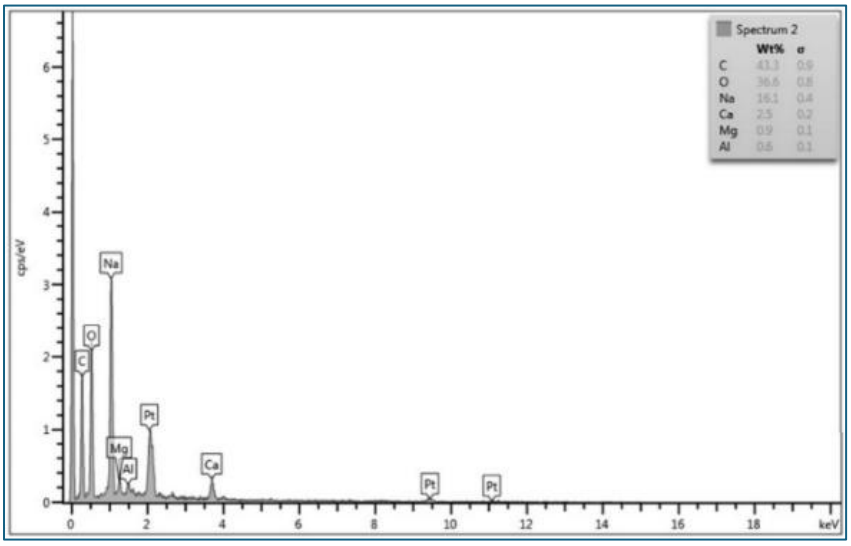


Figure 6. EDS Elemental analysis of chitin

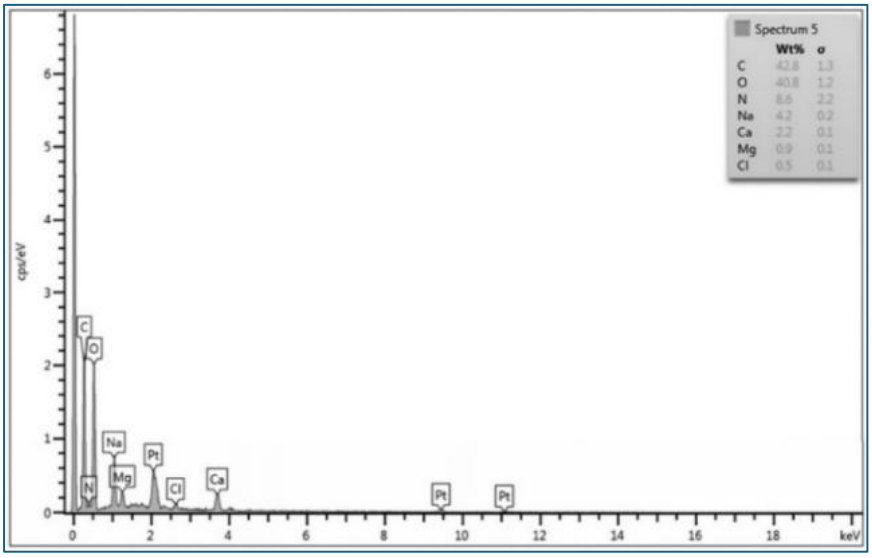


Figure 7. EDS Elemental analysis of chitosan

3.2 X-Ray Diffraction (XRD) Analysis

XRD was used to measure the crystallinity of the powder of extracted chitosan. The scan rate used was 1 o/min and scan angle was between 2° -40°. The results of analysis of the crystalline and structural properties are shown in Figure 8. XRD pattern shows that the chitosan powder obtained is crystalline with a diffraction peak at a 2θ-value of 10-15 and 25-30. The maximum was 1738.45-194.25 at 2θ position of 9.1238-27.9082. These findings, in accordance with previously done work, indicate that the chitosan samples retained their primary crystalline forms. More peaks were found probably because of the circumstances of the experiment and the character of the solvents demineralized and deproteinized. The appearance of the reflection peak at 9.31° confirmed the presence of extracted chitin and the appearance of the chitosan peak at 20° is related to the previous research studies [29].

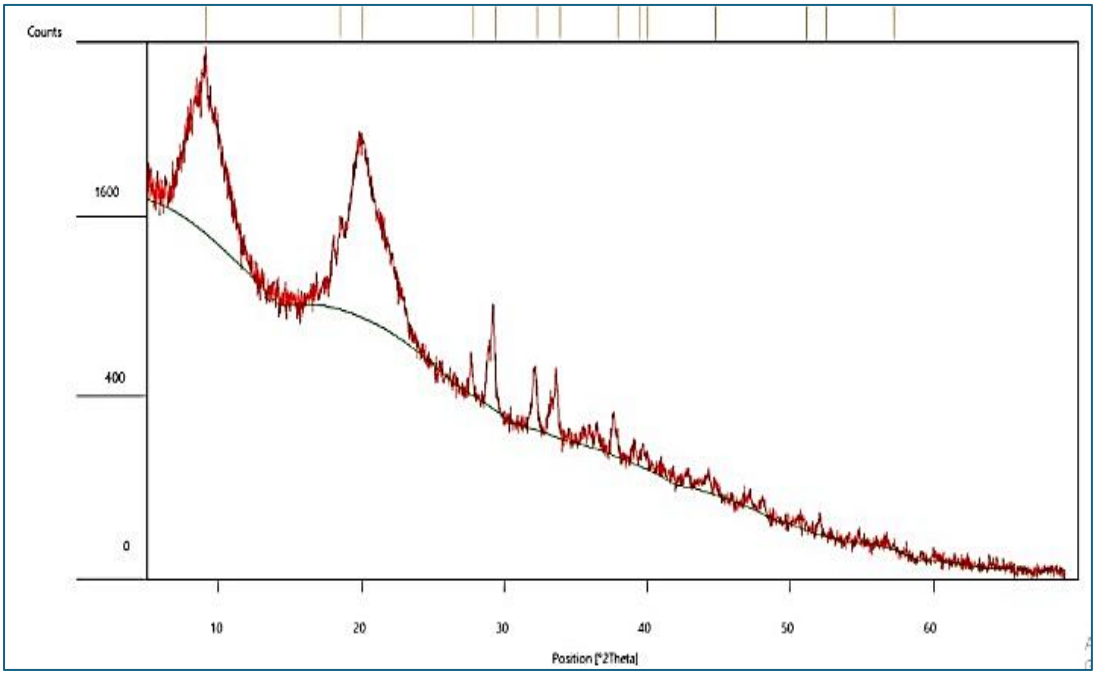


Figure 8. X-Ray Diffractogram Analysis of Chitin and Chitosan Powders

3.3 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR spectra of chitin and chitosan were all recorded in the 400-4000 cm^{-1} range at a resolution of 4 cm^{-1} and the functional groups and vibration bands of the chitosan powder

depicted in Figure 9 and Figure 10 were identified. The typical peaks were seen at 3469 cm^{-1}, O-H, 2930 cm^{-1}, C-H, 1659 cm^{-1}, C=O, 1380 cm^{-1}, CH2-H, 1580 cm^{-1}, CH_2-OH. Between 1600 cm^{-1} and 800 cm^{-1}, the N-H and O-H bands of chitosan were found. These FTIR findings agree with the literature of the past [30]. Physical analysis of chitin sample spectrum shows that the sample has calcium as the major component with a highest intensity of 92.89. Smaller almost flat peaks, which arise following the deacetylation process, are the impurities in the sample. The amount of calcium in the sample was detected to be 80.22% [31].

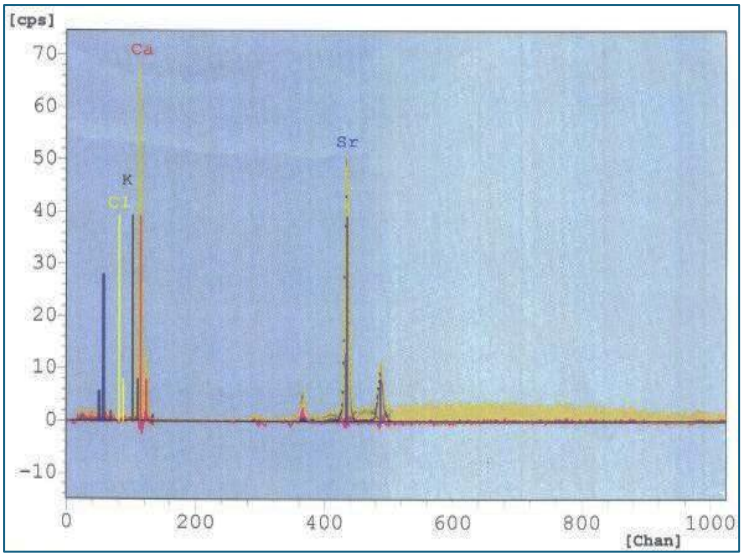


Figure 9. Spectral analyses Chitin

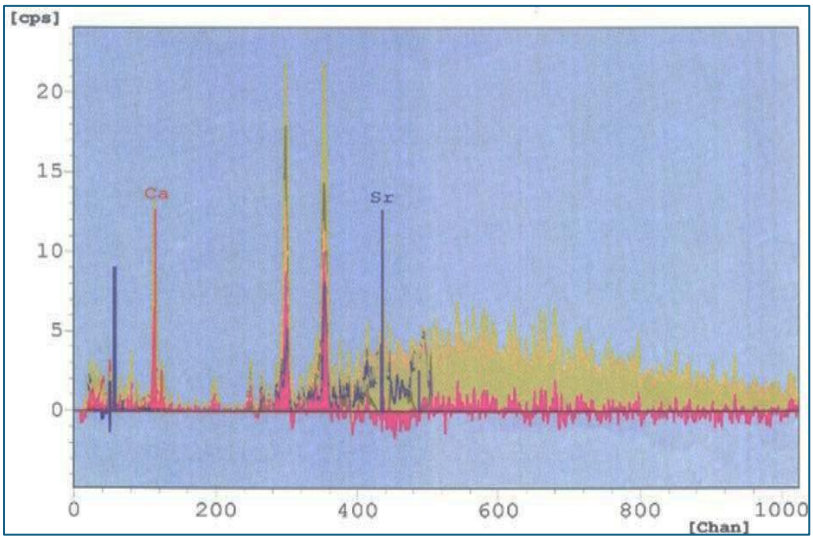


Figure 10. Spectral analyses of Chitosan

3.4 Chitosan application as edible coating

Enrichment of antimicrobial chitosan test results with the highest inhibition was furthered at the stage of applying edible coating to apples. The test parameters that were used in establishing the shelf life of the apples were weight loss, reducing sugars, vitamin C, and flavonoids. The apples in this study included red apple, green apple and Fuji Apple.

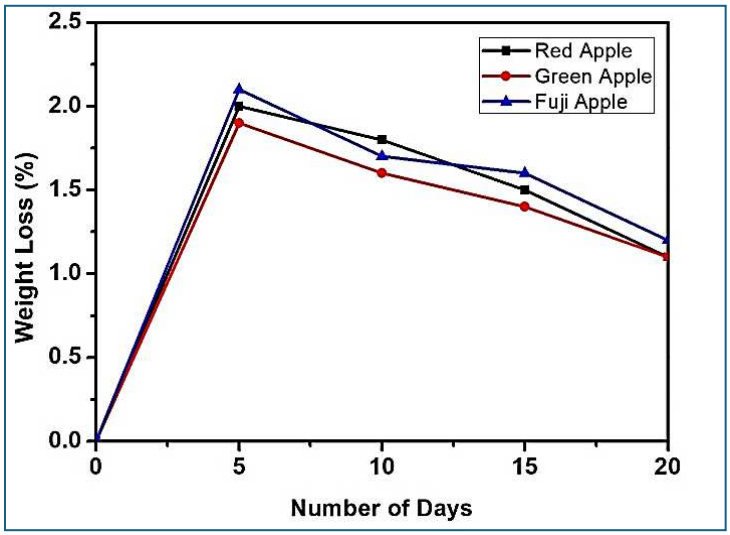


Figure 11. Weight loss Vs Number of Days

One of the indicators of the quality of apples during storage is weight loss. The process of losing weight happens because of physicochemical processes and postharvest metabolic processes that lead to moisture loss in the fruit with time. Figure 11 illustrates the outcomes of weight loss following the storage of apples with maturity level of 3 -5 days intervals during the next 20 days, day 01 to day 20. It was revealed that the mean percentage of weight loss of apples stored 20 days in chitosan coated treatment was 1.60% lower than that of uncoated apples (control), which was 1.95%. It refers to the fact that the coating of chitosan can achieve a reduction in the weight loss of apples stored. The process of loss of weight in apples is usually affected by the time and level of ripeness of the fruit [32]. Chitosan is an edible coating that can effectively block oxygen diffusion into the fruit and therefore in a way the respiration process is slowed down. Therefore, the amount of carbon dioxide (CO_2), a by-product of respiration, leads to reduced moisture evaporation/transpiration of the fruit. Transpiration makes the cells in the apple become loose

and gives it bigger air spaces resulting in shriveling. The quality characteristics of apples are valuable to the apple industry as this cellular change influences air volume, turgor pressure, and fruit firmness. Organoleptic testing is also a significant part of testing the quality of apples since it is a process that incorporates human sensual assessment of product acceptability. Sensory analysis is subjective, but necessary. The visual features of apples, especially their color, were rated on the visual scale of 1-5. This is either a scale of 1 (green dominates), scale 2 (green yellow), scale 3 (yellow), scale 4 (green red), and scale 5 (red). The detailed data is displayed in Table 3.

TABLE 3. Organoleptic test results

Panelist No.	Red Apple (Score 1-5)	Green Apple (Score 1-5)	Fuji Apple (Score 1-5)
1	5	1	4
2	4	2	3
3	5	1	4
4	5	2	3
5	4	1	4
6	5	2	4
7	4	1	3
8	5	2	4
9	4	1	3
10	5	2	4
11	5	1	4
12	4	2	3
13	5	1	4

Assessed by the results of the sensory tests, it was concluded that apples that were not coated with chitosan edible changed color more quickly than those coated with chitosan edible. The apples that were uncoated changed color faster as time lapsed than their counterparts who were coated. This shows that chitosan will be able to delay the process of ripening as demonstrated by the delay in apple color changes.

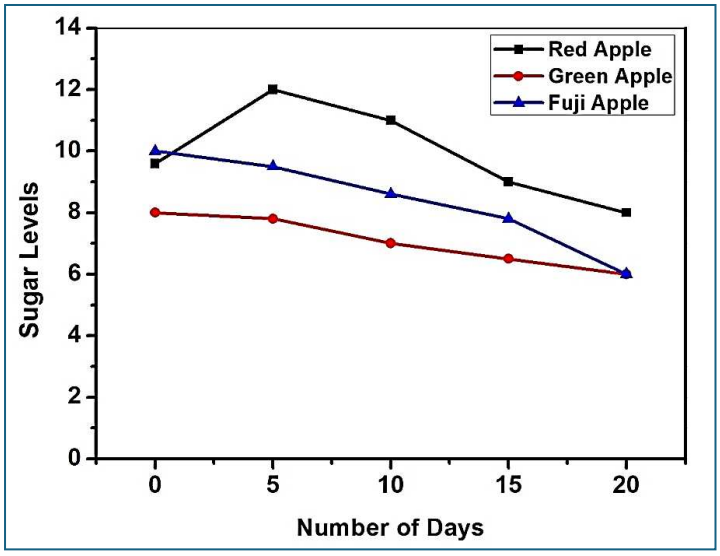


Figure 12. Sugar level (%) Vs Number of days

The reduction of sugar analysis was done to find out the sugar level in the apples, which is in terms of reducing sugars. According to the results presented in Figure 12, the average content of the total sugar in the apples covered with edible chitosan was 12.46 per cent (12.19 per cent more than in uncovered apples). This implies that chitosan coating is used to maintain sugars in apples during storage which could help preserve sweetness and flavor [33].

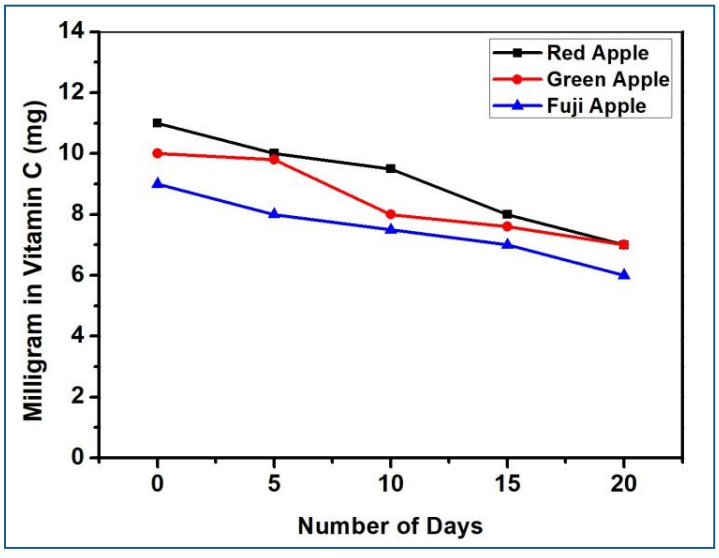


Figure 13. Sugar level (%) Vs Number of days

Reducing sugar analysis seeks to establish the amount of sugars found in tomatoes as calculating reducing sugars. The average total sugar reduction of tomatoes coated with edible chitosan was 11.46% as it was demonstrated in Figure 13 and it was 11.19% more than that of uncoated apples. Reducing sugar analysis seeks to establish the quantity of sugars found in tomatoes by analyzing reducing sugars. Figure 13 indicates an average total sugar decrease of the tomatoes with chitosan edible coating was 11.46% and 12.19 higher than the tomatoes without any coating apples [34].

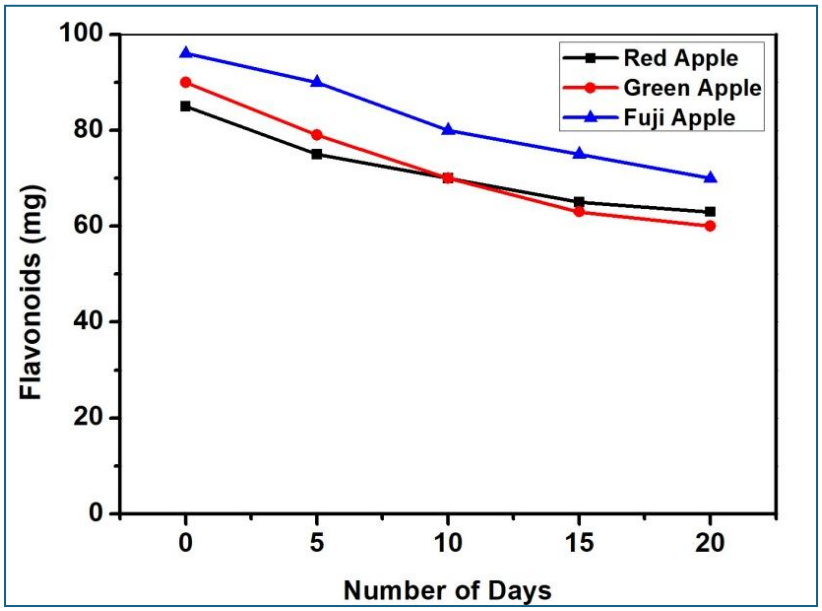


Figure 14. Flavonoids in apple with number of days

The chitosan-coated apples contained greater quantities of flavonoids than uncoated apples and this is illustrated in Figure 14. The acidity of fruits is a significant determinant of shelf life because the higher the acidity of a product, the more storage time it has. The flavonoids in the apples reduced with storage, and this has to do with the respiration rate of the apple fruit. Uncoated apples showed greater rate of respiration and thus it degraded organic acids faster, which is employed in the storage as source of energy. Acidity usually decreases with a rise in storage time because of this metabolic activity [35][36].

4. Conclusions

Research shows that chitosan could be used successfully on fruits without any change in their natural characteristics and is therefore a good substitute in maintaining long shelf life and preventing the growth of microorganisms. Still greater concentrations of chitosan have been found to be even more effective in such usages. Though there are still issues, there is a bright future for marketing biodegradable packaging films. Large-scale production is relatively cheap in terms of the raw materials and capital required and hence it is economically viable. Shrimp shell waste is especially used to obtain chitosan which is used to make biodegradable packaging films in its natural form or chemically modified. The application of eco-friendly materials that are completely biodegradable is important to ensure that our future generation will have a sustainable environment in the ecological aspect. Multi-component coating and films are becoming a popular subject due to their superior functionality and ecological issues, which is a developing trend in the field of sustainable packaging. The content of organic acids in climacteric fruits such as apples reduces very fast during storage because of the increased respiration rates which use organic acid as energy source. Nevertheless, control apples had a higher total acidity compared to apples covered with chitosan. This means that the chitosan coating is successful in containing the rate of respiration, reducing the rate at which the organic acids are used up thereby maintaining the acidity during the storage.

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