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CHEMISTRY DEPARTMENT

EXTRACTION OF CHITIN AND CHITOSAN FROM COMMERCIAL MUSHROOMS WASTE: A Comparative Study on the yield of Chitin and Chitosan from *Pleurotus Ostreatus* and *Agaricus Bisporus* mushrooms

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APPROVAL FORM

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I certify that this dissertation meets preparation guidelines as presented in the faculty guide and instructions for typing dissertation.

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Is my work and the sources used have been completely referenced.

DEDICATIONS

This thesis is dedicated to my mother Mlilo Sibongile and family who have always been there for me facilitating its success.

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ABSTRACT

Chitin and Chitosan from two species of mushrooms *Pleurotus Ostreatus* and *Agaricus Bisporus* were extracted through three procedural steps that are 1. deprotenisation where alkaline soluble proteins are removed using 4 M NaOH solution and refluxing with ethanoic acid to remove acid soluble proteins, 2. Decolourisation was done using 1% KMnO4 followed by 1% oxalic acid, and then 3. Deacetyation of chitin using 60% NaOH solution for 2 hours. Deacetyation is done to remove the acetyl groups in chitin and convert ß(1→4)- N-acetyl-D-glucosamine to α(1→4) linked 2-amino-2-deoxy-ß-D-glucopyranose (Chitosan). The effect of different parameters was investigated in the deproteinisation stage as follows:-temperature was investigated by using 80 ºC and then 100 ºC using 4 M NaOH for 3 hours, with 80 ºC maintained using a water bath, concentrations of 2 M and 4 M NaOH were used at 100 ºC for 3 hours, time of boiling with 4 M NaOH at 100 ºC was investigated at 1 hour and 3 hours. Crude Chitin produced using the different parameters was analysed using the FT IR. A yield of 11.6% was produced for *Pleurotus ostreatus* Chitin and 13.3% for *Agaricus Bisporus* Chitin. The corresponding Chitosan yields were 73% and 74.2% respectively, for the product from 4M NaOH, 100 ºC, 3 hours. Best Chitin quality was obtained from Sample B (*agaricus bisporus*) in terms of quality and resolution of FTIR spectra although it had an off white colour as compared to the sample A (*pleurotus ostreatus*). The bulk density was a bit lower for sample B as compared with sample A , that is, 0.344 g/cm^3 and 0.400 g/cm^3 respectively. The FTIR spectra gave the expected functional groups for both Chitin and Chitosan under all the conditions/parameters tested**.** Functional groups that are common to both Chitin and Chitosan like the OH bend, C-O-C bridge, CH and C-C stretches were observed, however for Chitin the C=O stretch was also obsevered which confirmed quality of product. The NH_2 stretch also confirmed the quality of Chitosan produced. These results concur with theory and literature.

CONTENTS

LIST OF ABREVIATIONS AND SYMBOLS

FT-IR …… Fourier Transform infrared spectrometer

GlcNAc …… Acetylglucosamine

GlcN……... Glucosamine

PCBs …… Polychlorinated biphenyls

DDA ……Degree of deacetylation

USEPA……. United State Environmental Protection Agency

LIST OF FIGURES **PAGE**

LIST OF TABLES **PAGE**

CHAPTER ONE: INTRODUCTORY CHAPTER

1.1 Introduction

Chapter 1 is an introductory chapter which focuses mainly on spelling out the goals of the study. The background to the study and significance/justification of the study are pointed out clearly in this chapter. The study is carried out in order to come up with solutions to an identified problem and cover a research gap. The statement of the problem is therefore stated in this chapter as well as limitations and delimitations of the study.

1.2 Background to the Study

Chitin is a chain of ß-1,4- N-acetylglucosamine (poly- GlcNAc) molecule, a natural biopolymer which has caught interest of most researchers due to its variety of uses that are paramount to life hence it is an inevitable substance. Chitosan is produced as a chitin derivative by alkaline de-acetylation of chitin (poly-GlcN) and it has more valuable applications. Chitin and Chitosan have a variety of applications in water purification, agriculture, food and pharmaceutical industries (Wu, 2004). It is therefore a good adsorbent for grease, filtration device to remove dyes and metal ions in water treatment, dietary supplements/weight management, cosmetics and agriculture. Kim and Park, (2010) state that Chitin and its derivatives have generated attractive interest in various fields such as biomedical, pharmaceutical, food and environmental industries- application in drug delivery systems, immune-stimulating and anticancer effects, antimicrobial, anti-inflammatory effects and antioxidant activity.

Pollution of water has become a major area of concern in Zimbabwe yet the process used for water treatment involves settling and filtration, coagulation and chlorination to kill germs and remove suspended solids as well as precipitates of metal ions. The process cannot remove dissolved metal ions in water hence the need for natural adsorbents like Chitin and Chitosan. Mining, effluent from industries, sewage disposal, oils and dyes are the major sources of water pollution and they release metals such as Zn, Cu, Fe, Co, and Ni as well as CN^- and Hg. (Mlambo, 2013) . The use of commercially available chitosan for potable water purification has been approved by the United State Environmental Protection Agency (USEPA) up to a maximum level of 10 mg/L. Chitosan, carboxymethyl chitosan, and cross-linked chitosan have been shown to be effective in the removal of Pb^{2+}, Cu^{2+}, and Cd^{2+} from drinking water (Kittur and Tharanathan, 2003).

Fe if consumed can cause severe stomach pains, brain and liver damage. Zn may lead to neurological damage, anaemia, bone marrow failure and central nervous system damage. Ni leads to skin rash, bronchitis, asthma and lung infections. CN^- and Hg are used in mines but when consumed they cause kidney and brain damage, gene damage and other serious health problems. Lead is one of the most dangerous, a potential toxic metal in marine ecosystem. The toxicity level may affect growth, and enzyme activity and even respiration of organisms (Muhaemin, 2005). The chelating and adsorption ability of the inexpensive natural biopolymer chitin and its derivatives can best be employed in removal of these dangerous metals from the environment.

Polluted water affects aquatic life, humans or animals as well as plants. Chitin and chitosan would be good for water treatment because they can adsorb metal ions (like Pb^{2+}, Ni^{2+}, Fe^{3+}, Cu^{2+}, Cr^{2+}, Co^{2+}, and Zn^{2+}), dyes, grease, and as a filtration device (Paul, 2005). The major advantages of chitin and chitosan are that they are recyclable, non-toxic, biodegradable and safe (Wu, 2004). In recent years studies on polymers which bind metals ions have increased significantly (Deans and Dixon, 1992) in (Hadi, 2013). Hadi, (2013) continues to say this approach is attractive since only the toxic metal ions can be removed whilst the harmless ions can be released into the environment. Use of mushroom waste will be an excellent way of adding value to the waste material rather than throwing away such a useful material and increasing amounts of solid waste. Synthesis of chitin and chitosan is less hazardous and user friendly. It can be done in any ordinary laboratory hence more economic and feasible.

Kittur and Tharanathan, (2003) argue that 'the largest single use of chitosan is the clarification of waste and effluent water. Better awareness of the ecological and health problems associated with heavy metals and pesticides and their accumulation through the food chain has prompted the demand for the purification of industrial water prior to their discharge for use'. The NH_2 group of chitosan forms dative covalent bonds with metal ions. It is used in metal ion complexing.

Phenol is one major pollutant present in the wastewaters from several industrial activities: coal mining, petrol refining, pharmaceutical production, founding and steel and iron manufacture, and the tanning and finishing of leather (U.S.EPA, 1980). Commonly used conventional treatments (biological, chemical oxidation and adsorption) often fail to generate final effluents with the required discharge quality at affordable costs (Bevilaqua, 2002). Chitin and Chitosan being natural and abundant copolymers that have adsorption and coagulant properties can be

used at affordable costs since they are sourced from readily available resources specifically on waste materials of consumed sea foods and fungi as well as other locally available sources.

Chitin as a natural biopolymer can be used with little resistance for medical purposes.
A high total cholesterol level, Poor kidney function, Intestinal disorders, skin grafts and a need to manage weight are signs that one may benefit significantly from chitin (xtend-life, 2000-2017).
Chitin and Chitosan can be extracted from shells of different crustacean organisms, insects, molluscs and fungi (Wu, 2004), however, this research seeks to provide a low cost extraction of chitin and chitosan from locally grown commercial mushroom waste, which can be used for treatment of water, health (medical uses) and other uses stated above. The two types of mushrooms, that is *Pleurotus Ostreatus* and *Agaricus Bisporus* will be used.

1.3 Statement of the Problem.

Many lives have been lost from causes that can be solved within the communities. Aquatic lives are lost mostly due to water pollution (loss of aquatic life means an imbalance in the ecosystem and this eventually has a negative impact on the environment and climate in general). The country has been experiencing human deaths due to cholera and heavy metal contamination. Some lives are lost due to high blood pressure and cholesterol management problems. Agriculture industry is producing low yields yet Zimbabwe's economy is based basically on agriculture. Mushrooms are a class of fungi that contain chitin which can be extracted at low costs and used together with its derivatives to solve most of these life threatening situations. The research seeks to extract the natural biopolymer chitin from two types of mushrooms *Pleurotus Ostreatus* and *Agaricus Bisporus*. Comparing the amount of yields from these two will help identify the type that has more of this valued polymer for economic reasons.

1.4 Aim.

The study aims to extract and compare the amount of chitin and chitosan from *Pleurotus Ostreatus* and *Agaricus Bisporus* mushroom waste.

1.5 Objectives.

The objectives of the study are:

1. To extract chitin from *Pleurotus Ostreatus* and *Agaricus Bisporus* mushrooms.

2. To compare the particle size and yield of chitin extracted from *Pleurotus Ostreatus* and *Agaricus Bisporus* under different temperature, concentration and reaction times.

3. To produce chitosan by homogeneous de-acetylation of chitin.

4. To characterise Chitin and Chitosan.

1.6 Purpose of Study/Justification.

Levels of pollution, health problems like obesity and kidney problems, and poor agricultural yields are still escalating in Zimbabwe. This calls for the need for scientists to research more on locally available, more economic and environmentally friendly substances that can be used to mitigate the national disaster. Chitosan is still an underutilized biopolymer in various applications, for example, in agriculture (Kittur and Tharanathan, 2003). Kittur and Tharanathan, (2003) continue to say, research at Washington State University has shown that the coating of wheat seeds with chitosan results in increased crop yields, a practice (allowed by the USEPA) that has been adopted by many so far. Chitin and chitosan have been proven to be very useful but too expensive and in low quantities. Extracting chitin from mushroom waste will be a much economic and feasible procedure to embark on.

The ever-increasing water pollution means very high costs in water treatment. This has necessitated the production of cost effective methods for removing dyes, grease and metal ions from water to save life. It is imperative to make use of locally available natural biopolymers which will work without further pollution of the environment or side effects on health.

Chitin derivatives are even more useful with improved characteristics. Recently researchers have proven that after primary derivation followed by graft modification, chitosan would obtain much improved water solubility, antibacterial and antioxidant properties. Grafting chitosan is also a common way to improve other properties such as increasing chelating or complexation properties, bacteriostatic effect or enhancing adsorption properties (Alves and Mano, 2008). This study aims to extract natural eco-friendly, nontoxic, recyclable and biodegradable

biopolymer chitin and chitosan from wastes of locally grown mushrooms, *Pleurotus Ostreatus* and *Agaricus Bisporus*, and then comparing the product yield from each type.

1.7 Limitations of Study

Time was the biggest constraint together with financial challenges. The researcher as a practising classroom practitioner had limited time to visit farmers and also the university Laboratories. The researcher also had some challenges in getting the species *Pleurotus Ostreatus* from Bulawayo farms as most of them were specialising with *Agaricus Bisporus*.

1.8 Delimitations

The mushroom stalks were collected from local farms in Bulawayo, South - Western Zimbabwe and the extraction process was done from Bindura University of Science Education Laboratories.

1.9 Summary

In summary, it has been noticed that there is really a problem in our communities which needs to be solved with low cost methods especially in water treatment and medicine. Chitin and Chitosan as products from natural and readily available sources can be extracted in large quantities in order to solve the societal problems indicated in this chapter especially if they can be extracted from waste substances in order to make good use of wastes instead of just keeping large heaps of pollutants.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter involves compilation of literature about Chitin and Chitosan, methods of extraction, sources, applications and their characterisation. The main objective of this chapter is to find out from literature the research that have been done about the subject in question in order to find out the research gaps that will then lead to the need for carrying out this project. The chapter will begin by the study of the chemistry of Chitin and Chitosan.

2.2 Chemistry of Chitin and Chitosan

In 1823, Antoine Odier isolated the alkaline insoluble fraction from cuticles of insects (May bus elytra) by repeated treatment with hot KOH solutions and he gave the name of the alkaline-insoluble fraction as Chitin, from Greek word "chiton" (tunic, covering or envelope) (Nwe, Furuike and Tamura, 2011).

In 1894, Hoppe-Seyler treated the shells of crabs, scorpions and spiders with KOH solution at 180 ºC and the resultant dissolved in dilute acetic acid and hydrochloric acid solution. This product could be recovered by addition of alkaline to the reaction solution and he gave the name of product as "Chitosan". Later on Chitin and Chitosan are known as copolymers of N-acety-D-glucosamine and D-glucosamine units' (Nwe etal, 2011)

Chitin is a white, hard, inelastic, nitrogenous polysaccharide composed of N-acetyl-D-glucosamine residues linked by ß(1→4) glycosidic bonds. It is structurally identical to cellulose, but it has acetamide groups (-$NHCOCH_3$) at the C-2 positions. The derivative of Chitin, Chitosn is a linear polymer of α(1→4) linked 2-amino-2-deoxy-ß-D-glucopyranose and is derived by N-deacetylation of Chitin (Dutta and Tripati, 2004).

2.2.1 Chemical Properties of Chitosan

Chitosan exhibit a number of chemical properties that make it suitable for several biomedical applications. These properties include the following:-

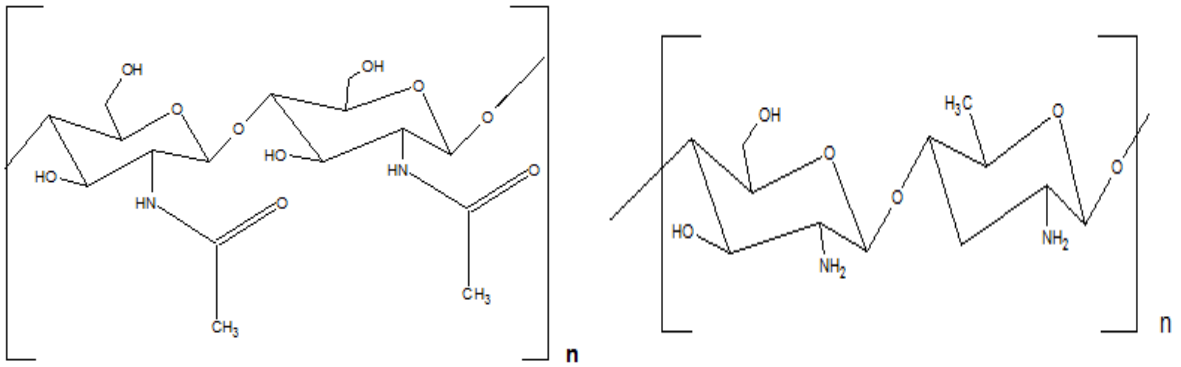
1. Chitosan is a linear polyamide.

2. It has reactive amino groups (-NH_2).

3. There is availability of reactive hydroxyl groups (-OH)

4. It has chelating ability for many transitional metal ions (Pokhrel, 2015). Chitin have reactive hydroxyl (-OH) and amide (-NHCO-) groups. These reactive groups are responsible for the adsorption and chelating abilities of chitin and chitosan as they can interact efficiently with different ionic species.

Chemical structures of Chitin and Chitosan are shown in figure 2.1 below. Cleaving off the acetyl group from Chitin gives Chitosan.



A B

Figure 2.1:- Chemical structures of chitin (A) and Chitosan (B)**.**

Chemically, chitin is deemed as an inert material with a poor reactivity since it is insoluble in ordinary solvents such as water, alcohols, acetone, hexane, deluded acids, and concentrated alkalis, among others (Alvarez and Ospina, 2015).

2.3 Sources of Chitin and Chitosan

Chitin is a compound found in fungi and shells of crustaceans and mollusks, in the backbone of squids and in the cuticle of insects (Wu, 2004). Different sources have different Chitin content. Factors affecting Chitin content are the species, peeling conditions during processing, part of the organism, state of their nutrition, and stage of reproductive cycle. (Paul, 2005).

Chitin is the main component of the invertebrates belonging to protostomia. The dry organic matter of their cuticle can contain up to 80% chitin (Pal and Verma, 2014).

Figure 2.2 below shows pictures of some common sources of chitin and chitosan.

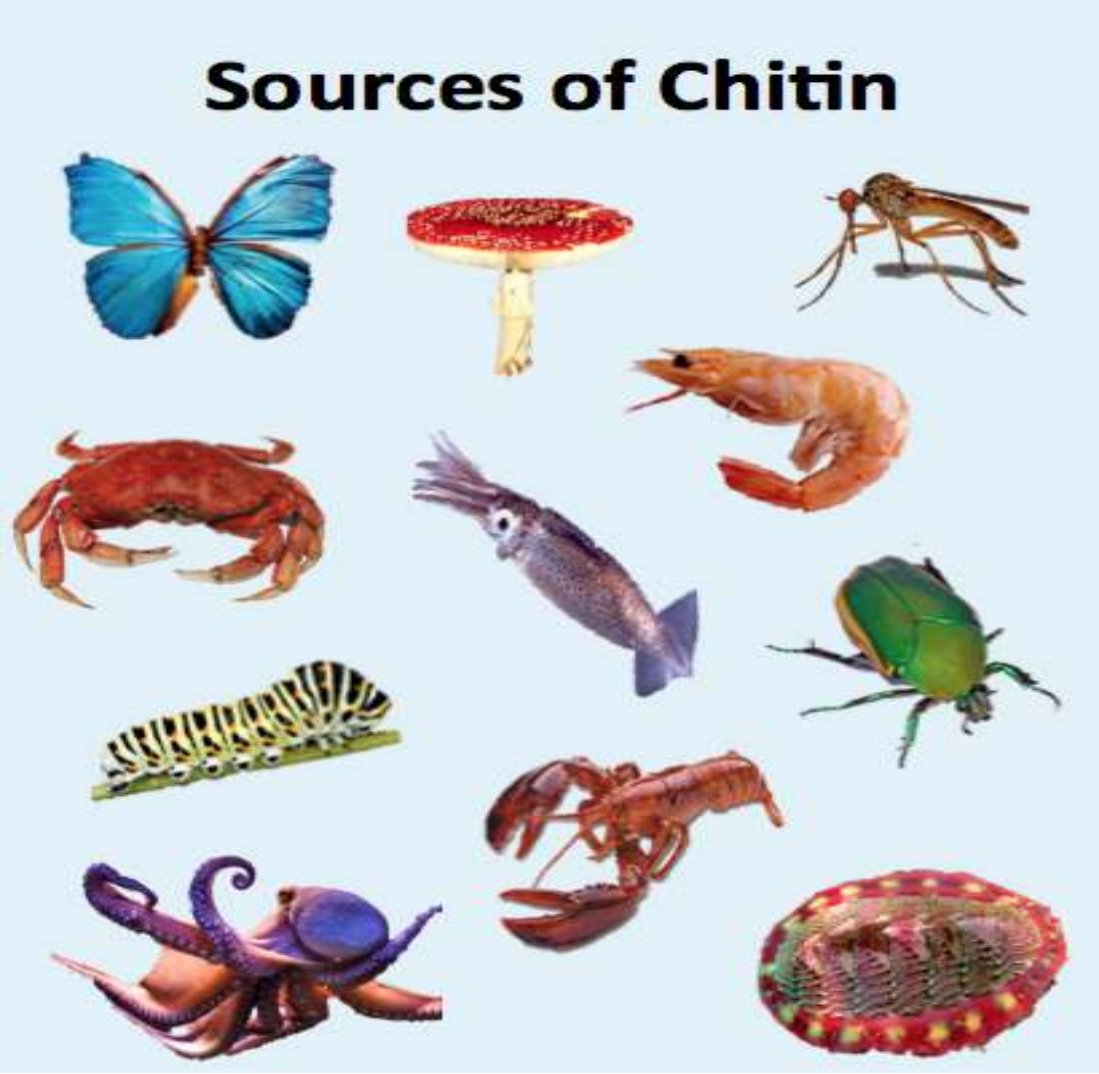


Figure 2.2 Picture showing some sources of Chitin (Rosenzweig, 2015).

Table 2.1 below shows Chitin Content of selected Crustacean, Insects, Molluscan Organs, and Fungi.

Table 2.1:- Chitin Content of selected Crustacean, Insects, Molluscan Organs, and Fungi (Paul, 2005).

TYPE	CHITIN CONTENT %	TYPE	CHITIN CONTENT %
Crustaceans		**Insects (continued)**	
Cancer (crab)	71.2[c]	*Pieris* (sulfur butterfly)	64.0[c]
Carcinus (crab)	64.2[b]	*Bombyx* (silkworm)	44.2[c]
Paralithodes (king crab)	35.0[b]	*Calleria* (wax worm)	33.7[c]
Callinectes (blue crab)	14.0[a]	**Molluscan Organs**	
Crangon (shrimp)	69.1[c]	Clamshell	6.1
Alasakan shrimp	28.0[d]	Oyster shell	3.6
Nephrops (lobster)	69.8[c]	Squid, skeletal pen	41.0
Homarus (lobster)	60-75[d]	Krill, deproteinised shell	40.2
Lepas (barnacles)	58.3[c]	**Fungi**	
Insects		*Aspergillus niger*	42.0[e]
Periplaneta (cockroach)	2.0[d]	*Penicillium notatum*	18.5[e]
Blatella (coackroach)	18.4[c]	*Penicillium chrysogenum*	20.1[e]
Colcoptera (beetle)	27-35[c]	*Saccharomyces cereviseae*	2.9[e]
Diptera (truefly)	54.8[c]	*Mucor rouxii*	44.5
		Lactarius vellereus (mushroom)	19.0

a. Wet body weight, b. Dry body weight, c.Organic weight of cuticle, d. Total dry weight of cuticle, e. Dryweight of the cell wall.

2.4 Types of Commercial Mushrooms

There is a variety of commercial mushrooms that include the ones shown in the table 2.2 and the pictures of these types of mushrooms are shown in figure 2.3 below (Filippone, 2017).

Table 2.2: Types of commercial mushrooms

Common name	Botanical name
Button	*Agaricus Bisporus*
Oyster	*Pleurotus Ostreatus*
Flat mushroom	*Agaricus Bisporus*
Enoki mushroom	*Flammulina velutipes*
Shiitake mushroom	*Lentinus edodes*
Swiss brown mushroom	*Agaricus Bisporus*

Figure 2.3 Image showing types of commercial mushrooms

The types used for the purpose of this study are *Pleurotus Ostreatus* and *Agaricus Bisporus,* these are readily available from local farmers in Zimbabwe.

2.5 Extraction of Chitin and Chitosan

The extraction procedure depends on the source used. Generally there are four steps which are:-

i. Deproteinisation- Alkaline removal of proteins using 4% NaOH followed by reflux in ethanoic acid to remove acid soluble proteins

ii. Demineralisation- Acid treatment using HCl to remove inorganic mineral substances like Calcium Carbornate

iii. Decolouration- Use of 1% $KMnO_4$ followed by 1% oxalic acid

iv. Deacetylation. Alkaline treatment with 40-50% NaOH (Dutta, 2004).

Demineralisation is not done with mushroom Chitin because these have very small quantities of minerals. This acid treatment stage is done for sea organisms (crustacean shells) that contain large amounts of calcium carbonate. (Muhaemin, 2005). 'Elimination of inorganic impurities (demineralization) is an unnecessary process in the extraction of chitin from fungi' (Alvarez and Ospina, 2015). Three extraction procedures are '(1) alkaline treatment to remove protein and alkali soluble polysaccharides, (2) acid reflux to separate chitin and chitosan, and (3) precipitation of chitosan under alkaline conditions' (Wu, 2004). The stages of extraction of Chitin and Chitosan from mushroom stalks are summarised in the following schematic diagram.

Figure 2.4 below shows the schematic representation of extraction of mushroom chitin and chitosan.

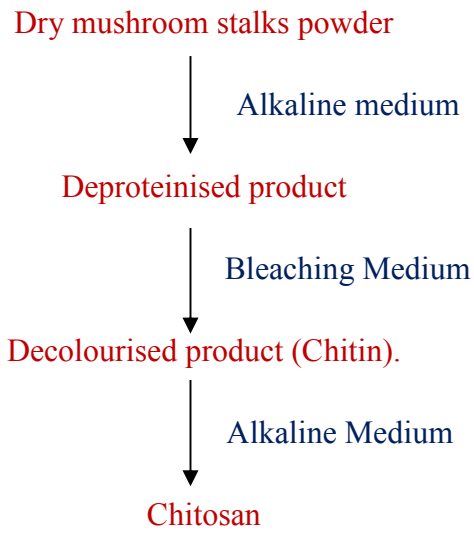


Figure 2.4 Schematic representation of extraction chitin and chitosan from mushrooms.

2.6 Applications of Chitin and Chitosan to Daily Life

During the last few decades, chitosan and chitooligosaccharides, were introduced into a variety of biomedicals including wound dressings and drug delivery systems. Chitin and its derivatives have delivered biological potential for a wide range of applications such as in the food and medical field, agriculture and aquaculture, dental and cosmetics, wastewater and membranes (Kim and Park, 2010).

2.6.1 Wound Healing

Chitin and chitosan have many distinctive biomedical properties; perhaps the most significant of which is their ability to promote wound healing, combined with their non-allergenic nature and antimicrobial activity this makes them ideal candidates for wound healing products, such as dressings, surgical sutures and artificial skins for burns victims, (Watson, 2008).

Watson, (2008) further points out that modified chitosans possess useful biomedical properties in that they promote ordered tissue reconstruction, vascularization and results in little scar formation. It is thought that this is due to their susceptibility to the hydrolytic action of lysozyme and N-acetyl-ß-D-glucosaminidase enzymes. These enzymes degrade the material, forming chito-oligomers which are capable of macrophage stimulation and exert a favourable influence on collagen deposition.

2.6.2 Treatment of Water

As a polymer, chitosan's natural tendency is to form long chains of molecules with positive charges, which act like hooks. These natural hooks catch organic materials, such as oils, detergents, and other contaminants dissolved in water. The material then coagulates to form flakes that are easily filtered out. Filtration companies are using chitin in clarifiers to help filter particulates and chemicals from water. In Japan, chitosan was first used for wastewater treatment because of its metal-binding properties. It is also good for cleaning up toxic organic compounds, such as PCBs. (Paul, 2005).

Chitosan has been used to remove mercury and fluoride in drinking waters (Adhami, 2013). Watson, (2008) highlights that 'Chitosan has been shown to be more effective at scavenging heavy and toxic materials than other natural materials having a binding capacity of more than 1 mmol/g for heavy and toxic metals'. He further argues that 'The removal of these heavy metal ions by binding to a biopolymer offers advantages over other processes due to the reduced cost of materials, ease of operation and selectivity over the alkaline materials'.

2.6.3 Food and Agriculture Industry

Alkyl glycosides of N-acetyl-D-glucosamine have been shown to promote the growth of bifidobacteria; which limits the growth of other types of micro-organisms and generates lactase, which is required for the digestion of lactose. In studies on rats and chickens, it was shown that animals fed with whey containing chitinous products had higher weights than those without, suggesting it could be used in animal feed (Watson, 2008).

Seeds treated with chitosan are larger and stronger and more resistant to fungal diseases (Paul, 2005).

2.7 Other Properties of Chitin and Chitosan

"Most of the naturally occurring polysaccharides viz. Cellulose, dextrin, pectin, alginicacid, agar, agarose and carrageenan are naturally acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides. They have several properties such as solubility in various media, solution, viscosity, metal chelating, and structural characteristics. The chemical properties of chitosan are due to the presence of linear polyamine and reactive amino groups which can chelate many transitional metal ions. Chitin also has biological properties like biocompatibility, accelerate the formation of osteoblast which is responsible for bone

formation, haemostatic, fungistatic, spermicidal, antitumor, anticholesteremic, and accelerate bone formation" (Pal and Verma, 2014).

2.8 Advantages and Disadvantages of using Chitin and Chitosan

Chitin and chitosan are natural biopolymers from a variety of sources hence are readily available, non-toxic, biodegradable, recyclable, environmentally friendly and the extraction procedure is not complicated.

The properties that make chitosan commercially relevant are its biodegradability, biocompartibility and the ability to transform into gels, beads, fibres, colloids, powders and capsules (Majekodunmi, 2016). These properties make Chitosan a valuable substance hence its variety of advantages.

The only noticed disadvantage of chitosan in literature is found in its property as a dietary fibre. 'If chitosan really can prevent the fat absorption (as indicated in the recent report), it can also prevent the absorption of other fat-soluble substances (e.g. fat-soluble vitamins and essential fatty acid) and negatively-charged substance (chitosan is positively-charged)' (Zhion.com, 2004).

2.9 Chitin and Chitosan Characterisation

The crystallinity of chitin can be determined by x-ray diffraction. However, a good estimation of crystallinity development in chitin samples has been achieved with FT-IR. FT-IR is a technique that gives information about the functional groups of a polymer hence good for polymer identification and analysis. It is a powerful tool used in organic and polymer chemistry. Band at 1379 cm^{-1} has been assigned to CH bending with some OH-bending contributions. The ratio of intensities of the bands at 1379 cm^{-1} and 2900 cm^{-1} has been suggested as the crystallinity index for chitin and chitosan (Wu, 2004).

The use of FTIR has a number of advantages. It is reasonably rapid and importantly does not require the samples to be solubilised, which means that it can be applied to chitinous materials with a wider range of DDA. Additionally, an accurate mass of the sample is not required therefore the purity of the sample does not need to be determined, providing that the impurities do not interfere with the probe and reference absorbance bands (Watson, 2008).

A method for calculating the DDA use equation 2.1 below:

$$\%DDA = \left\{ \left[\frac{A1655}{A3450} \right] + \left[\frac{A1630}{A3450} \right] - 0.13 \right\} \times 85.5 \quad \text{(Equation 2.1)}$$

2.10 Solubility Test of Chitosan

'In common, it is justified that the main physical differences between chitin and chitosan is the ability of chitosan to be soluble in organic acid such as acetic acid. Chitosan with higher content of protonated amino group are ready to form well-ordered arrangement in Van der Waals force and hydrogen bond which exceed its tendency for intramolecular chemical bonds. This explains its solubility in acidic chemical and partial solubility in hydrogen containing solvent' (Kewboonruang, 2016). The solubility of chitosan in acetic acid is a measure of its purity. 'Chitosan, unlike chitin has high content of highly protonated free amino groups that attract ionic compounds. This is why chitosan is soluble in inorganic acids' (Majekodunmi, 2016).

2.11 Summary

Chitin and Chitosan are nitrogenous polysaccharides that can be extracted from a variety of sources that include insects, molluscan organs, crustacean and fungi. The polymer has a number of physicochemical properties that give rise to its variety of applications. The procedure for extracting Chitin and Chitosan from mushroom waste is quite simple involving an alkaline extraction step and decolourisation. An FTIR technique can be used to identify functional groups after extraction (for characterisation). Chitosan is produced from alkaline treatment of Chitin to remove the acetyl groups. Chitin and Chitosan can be used in water treatment, agriculture, as an antibacterial (wound healing) and other uses.

CHAPTER 3: METHODOLOGY

3.1 Introduction

Chapter 3 looks at the methodology used in sampling, extraction of Chitin and Chitosan as well as the characterisation method which was used. Apparatus that was used in the investigation are also stated in this chapter as well as reagents and chemicals. The FTIR was used for characterisation of chitin and chitosan.

3.2 Sampling and Preparation of Mushrooms

Pleurotus Ostreatus and *Agaricus Bisporus* stalks were collected from mushroom farmers at the Youth Arena, Mabuthweni, Bulawayo, Zimbabwe. The stalks were washed 4 times with tape water to remove soil and then spread on plastic sacs and allowed to dry in the sun for 10 days. The dried samples were then ground into powder using mortar and pestle. The two sample powders were then weighed and kept in plastic containers before used for extraction of chitin and chitosan at Bindura University of Science Education Chemistry Laboratories. Figure 3.1 A below shows the sample stalks of *agaricus bisporus* that were used and figure 3.1 B shows the *pleurotus ostreatus* whose wastes were used.

A B

Figure 3.1 Pictures of Sample stalks of *Agaricus Bisporus (A)* and *Pleurotus Ostreatus(B)*

3.3 Chemicals and Reagents

Chemicals used were all of analytical reagent grade and were used without further purification. Sodium hydroxide 4M and Concentrated NaOH(40-50%) - was purchased from Glassworld (Pvt) Ltd, Ethanoic acid- purchased from ACE, oxalic acid 1% - purchased from Merck, Potassium permanganate 1% - purchased from ACE(Pvt) Ltd.

3.4 Equipment

Drying oven Heraeus D-6450 Hanau was used to dry samples at 80 ºC. A Thermo Fisher Scientific Nicolet iS5 MIR FTIR spectrophotometer equipped with an ID 7 ATR diamond accessory (Omnic software), was used to record spectra.

3.5 Extraction of Chitin from *Pleurotus Ostreatus* and *Agaricus Bisporus* Powders

The extraction of Chitin was done according to the method by Kurita extracted from (Ifuku, 2011) who says the two steps systems are Deproteinisation and then Decolouration. Deproteinisation involves removal of proteins using 4M NaOH at the ratio 1:10 (w/v) at different temperatures ranging from 60-100 ºC for 3hours. Sodium hydroxide was used to dissolve, hydrolyse, and remove proteins and alkali-soluble glucans. The product was then washed with deionised water thrice before refluxing with 10% ethanoic acid at 60 ºC for 6 hours. At this stage, it is known that partial neutral saccharides and acid-soluble protein compounds are also separated. The product was then centrifuged and the precipitate washed with distilled water until neutrality and then dried.

Decolourisation was done using a method stated by (Chang, 1982), cited in (Mau and Ming, 2006) with some alterations. The precipitate is decoloured using 1% potassium permanganate for 1 hour, and then reacted with 1% oxalic acid for 12 hours (overnight).

3.5.1 Effect of Temperature

The effect of temperature was investigated by using a variety of temperatures as follows 80 ºC and then 100 ºC and then comparing particle size/crystallinity of Chitin extracted at these different temperatures. The concentration of NaOH solution used for each sample was 4 M and boiling was done for 3 hours. The 80 ºC temperature was maintained using a water bath.

3.5.2 Effect of NaOH Concentration

Concentrations of NaOH were varied in order to study the effect of its concentration on Chitin extraction. Concentrations of 2 M and 4 M were used each at 100 ºC and boiled for 3 hours. 2 M was prepared by dissolving 80 g of NaOH pellets in 1 L of deionised water whilst 4 M was prepared from 160 g of NaOH in 1 L.

3.5.3 The Effect of Reaction Time

The time taken for boiling the mushroom powder in NaOH was varied with one sample boiled for 1 hour and the other boiled for 3 hours, 4 M concentrations were used at 100 ºC and then the crude chitin from each sample analysed with the FT-IR.

Figure 3.2 below shows the summary of the study of the effect of parameters (concentration, time, and temperature) in Chitin extraction.

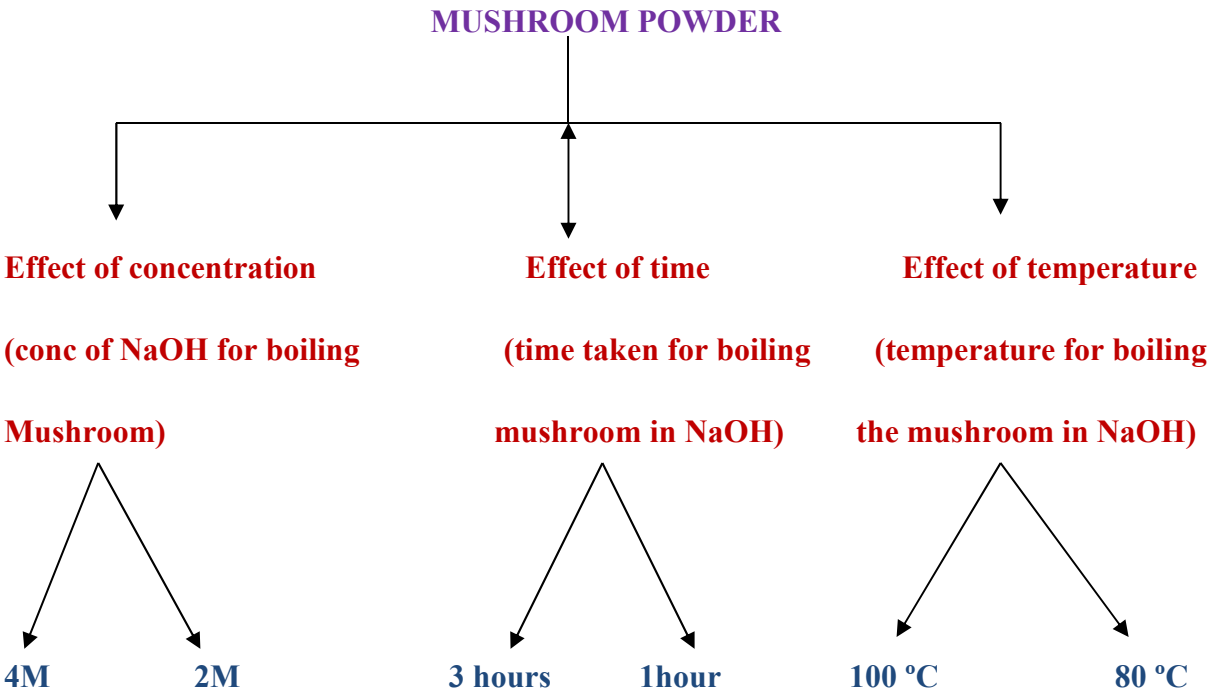


Figure 3.2 Summary of the effect of parameters in Chitin extraction.

3.6 Deacetylation of Chitin – Production of Chitosan

This was done to remove the acetyl group in Chitin and thereby producing Chitosan. Alkaline deacetylation was done (by heating chitin which was produced from 4 M NaOH, boiled for 3 hours at 100 ºC) in concentrated NaOH (60%) at the ratio 1:10 (w/v) at temperatures of 100 ºC for 2 hours. The product was then centrifuged and the precipitate washed with hot water at a temperature of 80 ºC and then dried.

3.7 FTIR Characterisation of Chitin and Chitosan.

The (4000-400 cm^{-1} wavenumber range) FT-IR was used with the sample put on the diamond disc at room temperature and 16 scans were conducted. The spectra were then recorded and analysed.

3.8 Summary

Chitin and Chitosan were extracted from mushroom waste powder by boiling in NaOH. The following parameters were studied:- effect of concentration (2 M and 4 M), effect of reaction time (1 hour and 3 hours) and the effect of temperature (80 and 100 ºC). Decolourisation was done using 1% oxalic acid and potassium permanganate and the chemicals used were analytical grade. An FTIR was used for characterisation.

CHAPTER 4: RESULTS

4.1 Introduction

In this chapter, the results of the investigation will be presented in tables and graphs. The chapter will begin by showing pictures of the crude products. The percentage yield will be presented in a table whilst the FTIR spectra that were collected are also presented. Sample *Pleurotus ostreatus* will be referred to as sample A and *Agaricus Bisporus* as sample B.

4.2 Crude Products

Figure 4.1 below shows crude Chitin before washing.

A B

Figure 4.1 Picture of Crude Chitin before washing, (A – *agaricus bisporus* and B – *pleurotus ostreatus)*

Figure 4.2 below shows a picture Crude Chitin from left- *Pleurotus ostreatus* (sample A) and *Agaricus bisporus* (sample B) after decolourisation.

Figure 4.2 Picture of Chitin after decolourisation.

4.3 Chitin and Chitosan Yield

A yield of 11.6% was produced for *Pleurotus ostreatus* (sample A) Chitin and 13.3% for *Agaricus Bisporus* (sample B) Chitin. The corresponding Chitosan yields were 73% and 74.2% respectively (for the product from 4M NaOH, 100 ºC, 3 hours).

Table 4.1 below shows the percentage yield of Chitin produced under the different parameters (temperature, reaction time, and concentratin)

Table 4.1: Effect of parameters on the Yields of Chitin

Parameter		**%-Yield on Chitin A sample**	**%-Yield on Chitin B sample**
Temperature	80 °C	9.85 ± 0.0025	11.1 ± 0.01134
	100 °C	11.6 ± 0.0050	13.3 ± 0.0023
Reaction time	1 hr	11.4 ± 0.0130	12.7 ± 0.0023
	3 hrs	11.2 ± 0.0024	12.9 ± 0.0312
Concentration	2 M	11.4 ± 0.0015	12.9 ± 0.0025
	4 M	11.6 ± 0.0210	13.1 ± 0.0140
Appearance		White	Off white

Table 4.2 below shows the percentage yields of Chitosan from sample A and sample B.

Chitosan A Sample	**Chitosan B Sample**
73 ± 0.234%	74.2 ± 0.017%

4.4 Bulk Density

The bulk density of the powdered mushroom samples was measured and results obtained are as shown in table 4.3 below.

Table 4.3 Bulk density of the samples

Sample A	0.400 ± 0.0211 g/cm^3
Sample B	0.344 ± 0.0034g/cm^3

4.5 FTIR Spectra for Chitin Samples

Figure 4.3 below shows FTIR spectra for Chitin A (*pleurotus ostreatus*) sample at the different conditions

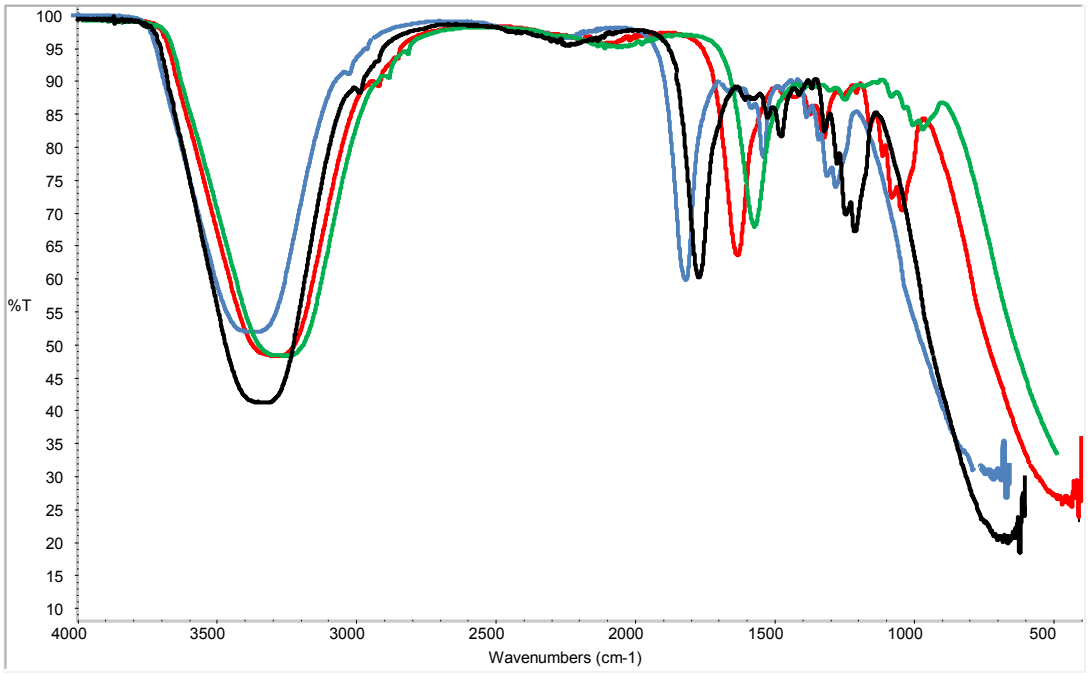


Figure 4.3 FTIR spectra for Chitin A

Key :- 4 M, 100 ºC, 3 hours

2 M, 100 ºC, 3 hours

1 hour, 100 ºC, 4 M.

80 ºC,3 hours, 4 M.

Figure 4.4 below shows spectra for Chitin B (*Agaricus bisporus*) sample at different conditions

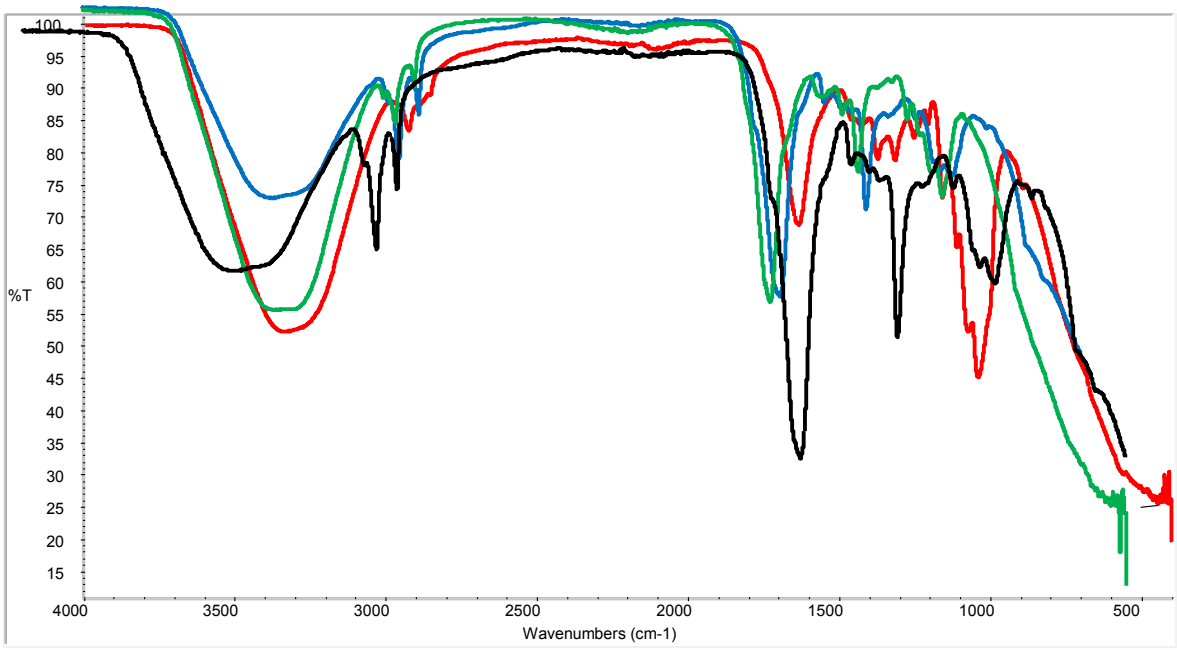


Figure 4.4 FTIR spectra for and Chitin B

Key :-

4M, 100 ºC, 3 hours

2M, 100 ºC, 3 hours

1 hour, 100 ºC, 4M.

80 ºC,3 hours, 4M.

Table 4.4: Results of FTIR Analysis of Chitin

Absorption Band	Chitin A sample (cm^{-1})	Chitin B Sample (cm^{-1})
OH	3281.50	3349.29
N-C=O	1624.27	1618.57
C-C stretch	1319.76	1321.84
C-O-C	1043.78	1025.71

Table 4.5: Chitin A and B Results summary of wavenumbers (cm^{-1}) and their functional groups-stretches and bands on the FTIR spectra.

	Pleurotus ostreatus (ChitinA)	*Agaricus bisporus* (ChitinB)
CONDITION	**Wave number (cm^{-1}) and functional group**	**Wave number (cm^{-1}) and functional group**
Chitin 4 M NaOH, 100 ºC, 3 hours.	3281.50 = O-H bend 1624.27 = Amide C=O stretch 1319.76 = C-C stretches 1043.78 = C-O-C bridge	3349.26 = O-H bend 2917.41 = symmetric CH_3 (sp^3) 2848.67 = assymmetric CH_2 1618.57 = Amide N-H bend 1321.84 = C-C stretches 1025.71 = C-O-C bridge
Chitin 2 M NaOH, 100 ºC, 3 hours	3280.61 = O-H bend 1629.58 = Amide C=O 1319.73 = C-C stretches 1038.19 = C-O-C bridge	3331.48 = O-H bend 2919.21 = symmetric CH_3 1631.04 = Amide C=O bend 1369.61 = C-C bend 1312.94 = C-N stretch 1249.96 = pyranose ring 1154.19 = alcohol C-O stretches 1036.45 = C-O-C bridge
Chitin 1 hour, 4M NaOH, 100 ºC	3267.46 = O-H bend 1634.84 = Amide C=O bend 1045.20 = C-O-C bridge	3335.63 = O-H bend 2916.75 = symmetric CH_3 2849.00 = assymmetric CH_2 1623.84 = Amide N-H bend 1320.02 = C-C stretches 1031.43 = C-O-C bridge
Chitin 80 ºC, 4 M NaOH, 3hours	3290.10 = O-H bend 1632.67 = Amide C=O bend 1318.42 = C-C stretches 1040.15 = C-O-C bridge	3344.17 = O-H bend 2917.33 = symmetric CH_3 2849.52 = assymmetric CH_2 1618.12 = Amide N-H bend 1321.81 = C-C stretches

1024.18 = C-O-C bridge

4.6 FTIR Spectra for Chitosan

Figure 4.5 below shows the FTIR spectra for *Pleurotus ostreatus* and *Agarisus bisporus* Chitosan.

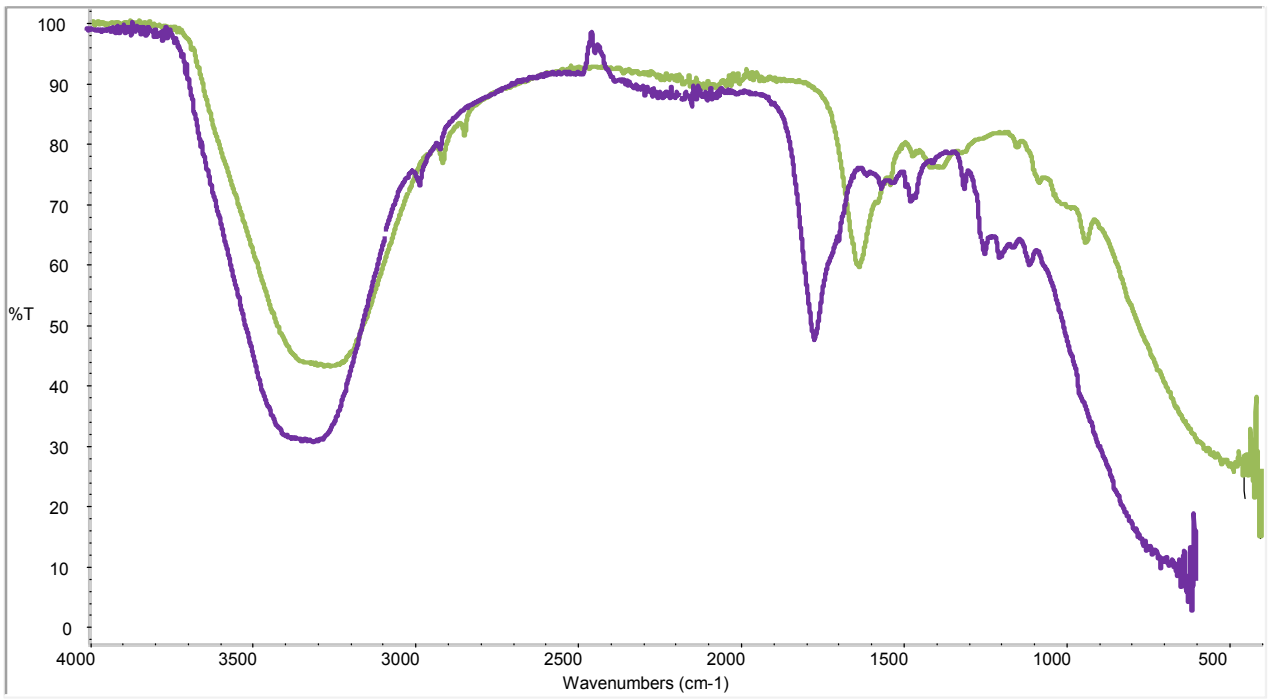


Figure 4.6 FTIR spectra of Chitosan from Sample A and sample B

Key :- Chitosan *pleurotus ostreatus* (Sample A)

Chitosan *agaricus bisporus* (Sample B)

Table 4.6: Results of FTIR Analysis of Chitosan

Absorption Band	**Chitosan A sample cm^{-1}**	**Chitosan B sample cm^{-1}**
OH	3259.77	3259.73
N-H	1635.08	1634.07
C-C stretch	1321.56	1392.29

Table 4.7: Chitosan A and B Results summary of wavenumbers (cm^{-1}) and their functional groups-stretches and bands on the FTIR spectra.

	PLEUROTUS OSTREATUS (ChitosanA)	*AGARICUS BISPORUS* (ChitosanB)
CONDITION	**Wavenumber (cm^{-1}) and ±functional group**	**Wave number (cm^{-1}) and functional group**
Chitosan produced from Chitin 4 M NaOH, 100 ºC, 3 hours	3259.77 = O-H bend 1635.08 = N-H stretch for NH_2 1321.56 = C-C stretches	3259.73 = O-H bend 1634.07 = N-H stretch for NH_2 1392.29 = C-C stretches 935.52 = CH_3COH group

4.7 FTIR wavelengths from a Standard (Commercial Chitin and Chitosan)

Table 4.8 below shows the standard FTIR absorption wavelengths of Chitin and Chitosan (Rumengan, 2014)

Table 4.8 FTIR absorptions for Standard Chitin and Chitosan

	Chitin	***Chitosan***
Group	**Wavelength cm^{-1}**	**Wavelength cm^{-1}**
OH	3448	3450.0
NH stretching	3300-3250	3335.0
C-H	2839.1	2991.1
C=O	1680-1660	-
N-H bending	1580-1530	1655.0 NH_2 cutting
CH_3	1419.5	1419.5
C-O-C	1072.3	1072.3
N-H	750-650	715
NH_2	-	850.0 - 750.0

4.8 Summary

A yield of 11.6% was produced for *Pleurotus ostreatus* (sample A) Chitin and 13.3% for *Agaricus Bisporus* (sample B) Chitin. The corresponding Chitosan yields were 73% and 74.2% respectively (for the product from 4 M NaOH, 100 ºC, 3 hours). Main functional groups in Chitin and Chitosan were identified by the bands and stretches in the FTIR spectra shown on the graphs above and results were summarised in tables. The bulk density of samples was 0.400 g/cm^3 for sample A and 0.344 g/cm^3 for sample B.

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

In this Chapter, the results presented in Chapter 4 are discussed in relation to what is in literature and the standards. Conclusions are drawn from the data outcome and recommendations are done. It is realised that the results from the FTIR concur with theory and the best conditions for extraction of Chitin from mushroom waste are 4 M NaOH, 100 ºC temperature and 3 hours reaction time although generally, these parameters have very slight effects since they all produced the expected functional groups which were picked by the FTIR.

5.2 Discussion

Chitin is a chain of ß-1,4- N-acetylglucosamine (poly- GlcNAc) molecule, a natural biopolymer which was extracted by removing alkali and acid soluble glucans from mushroom *(pleurotus ostreatus* and *agaricus bisporus)* wastes. Chitosan (poly-GlcN) was produced as a chitin derivative by alkaline de-acetylation of chitin and it has more valuable applications. The extraction of Chitin was done under different parameters namely, concentration, reaction times and temperature to study their effect on the quality and particle size of the Chitin product. Ideal Chitin should be crystal white.

5.2.1 Yield of chitin and chitosan

A yield of 11.6% was produced for *Pleurotus ostreatus* (sample A) Chitin and 13.3% for *Agaricus Bisporus* (sample B) Chitin. The corresponding Chitosan yields were 73% and 74.2% respectively (for the product from 4 M NaOH, 100 ºC, 3 hours. This was quite a good yield for the mushroom waste samples following that literature says the highest yield is 19% (Wu, 2004). *Agaricus bisprus* gave a higher yield as compared with *pleurotus ostreatus* but in terms of colour, the later (Chitin) was whiter with bigger particle sizes. Considering the bulk densities, Sample *Pleurotus ostretus* had a greater density. This sample was cultivated in pockets of bags whilst sample agaricus bisporus was cultivated in compost. Growing practices, can provide plenty of raw materials for fungal chitin and chitosan production (Wu, 2004) .

5.2.2 Characterisation of Chitin

The FTIR spectra gave the expected functional groups for both Chitin and Chitosan under all the conditions/parameters tested**.** The FTIR spectra for Chitin show a broad OH bend ranging from 3267.46 cm^{-1} - 3290.10 cm^{-1} for Chitin sample A whilst Chitin sample B ranges from 3331.48 – 3349.26 cm^{-1}. This agrees with the standard from literature which shows this peak at 3448 cm^{-1} (Rumengan, 2014), however Chitin sample B is more closer to the standard. Chitin differs from chitosan by one functional group the acetyl group, the spectra results show an amide C=O stretch at 1634.84 cm^{-1} for sample A and 1631.04 cm^{-1} for sample B. The standard has this peak at 1680 – 1660 cm^{-1}. The other characteristic peaks are the C-H stretch at 2919 cm^{-1} for sample B, bands near 2900 are reported as representative bands for Chitin (Alvarez and Ospina, 2015). There is a C-O-C bridge stretch ranging from 1034.84 – 1047.78 cm^{-1} for sample A and 1024.18 – 1036.45 cm^{-1} for sample B, this stretch is at 1072.3 cm^{-1} for the standard in literature (Rumengan, 2014). Generally, the results observed for Chitin sample A and Sample B concur with literature meaning the extraction process was a success for all the parameters studied in this project.Chitin sample B however showed more resolved peaks than sample A including a pyranose ring stretch at 1249.96 cm^{-1} and the C-N stretch at 1312.94 cm^{-1}.

Effect of concentration

Extraction of Chitin was done at 2 M and 4 M concentrations of NaOH in order to analyse the effect of concentration. The temperature was 100 ºC and the reaction time was 3 hours for both experiments on effect of concentration. 4 M gave a higher yield although generally they both produced the desired product.The high yield in 4 M agrees with the literature which says 4% NaOH is the most successful to extract Chitin (Hossain, 2014). The FTIR was able to pick most of the charactristic functional groups, however, 2 M showed more resolved peaks than 4 M, and is the only condition that gave the stretch of the pyranose ring at 1249.96 cm^{-1} and the C-N stretch at 1312.94 cm^{-1} which means it produced better quality of Chitin. It is threfore realised that lower concentration conditions are more gentle, given more time 2 M would yield the best results. The article in literature confirms this conclusion by saying 1.5 M NaOH solution yielded better quality Chitin and Chitosan (Rokshana, 2005).

Effect of reaction time

This was done by boiling the samples in NaOH for 1 hour and also doing the same for 3 hours with concentration and temperature the same for both (4 M and 100 ºC). Varying reaction times had a very slight diference in the yield of Chitin product. Their FTIR spectra were also closely similar with Chitin sample A having more peaks at 3 hours boiling time that in the 1 hour experiment which had no C-C stretch peak. This is similar to literature which says 'the reaction times did not affect the yields of chitin and average yields were 13.64, 12.97, and 12.92% for 60, 90, and 120 min, respectively' (Yen, 2006).

Effect of temperature

The effect of temperature in extraction of Chitin was studied by heating the samples in NaOH at 100 ºC and 80 ºC. The later was maintained by using a water bath. The other parameters were the sme for both samples, that is reaction time of 3 hours and concentration of 4 M. The highest yields were obtained at 100 ºC which meant it was the most conjucive temperature for the experiment. The FTIR gave closely similar results for both temperatures with exactly the same peaks observed which means the quality of product is independent of temperature.

5.2.2 Characterisation of Chitosan

Chitosan functional groups are similar to those of Chitin except the C=O where Chitosan have the NH_2 instead. The OH bend for chitosan A was obseved at 3259.77 cm^{-1} and 3259.73 $cm^{-1}$for Chitosan sample B, the standard Chitosan has this bend at 3450.0 cm^{-1} (Rumengan, 2014). The NH stretch for NH_2 is observed at 1635.08 cm^{-1} for sample A and 1634.07 cm^{-1} for sample B whilst the standard have this peak at1655.0 cm^{-1}. The C-C peak is observed at 1321.56 cm^{-1} and 1392.29 cm^{-1} for samples A and B respectively. Chitosan sample B has another characteristic peak at 935.52 cm^{-1} for CH_3COH. The Chitosan FTIR results also agree with the literature which means the extraction method was efficient.

5.3 Conclusion

Following the results and discussions given in this thesis, it can be concluded that the best Chitin quality was obtained from Sample B (*agaricus bisporus*) in terms of quality and resolution of FTIR spectra although it had an off white colour as compared to the sample from A(*pleurotus ostreatus*). The bulk density was a bit lower for sample B as compared with sample A , that is, 0.344 g/cm^3 and 0.400 g/cm^3 respectively. The percentage yield was however greater for sample B 13.3% than sample A 11.6% and the corresponding Chitosan yields were 74.2% and 73% respectively. These highest yields were obtained at 4 M concentration of

NaOH , reaction time of 3 hours and temperature of 100 ºC, however the best FTIR result was obtained at concentration 2 M. Particle size and colour were similar under all the different conditions, these only depended on the type of mushroom which is further affected by the method of cultivation used. Chitin sample A gave the best colour and particle size.

5.4 Recommendations

Given the output from the experiments studied in this project, it is recommended that extraction of Chitin and Chitosan from mushroom waste is best done at 4 M concentration of NaOH, 100 ºC temperature, and 3 hours reaction time. 2 M concentration of NaOH is the best when it comes to quality of the Chitin of which given more reaction time than 3 hours it is likely to give the best results. *Agaricus bisporus* type contains more Chitin than *pleurotus ostreatus* however for further study, it is best to compare the samples that are bred under the same conditions, that is, the type and amount of manure during cultivation as well as the incubation temperature, amount of water and the time of harvest.

5.5 Summary

This chapter concentrated at discussion of results from the experiment and comparing the experiment conditions vis the yield in terms of both quality and quantity. It was noticed that extraction of Chitin and Chitosan from mushroom waste is best done at 4 M concentration of NaOH, 100 ºC temperature, and 3 hours reaction time for higher yields. 2 M concentration of NaOH gives the best quality as confirmed by the FTIR results.

REFERENCES

Adhami, S. T. (2013). Chitosan Optimisation as a Drinking Water Treatment in Developing Countries. *Exploring Properties of Chitosan to Purify Water*. Retrieved from *curca.buffalo.edu.*

Alvarez, S.P.O and Ospina R.N. (2015). Isolation of chitosan from Ganoderma lucidum mushroom. *BIOMATERIALS SYNTHESIS AND CHARACTERIZATION.Journal of Mater Science:Mater medicals 26,135.*

Alves, M. N. and Mano J. F. (2008). Chitosan derivatives obtained by chemical modifications for biomedical. *International Journal of Biological Macromolecules 43, 401–414*.

Bevilaqua, J. V. (2002). PHENOL REMOVAL THROUGH COMBINED BIOLOGICAL AND ENZYMATIC TREATMENTS. *Brazilian Journal of Chemical Engineering 19 (2)*, 151-158.

Dutta, P. K, Dutta J and Tripathi V. S. (2004). Chitin and Chitosan: Chemistry and applications. *journal of Scientific and Industial Research* 63,20-31.

Filippone, P. (2017). Common Types of Mushrooms in Recipes. *Edible Mushroom Varieties With Pictures*. 09/21/17 retrieved from *www.thespruce.com.*

Hadi, A. G. (2013). Synthesis of Chitosan and Its Use in Metal Removal. *Chemistry and Materials Research 3(3),* 2224-3224.

Hossain, M. (2014). Production and characterization of chitosan from shrimp waste. *J. Bangladesh Agricultural University 12(1)*, 153-160.

Ifuku, S , Nomura R,Morimoto M and Saimoto H . (2011). Preparation of Chitin Nanofibers from Mushrooms. *Materials 4*, 1417-1425.

Kewboonruang, S. (2016). Comparative Studies on the Extraction of Chitin – Chitosan from Golden Apple Snail Shells at the Control Field. *Journal of Polymer and Textile Engineering 3*, 34-41.

Kim, M and Park B.K. (2010). Applications of Chitin and its derivatives in Biological medicine. *International Journal Molecular Science 11(12),* 5152-5164.

Kittur, S.F and Tharanathan R. N. (2003). Chitin — The Undisputed Biomolecule of Great. *Critical Reviews in Food Science and Nutrition, 43(1):61–87* .

Majekodunmi, S. O. (2016). Current Development of Extraction, Characterization and Evaluation of Properties of Chitosan and Its Use in Medicine and Pharmaceutical Industry. *American Journal of Polymer Science* , 6(3), 86-91.

Mau, J, L and Ming, T Y (2006). Preparation of fungal chitin and chitosan from shiitake stipes. *Fungal Science. 21(2)*, 1-11.

Mlambo, M. (2013, april 7). *Zimbabwe's riiver system heavily polluted.* Retrieved jan 26, 2017, from *http://www.thestandard.co.zw.*

Muhaemin, M. (2005). Chelating ability of crab shell particles and extracted acetamido groups(CHITIN and CHITOSAN) from Portunus sp TO LEAD (Pb2+). *Journal of coastal Development 9(1)*, 1-7.

Nwe, N , Furuike T and Tamura H. (2011). Chitosa from Aquatic and Terrestrial Organisms and Micro organisms: Production, Properties and Applications. *In: Biodegradable materials*, 978(1),30-50.

Pal, J and Verma O.H. (2014). Biological Method of Chitin Extraction from Shrimp Waste an Eco-friendly low Cost Technology and its Advanced application. *International Journal of Fisheries and Aquatic Studies,* 1(6), 104-107.

Paul, St. (2005). Green savior-Chitin and Chitosan. *Journal of Agricultural and Food Chemistry Vol 53*, 3888-3894.

Pokhrel, S. (2015). Applications of Chitin and Chitosan in Industry and Medical Science: A Review. *Nepal Jounal of Science and Technology 16(1)*, 99-104.

Rokshana, N. (2005). Extraction of Chitin and Chitosan from Shrimp(Metapenaeus monoceros) Shell by Chemical Method. *Pakistan Journal of Biological Sciences 8(7)*, 1051-1054.

Rosenzweig, R. (2015). Natural Sources of Chitin. *Water Purification for Developing Countries*.Retrieved from *https//experiment.com* Feb 2015

Rumengan, I. F. (2014). Structural Characteristics of Chitin and Chitosan Isolated from the Biomass of Cultivated Rotifer, Brachionus rotundiformis. *International Journal of Fisheries and Aquatic Sciences, 3(1)*, 12-18.

Watson, H. R. (2008). The isolation of useful bioproducts remaining from the large-scale fermentation of penicillium chrysogenum. *Durham theses,retrieved from http://etheses.dur.ac.uk/2078/*.

Wu, T. (2004). Production and Characterization of Fungal Chitin and Chitosan. *Trace: Tennessee Research and Creative*, (8), 1-72.

xtend-life. (2000-2017). *Health Benefits and Uses of Chitin.* Retrieved from *www.xtend-life. com*.

Yen, M. (2006). Preparation of fungal chitin and chitosan from Shiitake Stripes. *Fung. Sci. 21(1, 2)*, 1-11.

Zhion.com. (2004). CHITOSAN, *Chitosan has been popular in nutritional industry for years. What makes it so popular in the industry? What is its benefit?*. *R*etrieved from *www.zhion.com/Index.html.*