Beneficiation of chicken feathers and sawdust waste biomass: extraction of keratin and cellulose nanocrystals for use as binders in particleboard production.

By

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Chemical Engineering, School of Engineering of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Howard Campus, Durban, South Africa. The research was financially supported by: Bio-refinery Industry Development Facility (BIDF), Council for Scientific and Industrial Research (CSIR), and the Department of Science and Technology (DST), Waste Research Development and Innovation Roadmap, and the Bio-refinery Consortium research projects. The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Professor B.B. Sithole (Supervisor)

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DECLARATION 1: PLAGIARISM

I, Olajumoke Deborah Fagbemi, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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a) their words have been re-written but the general information attributed to them has been referenced;

b) where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) this dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections.

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DECLARATION 2: PUBLICATIONS

This thesis involves a compilation of manuscripts. Each chapter is an individual entity prepared as per the journals' specifications hence some repetition between chapters has been inevitable. The first author (student) conducted all experimental work, data collection and manuscript preparation, guided by the second (supervisor) and/or third author. The * indicates corresponding author.

Chapter 2

1. **Fagbemi, O.D**., B. Sithole. Sustainable Sources of Bio-Adhesives For Application in the Wood Products Industry: A Review (In process of submission)

Chapter 3

1. **Fagbemi, O.D.,** B. Sithole, and T. Tesfaye, Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques. Published in *Journal of Sustainable Chemistry and Pharmacy, 2020. 17: p. 100267.*

Chapter 4

1. **Fagbemi, O.D**., B. Sithole. Beneficiation of waste chicken feathers as a formaldehyde-free bio-adhesive for particleboard production. Waste and Biomass Valorisation (Submitted and under review in *Journal of Adhesion Science and Technology*)

Chapter 5

2. **Fagbemi, O.D**., B. Sithole, and J. E. Andrew. Cellulose Nanocrystals Extraction and the effect of different organic cross-linkers on its application as bio-binder for particleboard production (Submitted and under review in *Cellulose*)

Conference Presentations:

1. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2017. Valorization of chicken feathers: Utilisation as a binder in the forest products industry. Oral presentation at "The South African Institution of Chemical Engineers (SAIChe), Durban, South Africa, August 30/2017.
2. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2018. Synthesis of Cellulose Nanocrystals based-bioadhesive for Forest Products Industries. Oral presentation at "The South African Institution of Chemical Engineers KwaZulu-Natal (SAIChE KZN) Branch Research Day" on the 15th of August, 2018

3. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2018. Synthesis of Cellulose Nanocrystals based-bioadhesive for Forest Products Industries. A poster presented at the "Annual College of Agriculture, Engineering and Science Postgraduate Research and Innovation Symposium" which took place on Thursday 25 October 2018 on the Westville Campus, T-Block, University of KwaZulu-Natal.
4. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2017. Valorisation of chicken feathers: Beneficiation of waste Chicken Feathers as a Binder. Oral presentation at "The third International Conference on Composite, Biocomposites and Nanocomposites" 7-9 November 2018, Port Elizabeth South Africa.
5. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2019. Beneficiation of Waste Chicken Feathers as Sustainable Source of Bio-adhesive for Wood Composites Industry. Oral presentation at "The International Conference on Biorefinery" 18-21 August 2019, Johannesburg, South Africa.
6. Fagbemi Olajumoke Deborah, Tesfaye, T., Sithole, B., and Ramjugernath, D., 2019. Beneficiation of Waste Chicken Feathers as Sustainable Source of Bio-adhesive for Wood Composites Industry. A poster presented at the "Annual College of Agriculture, Engineering and Science Postgraduate Research and Innovation Symposium" which took place on Thursday 17 October 2019 on the Westville Campus, T-Block, University of KwaZulu-Natal.

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Date: 09/11/2020

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ABSTRACT

Abstract

The wood industry consumes large quantities of synthesis adhesives accounting for more than 65% by volume of the adhesives used worldwide. Synthetic adhesives are formaldehyde-based and that cause environmental pollution and affect human health. Hence, there is a growing interest in bio-adhesives sourced from natural sources: plant and animal, these could be a suitable replacement for environmental toxic formaldehyde-based binders. In addressing the problems mentioned above, from both economic and environmental points of view, this study focused on the beneficiation of waste chicken feathers generated by poultry slaughterhouses and waste sawdust from the sawmilling industry into binders to replace fossil-based binders and explore their use in the production of wood panel particleboards. The linear and interactive effect of process condition on the extraction efficiency of keratin protein were modelled and optimized. To the best of the author's understanding, the work presented here is first for South Africa as a country. Extraction processes with varying key process parameters were experimentally assessed for protein and keratin yield. The novel extraction procedure used a hybrid of two reducing agents; sodium hydroxide and sodium bisulphite, under mild concentrations to minimize the keratin protein structure's degradation. The extraction variables, optimised using Response Surface Methodology, were temperature (87°C), extraction time (111 minutes), sodium hydroxide (1.78%), and sodium bisulphite (0.5%). Analysis of the protein hydrolysate content showed the elemental composition of 13.85% N, 47.25% C, 6.90% H and 2.8% S, and a molecular weight range between 15 and 3 kDa; ideal characteristics for bio-binder applications. Keratin and cellulose nanocrystals were each evaluated separately as bio-adhesives for particleboard production. The efficiency of the formulated bio-adhesives and the mechanical strength performances of their fabricated particleboards were also assessed. Results showed that keratin on its own did not display significant binding properties; however, these were significantly improved by adding the citric acid-based polyamide-epichlorohydrin cross-linking agent. The fabricated particleboard's mechanical strength performance met the 1-L-1 grade specification of the American National Standards Institute.

Moreover, the beneficiation of extracted keratin protein hydrolysate from waste chicken feather with incorporated cellulose nanocrystals for bio-adhesive formulation and particleboard fabrication was investigated. The FTIR spectra confirmed the covalent bonding between the azetidinium of the citric acid-based polyamide-epichlorohydrin cross-linking and the hydroxyl groups of the keratin protein hydrolysate. The mechanical strength performance of the

fabricated particleboard met the specification for the 1-L-1 grade of the American National Standards Institute (A208.1). 6, 5 and 1184, 34 MPa, were the respective values obtained for modulus of rupture and modulus of elasticity of the panels made with keratin-based adhesive. Additionally, the keratin-based adhesive incorporated with cellulose nanocrystals as a filler enhanced the static bending and bonding strength properties of the formulated bio-adhesive.

Furthermore, the valorisation of wood sawdust into cellulose nanocrystals (CNC) for application as a binder in the manufacture of particleboard was also carried out. The cellulose nanocrystal extracted from wood sawdust using acid hydrolysis and an oxidizing agent, incorporated with crosslinking agents, viz., CNC-glyoxal, CNC-hexamine, CNC-polyamide-epichlorohydrin, and CNC-polyethylene to make cross-linked bio-binders. X-ray diffraction (XRD) indicated high crystallinity index (78%) of the CNC and typical nano dimensions of 2.1–10 nm for diameter and 150-350 nm for length as revealed by the transmission electron microscope (TEM). Thermogravimetric analysis (TGA) and differential thermogravimetric (DTG) showed high thermal stability (250 – 400 ℃) of the CNC. Significant mechanical strength performances of the particleboard panels were evident in the modulus of rupture (MOR) and the modulus of elasticity (MOE) of the CNC-binder fabricated particleboard. The panels met grade 1-L-1 specification of the American National Standards Institute A208.1. Similarly, the incorporation of cross-linking agents enhanced the static bending and bonding strength properties of the formulated CNC-binders. Hence, the research conducted in this thesis demonstrated the potential of bio-binders produced from waste biomass, viz., chicken feathers and sawdust to replace fossil-based binder.

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INTRODUCTION

General overview

The increased demand for wood products due to population growth has led to high adhesive consumption globally. This large demand is because large amounts of wood adhesives are used as bonding agents in wood composite industries. Consequently, the rise in the manufacture of reconstituted wood products such as particleboard has led to the increase in the uses of synthetic wood adhesives, which are usually formaldehyde-based and emit formaldehyde, which has been classed as a human carcinogen to the environment (Carvalho et al., 2014, McDevitt and Grigsby, 2014, Ülker and Ulker, 2019). Hence, there is a need to replace the resins with bio-adhesives sourced from natural biomass, which will be formaldehyde-free and have similar strength and binding properties as synthetic wood adhesives (Ghahri et al., 2021). Biomass from plants and animals such as lignocellulosic and animal protein sources contain materials and components that can be used as feedstock in bio-adhesives production (Pizzi, 2006). These biomass sources exist as large quantities of waste materials currently disposed of by environmentally unsustainable methods such as landfilling, incineration, or stockpiling on production sites. These materials are abundant, sustainable, renewable, and low cost. Beneficiation use of the eco-friendly feedstock into high-value chains such as binders for wood adhesives could significantly benefit industries that generate them. The synergistic relationship between the slaughterhouse waste bio-adhesive formulation and the wood industry could be made economically viable with the bio-refinery concept (Al-Salem et al., 2009). Various researchers have used different biological materials such as lignin, tannin, furfural, or soybean to either reduce the formaldehyde content in adhesives or even develop adhesives entirely from natural materials (Amaral-Labat et al., 2008, Alawode et al., 2020, Ostendorf et al., 2020) for the production of wood composite products such as plywood, particleboard, and oriented strand board (He, 2017). However, the binders have some drawbacks, such as low gluing strength, poor moisture resistance, and poor biodegradation characteristics (Zhang et al., 2018, Rosseto et al., 2019).

Furthermore, the prices of conventional binders are dependent on the oil market price. These oil prices are not reliable due to their fluctuating nature. Additionally, the depletion of fossil fuel reserves is a vital concern, making synthetic adhesives availability unpredictable in the

future (Alawode et al., 2019). The problems described above can be alleviated by replacing the synthetic adhesives with biological-based resins that can be modified to reproduce properties and the gluing characteristics of synthetic adhesives (Pizzi, 2006). Natural materials such as tannins, carbohydrates and proteins have been studied for wood adhesive potentials (Amaral-Labat et al., 2008). The most desirable and conventional natural-based adhesives are protein-based wood adhesives (Müller et al., 2007). As outlined earlier, natural proteins offer many advantages such as renewability, availability, and relatively low cost that qualify them as a possible adhesive source for industrial applications (Müller et al., 2007). Adhesive derived from animal hides and bones has been used from ancient times (Pearson, 2003). Nevertheless, only a few research has been carried out on animal sources of bio-adhesive from slaughterhouse by-products (such as waste from poultry meat processing).

The majority of the generated chicken feather waste in many countries is disposed of inappropriately, resulting in environmental problems such as air pollution (Chinta et al., 2013). On the other hand, research into their valorization has gained global attention and has necessitated searching for chicken feathers' best application. Waste chicken feathers are currently not exploited for valuable products at a commercial scale (Nuutinen, 2017). There is limited research on evaluating potential bonding properties of keratin protein extracts from chicken feathers waste, especially for wood composites. Keratin protein can therefore be extracted and used as a natural raw material for the production of films, fibres, hydrogels, binders, micro and nanoparticles for cosmetic, medical, textile, composite and other industrial applications (Khosa and Ullah, 2013).

Keratins are not soluble in conventional polar and non-polar chemical solvents due to their recalcitrant structure: an extensive disulphide cross-linking, tightly packed α-helices, and β-sheets in the polypeptide chain. A suitable and scalable pre-treatment process to extract these keratins is desirable. There are significant functional groups in the keratinous protein that should be considered during processing, regeneration, and modification of feather keratin for further applications. The type of pre-treatment technique and chemical solvent selected is based on the application to which keratin protein is to be used (Nuutinen, 2017). The most common method for the extraction of keratin is to use reducing agents in an alkaline solution. The extracted protein quality can be controlled via the solvent of extraction, dissolution time, and other physical parameters employed. These will reflect on the variability in the extraction process (Poole et al., 2011). Regardless of several studies devoted to the valorization of keratin-based materials such as chicken feathers, there are still challenges due to the recalcitrant nature of keratin to various solvents, enzymes, and physical conditions. Also, the potential adverse

effect (such as protein deformation and aggregation) of the chosen methods. Hence, an approach preventing detrimental changes of amino acids, leading to the keratin of desirable dissolution and ultimately improved protein yield is needed. Additionally, there is a shortage of scientific knowledge in the literature on optimized processes to extract keratin protein from waste chicken feathers.

Optimization

Process optimization is an essential factor in developing economically viable processes, owing to their impact on the techniques. Process optimization aids in reducing the cost profit-ratio to establish an industrial-scale production system (Schmidt, 2005, Singh et al., 2017). Process optimization is crucial to an industrial production process, in which even slight improvements can be essential for the commercialization of a process. Process performance is influenced by many process parameters, including the solvent choice, temperature and pH (Sinkiewicz et al., 2017). Modeling and optimization have been employed to improve processing using various modeling tools such as Response Surface Methodology (RSM).

RSM is a blend of stepwise mathematical and empirical techniques developed to improve and optimize chemical processes. This technique's merits include minimum experimental runs, less process time, assessment of relations between experimental factors and the target responses (Singh et al., 2017). Optimization is one of the essential procedures to develop a robust chemical process for industrial applications to improve keratin production (Malik and Rashid, 2000). Optimization experimental design is of immense importance in the chemical process with various interacting parameters due to the complexity of their interaction; therefore, a suitable experimental design must be employed to assess these parameters' impact in the process (Bezerra et al., 2008). Equally, the model would provide valuable suggestions on the analysis, design and operation of the process that will be of enormous importance in scaling up the process (Schmidt, 2005).

Rationale and significance

In the olden days, natural adhesives were used in the wood products industry but later displaced by synthetic, mostly thermosetting adhesives as natural binders had poor performance concerning water resistance and mechanical strength. Consequently, thermosetting adhesives are formaldehyde-based, but their use causes environmental pollution, and they are detrimental to human health once released to the environment. However, to curb these binding deficiency

and ecological problems, the synthetic adhesives should be replaced by revisiting the natural adhesives and modifying them to reproduce synthetic resins' behavior and performance. On the other side, there is waste biomass, which is being landfilled and incinerated; these include waste chicken feathers and sawdust. This disposal practice leads to environmental problems (Greenhouse gases emission, fire hazards and flue gas). The landfilled biomass should be valorized into valuable chemicals used to produce value-added bio-products such as bio-adhesives.

Waste chicken feathers are one of the significant waste generated from poultry slaughterhouses. An estimated 15 million tons of chicken feathers are available globally each year as a by-product of poultry processing. According to the USA Foreign Agricultural Service, the total domestic per capita consumption of chickens in the USA and some selected countries, including South Africa in 2019, is about 96,464 x 10^3 metric tons (USDA;, 2019). Similarly, there has been an increase in chicken production and processing in other African countries over the last decade. As a result of an increase in chicken meat consumption worldwide, the poultry meat processing industry generates large amounts of feather by-products that result in about 40 × 10^9 kg waste stream annually, with about 258 million tons from South Africa (Tesfaye et al., 2017).

Moreover, about five to seven percent of poultry's bodyweight is feathers. A chicken meat processing plant with a capacity of 50 000 birds generate about 2-3tonnes of dry feathers daily. Chicken feathers are considered a waste; currently, their uses are economically marginal, and they are difficult to dispose of, thereby leading to environmental pollution. Presently, the feathers from small and large-scale poultry industries in most countries are disposed of in landfills, burned or processed to make a low-grade animal feedstock. Meanwhile, feathers are bio-resources with a high protein content that is a good source of natural adhesive, and this is because it consists of about 91% keratin. Keratin is a hard protein that can be chemically extracted to produce a protein-based resin for wood composites industries. The use of these adhesives from waste chicken feathers can reduce the cost of wood panel production. It will also reduce the environmental pollution that can be caused by improper disposal of waste chicken feathers. The unique characteristics of waste chicken feather keratin have prompted interest in investigating this keratin for many potential applications (Frazer, 2004, McGovern, 2000). Poultry feathers are currently renewable protein resources, inexpensive, and abundantly available keratinous biomass, but with limited applications.

Another waste with the potential of been valorized for bio-adhesive is the wood sawdust. Sawdust is a waste product generated from the wood products industry when processed for

different applications. The literature revealed that every log of wood produced about 10% of wood sawdust during processing (Olufemi et al., 2012). Currently, a little amount of sawdust is utilized as wood compost or valorized as particleboard. Simultaneously, most of them are either stockpiled or landfilled, causing additional environmental problems. A valuable product such as cellulose nanocrystals (CNC) is a unique nanomaterial that could also be obtained from sawdust. These nanomaterials have several notable mechanical, optical, chemical, electrical and rheological properties (George and Sabapathi, 2015). These distinctive properties could be of immense value in the bio-adhesive formulation and gluing strength enhancement and efficiency. There are limited research works on evaluating potential bonding properties of nano-based bio-adhesive, especially for wood composites.

Aim and objectives

This study aimed to synthesize and characterize bio-adhesives from renewable and sustainable waste chicken feathers and wood sawdust for particleboard fabrication.

The specific objectives were:

- To optimize keratin protein extraction process from waste chicken feathers;
- To modify the keratin protein obtained in objective (i) for bio-binders application;
- To produce cellulose nanocrystals from wood sawdust for bio-adhesive production; and,
- To evaluate the binding performances of the produced bio-adhesives in particleboard fabrication

The organization of the thesis

This dissertation comprises six chapters and is based on publications referred to by their titles in the text. The publication outputs include those that are in print and those submitted for publication in high impact factor peer-reviewed journals. Each chapter is self-contained, containing a brief introduction for the study's motivation through literature, materials and methods, results and discussion, and conclusions.

Chapter 1 is an overview that offers background information and the rationale for the study, including the overarching aims and specific objectives of this study.

Chapter 2 consists of a literature review on background information on current wood adhesives used in the wood products industry and the challenge posed to the environment and the

alternative renewable feedstock sources. A peer-reviewed journal article has been written from this work.

Chapter 3 focuses on developing a hybrid pre-treatment technique and optimizing the operational condition for keratin extraction from waste chicken feathers using response surface methodology. A peer-reviewed publication had been generated from this work.

Chapter 4 focuses on bio-binder synthesis from the feathers keratin hydrolysate and its application for particleboard manufacture. A peer-reviewed publication has been derived from this work.

Chapter 5 is about the cellulose nanocrystals extraction and its application as a bio-binder for particleboard fabrication. A peer-reviewed journal article has been written from this work.

Chapter 6 summarises the whole dissertation, the various findings are drawn, and recommendations for future work are made.

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CHAPTER 2

PAPER 1:

SUSTAINABLE SOURCES OF BIO-ADHESIVES FOR APPLICATION IN THE WOOD PRODUCTS INDUSTRY: A REVIEW

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Abstract

The wood products industry consumes large quantities of adhesives accounting for more than 65% by volume of the adhesives used worldwide. The resins are primary bonding agents for wood composites production and are sourced from synthetic or natural resources. Synthetic adhesives are formaldehyde-based and emit formaldehyde, thus causing environmental pollution and adverse effects on human health. Hence, there is a continual interest in replacing them with bio-adhesives sourced from natural sources. This report is a critical review of challenges concerning synthetic adhesives used in the wood products industry and how these can be addressed by replacing the resins with bio-adhesives sourced from natural raw materials like plant and animal waste biomass, such as lignocellulosic and proteinaceous (keratinous and casein) sources. These biomass sources contain materials and components that can be used as adhesives or converted into bio-adhesives. The biomass exists in large quantities as waste materials currently disposed of by environmentally unsustainable methods such as landfilling, incineration, or stockpiling on production sites. These wastes can be beneficiated via bio-refinery technologies into high-value materials, including binders. Such usage can enable disposable of the biomass in an environmentally sustainable manner. Significant problems associated with the use of bio-adhesives are poor performance related to water resistance and bonding strength. However, these can be overcome through chemical modification, cross-linking agents and incorporating natural additives derived from lignocellulosic biomass.

Keywords: synthetic adhesives, bio-adhesives, bonding, wood composites, chicken feathers, keratin, casein, lignin, cellulose nanocrystals

2.1 Introduction

Chemical and natural bonding of wood components have played an essential role in the development and growth of the wood products industry and has been a critical factor in the efficient utilization of the timber resources (Frihart and Hunt, 2010).

The bonding agents, which are majorly adhesives and some other additives, are the basis for the industrial production of wood composites. An adhesive or glue is referred to as a compound in a liquid or semi-liquid state that adheres or bonds two items together (Mehdizadeh and Yang, 2013). The overall demand for adhesive has increased globally since 2009; in 2013, the global market volume was about 13 million tonnes (Zhao, 2017). The most significant amount of this demand came from Asia-Pacific (8 million metric tons), North America (3.1 million metric tons), Europe-Middle East and Africa (EMEA) (Statista, 2016, Zhao, 2017) this is with the projection of increment by 2019, as shown in figure 2. 1. The wood product manufacturers are the highest adhesive users, and they are responsible for more than 65% by volume of the adhesives used and demand in the world (Zhao, 2017) because the use of adhesives is a daily occurrence in many wood products industries, such as particleboard, fibreboard, plywood, and so on.

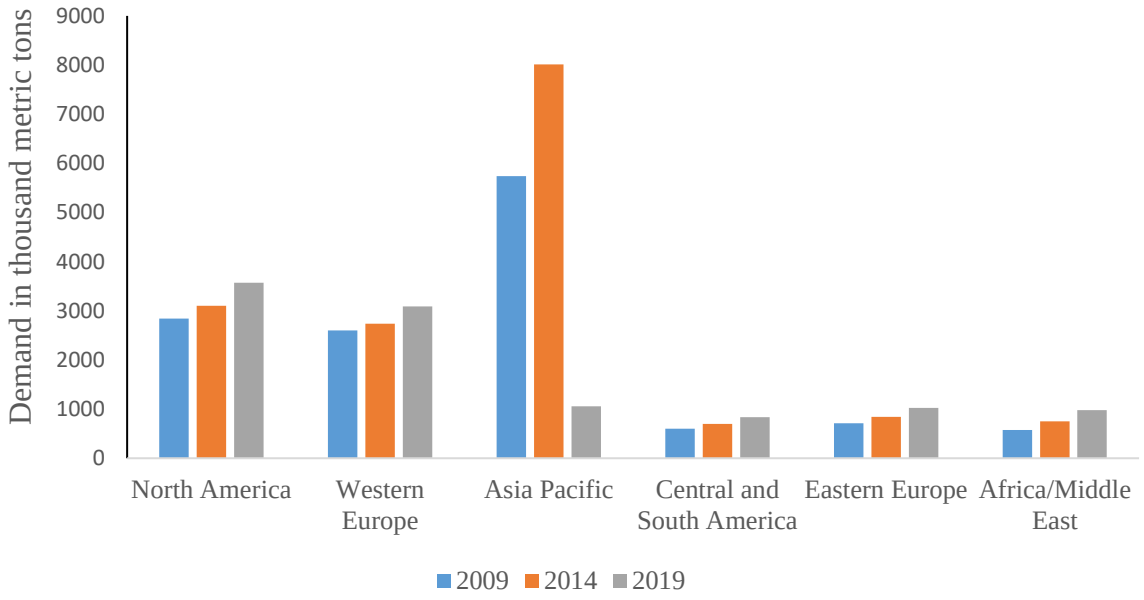

Figure 2. 1. Adhesive and sealant demand worldwide by region from 2009 to 2019 [adapted from (Statista, 2016)]

Wood adhesives from natural sources were predominant adhesives used in the wood products industry but displaced by synthetic resins derived from petrochemical sources (Conner and Bhuiyan, 2017). Although various political, environmental, and economic factors have always

caused the supply of petrochemicals to decrease dramatically, producing a related marked increase in the price of petroleum-based adhesives (Conner and Bhuiyan, 2017). However, most synthetic resins used in wood-based industries are formaldehyde emitters, which cause environmental pollution and are detrimental to human health. It is a human carcinogen, likewise known to cause various immediate health problems such as cough, headache, and mucous membranes (Kim et al., 2011). The real concerns are how to reduce chemical emissions of synthetic adhesives and follow government regulations, which are just starting to be put into place to relieve the public's environmental concerns (Pizzi, 2006). And this has led to continued interest in the natural sources of wood adhesives or starting materials for the production of bio-adhesives for wood products industry (Lambuth, 1989). However, bio-adhesives have had poor performance compared to synthetic, particularly with water resistance and bonding strength (Vnučec et al., 2017, Frihart, 2005). Hence, adhesives based on natural sources are concentrated on improving the mechanical properties and water resistance of the adhesive bonds. These involve using different methods like thermal treatment procedure, the addition of chemical or enzymatic modifiers, and the extraction of materials in a form containing a high proportion of protein (Vnučec et al., 2017). However, these adhesives' nature and composition depend on the expected performance of the bond strength and durability, especially in different environments (temperature, rain, humidity, mild, and so on). This review paper highlights the challenges posed by synthetic binders and possible replacement by bio-binders from different sources including waste chicken feathers.

2.2 Synthetic adhesives commonly used in the wood products industry and their challenges

There are two significant wood adhesives, synthetic and natural, because adhesives may come from either synthetic polymers or natural polymers (glue) (Conner, 2001). As stated by ASTM, a polymer is a compound formed by the reaction of simple molecules having functional groups that permit their mixture to produced to higher molecular weights under suitable conditions (Vick, 1999). In terms of delivery, glue resins are liquid and consist of linear or branched oligomeric and polymeric molecules in an aqueous solution and their partly dispersion in this form (Conner, 2001). Synthetic adhesives include either prepolymers (oligomers) or polymers synthesized from petro-chemical derived raw materials, classified as thermosetting and thermoplastic, which resins the polymer's nature after it has set or cured (Conner, 2001). Wood adhesives are classified into different groups based on their chemistry, kind, and applications

(Conner and Bhuiyan, 2017). The groups include natural polymers (glue) and synthetic polymers, which have two categories thermoplastic and thermosetting resins (Conner, 2001). The thermoplastic resins are polymers that soften when exposed to heat and solidify on cooling to room temperature; these polymers are generally soluble (Conner, 2001). Thermosetting resins are polymers that change irreversibly into an infusible, insoluble polymer network by curing (Horie et al., 2004). They constitute the primary type of adhesives presently used to bond wood in the wood processing industries (Conner, 2001). The wood composite industries' three important ones include amino resins, phenolic resins, and isocyanates (Conner, 2001). They harden by chemical reaction and are usually controlled by; amount of catalyst added, temperature of the glue line and moisture in the glue line (Conner, 2001).

2.2.1 Amino resins

Urea-formaldehyde resins are the key examples of the adhesives referred to as amino resins (Pizzi, 1994) and are among the main resins used in the wood products industry. It comprised about 80% of the amino resins produced worldwide (Conner, 1996). They are the condensation products obtained by formaldehyde reaction with nitrogen-bearing compounds such as urea, aniline, and amides (Sivasankar, 2008). The condensation reaction between urea and formaldehyde in an acidic or alkaline medium produced urea-formaldehyde (Sivasankar, 2008). The first product formed when urea reacts with formaldehyde during the resin production is mono-methylol and dimethylol ureas. Simultaneously, polymerization can occur from mono or dimethylol urea or possibly through both, forming long chains (Sivasankar, 2008). Urea-formaldehyde (UF) resins are the most used class of amino resin adhesives (Conner, 1996).

Over one hundred million metric tons of UF resins are produced annually, while more than 70% of this resin is utilized by the wood composite industry (Conner, 1996). The primary use of UF resins by the wood products industry is due to many advantages, which include low cost, ease of use under a wide variety of curing conditions, low cure temperatures, water solubility, and resistance to microorganisms, hardness, excellent thermal properties, non-flammability and lack of colour of the cured resin (Pizzi, 1994). Despite all these advantages, there are several disadvantages associated with UF resins such as formaldehyde release from wood-based panels that exceed the limit, and the form of a brittle adhesive layer (Zhao et al., 2011), lack of resistance to moist conditions, especially in the presence of heat (Pizzi, 1994).

Formaldehyde release of Urea-Formaldehyde is the major problem of these adhesives; it is mainly due to its remaining parts, followed by the unstable methylene ether during formaldehyde and urea synthesis reaction, its hot press and decomposition (Zhao et al., 2011). However, a lot of attention has been given to formaldehyde release of wood-based panels due to its possible health hazard on human beings and the environment (Roffael, 2006). Different methods have been employ to reduce the formaldehyde release of wood-based panels (Roffael, 2006). These methods include using unique resin formulations and condensation techniques to reduce the molar ratio of formaldehyde and urea in UF-resins, modification of urea-formaldehyde resins with the use of formaldehyde scavengers, as well as using optimal post-treatment methods to decrease the free formaldehyde and keep the other properties almost unchanged (Roffael, 2006). Significant changes in the resin formulation have been achieved for the past twenty years, especially for particleboard and medium-density fibreboard, by decreasing the F/U molar ratio of urea-formaldehyde resin (Roffael, 2006). A decrease in the molar ratio below a particular level leads to a reduction in the formaldehyde emission during the production of the wood-based panels and the subsequent formaldehyde emission. Still, the boards' mechanical strength properties are also affected, and the degree of hardening of the resins (Dunky, 2003). The low strength and the hardening degree problem also lead to an increase in the thickness swelling and water absorption of the wood products, making the boards susceptible to hydrolysis (Dunky, 2003). The imbalance in the reactant's molar ratio during the resin synthesis; is responsible for the problems mentioned above. Meanwhile, these have not been able to eliminate the problem of formaldehyde emission.

Costa et al. (2013) examine the performance of some formaldehyde scavengers in wood-based panels. The scavengers used were powder sodium metabisulfite, aqueous solutions of sodium, and ammonium bisulfite and urea. Formaldehyde emission of particleboards produced with the resins was evaluated; boards made with sodium metabisulfite modified resins exhibited formaldehyde content near wood levels and zero formaldehyde emissions. In contrast, those modified with ammonium bisulfite did not perform very well in internal bond and thickness swelling (Costa et al., 2013). It was reported that boards with a higher content of sodium metabisulfite showed zero-emission without deteriorating physicomechanical properties (Costa et al., 2013). Polyvinyl alcohol (PVA), aniline, and $CaCO_3$ nano-particle have been used to modify urea-formaldehyde. They were able to reduce formaldehyde emission partially but were unable to sufficiently improve urea-formaldehyde resins (Zhao et al., 2011). These have not been used for commercial applications. This work shows that renewable raw material needs to

be introduced. An in-depth study needs to be done on formaldehyde scavenger and as an alternative source of bio-adhesives.

2.2.2 Phenolic resins

Another general adhesive used in the wood products industry is phenol-formaldehyde (PF) resins or Bakelite; this is an essential member of the phenolic resin and are the second largest most used wood-based adhesives after urea-formaldehyde. Phenolic resins are the significant adhesives used for bonding wood panel products for exterior applications (Conner, 2001). For example, about 60 % of the total US demand for PF resins comes from the forest products industry (White, 1995). The PF adhesive resins are used primarily to produce plywood, oriented strand board, medium-density fibreboard, particleboard, and wafer board (Conner, 2001). Phenolic resins are condensation polymerization of phenol or phenolic derivatives like resorcinol, also aldehyde such as formaldehyde and furfural (Sivasankar, 2008). The phenol condensation polymerization with formaldehyde in acidic or alkaline catalyst forms formed phenol-formaldehyde (Sivasankar, 2008). While the initial reaction results in the formation of O- and P- hydroxyl methyl phenol, which react to form linear polymer navalac. During the molding reaction, hexamethylenetetramine [$(CH_2)_6N_4$] is added, which converts the fusible novalac into a hard infusible and insoluble solid cross-linked structure known as Bakelite (Sivasankar, 2008). They have excellent properties to resist weather, water, and temperatures, have high bond strength after their solidification process. They are commonly used in outdoor structures of wood-based panels (Zhao et al., 2011). Another advantage of phenolic resins is the very low formaldehyde emission in service after hardening due to the methylene bridges' stability between aromatic nuclei (Dunky, 2003). The disadvantages of phenolic resins are the distinctly longer press times necessary for hardening when compared to UF resins, the dark color of the glue line and the board surface as well as a higher equilibrium moisture content of the boards due to the hygroscopicity of the high alkali content of the board (Dunky, 2003). Also, its raw material comes from petroleum products also emit formaldehyde though in low quantity. Decreasing oil reserves and rising oil prices have brought many uncertainties to the use of phenol-formaldehyde resin and they are expensive to use.

Moreover, phenol is highly toxic, and formaldehyde is a hazardous chemical (Gardziella et al., 2000). The scientist has been committed to replacing phenol in the PF resins. Some of the substitutes are pyrolytic oil, lignin, tannin, wood liquefaction products, and biomass pyrolysis tar (Zhao et al., 2011). Nakos et al. (2001) replaced about 50% of phenol needed in PF resins

production with a phenol-rich pyrolytic oil and modified the synthesis process. The resin was used in the production of oriented strand board (OSB), and it was reported that a comparable result with commercial PF resins was achieved (Nakos et al., 2001). Another work investigated the substitution of phenol in the PF resins with organosolv lignin. The study reported that organosolv lignin was used to partially replace up to 30% of phenol that is generally used to produce conventional phenol-formaldehyde resins. The performance was tested in particleboard production (Cetin and Özmen, 2002a, Cetin and Özmen, 2002b). However, formaldehyde is still present in the resins formulation. The formaldehyde emission rate, which is of much health and environmental concern, was not determined in these studies. However, there is a necessity for more research work on sourcing alternative renewable raw material, which is not petroleum-based, and formaldehyde emitters.

2.2.3 Isocyanates resins

Isocyanates are essential chemicals used in the industrial mostly for injection molding and production of polyurethane foams. The phosgenation of amines produces isocyanates and all isocyanates contain two or more isocyanate groups (–N = C=O) per molecule (Conner, 2001). Polymeric diisocyanate (PMDI) is an essential adhesive in the wood products industry, especially for particleboard and oriented strand board production. PMDI synthesis begins with aniline condensation with formaldehyde in an acid solution and a complex blend of isomeric diamines and oligomeric polyamines is formed (Conner, 2001). This complex mixture is phosgenated to give polymeric diisocyanates (PMDIs) with various other resins to yield thermosetting wood adhesives. The wood products industry use PMDI as adhesive, rather than a purified diisocyanate (Conner, 2001). According to Pizzi (1994), the limitation for accepting this adhesive as thermosetting wood adhesives are due to the following factors: their fundamental characteristic of tending to bind the board to the hot press machine, requiring that the board surfaces be glued with a different type of adhesive. Also, their poisonousness and low vapor pressure, the inability to utilize them in plywood and the difficulty of diluting them with water are part of the constraint. However, these issues were in part addressed by the advent of emulsified diisocyanates (Ball and Redman, 1983), they are still prohibitive to use as a wood composite binder.

2.3 Environmental impact and cost implications of synthetic adhesives

There has been a growing concern over synthetic adhesives materials, which are considered environmentally unfriendly (McDevitt and Grigsby, 2014). They are termed unhealthy to the environment because even the wholly cured resins regarded as nontoxic and safe can also produce hazardous materials for both humans and the environment (Yang and Rosentrater, 2015, Adhikari and Ozarska, 2018). Below are some of how synthetic adhesives affect both the environment and the human being.

2.3.1 Impact due to raw material selection

The most common adhesives used in wood composite panels industries are formaldehyde-based derived from urea-formaldehyde (UF) and phenol-formaldehyde (PF) (Zhang et al., 2013). Many adhesives cure by chemical reactions and therefore are hazardous in the uncured state. Meanwhile, uncured adhesives can be harmful and require safety precautions, while cured resins are usually safe for human contact (Frihart and Hunt, 2010). Nevertheless, the urea-formaldehyde adhesive is exceptional as it can release low concentrations of formaldehyde gas from bonded wood products, especially under hot and moist conditions (Frihart and Hunt, 2010). Phenol (resorcinol)-formaldehyde adhesives, used to manufacture plywood, strand board, and laminated beams for exterior purposes, also contain formaldehyde. Phenol is highly toxic, and formaldehyde is a hazardous chemical that can react with the body's proteins to cause irritation and inflammation of membranes of the eyes, nose, and throat and could be a human carcinogen (Mao and Kim, 2013, Frihart and Hunt, 2010). However, exposure limits have to be strictly controlled (Gardziella et al, 2000). Diisocyanates are sensitizers that are capable of causing occupational asthma (Frihart and Hunt, 2010). They also are highly reactive chemicals that polymerize rapidly in contact with a strong alkali, mineral acids, and water (Frihart and Hunt, 2010). Because polymeric methylene diphenyl diisocyanate (pMDI) adhesives develop strong and durable bonds to wood, they have gained acceptance in composite wood products (Frihart and Hunt, 2010). Nonetheless, any isocyanate is potentially hazardous if mishandled. Still, the low vapor pressure of pMDI adhesives coupled with adequate ventilation to remove airborne pMDI on dust particles may permits manufacturing plants to operate safely (Frihart and Hunt, 2010).

2.3.2 Impact due to the manufacturing process

Adhesives manufacturing processes, such as machining, grinding, and synthesizing, could produce hazardous materials to both human beings and the environment. Phenol and substituted phenols, formaldehyde, and possible solvents are liberated during various operations, like those that need mixing, impregnation, and drying (Gardziella et al., 2000). The primary emissions during the curing of phenolic resins are phenol and formaldehyde, while ammonia is emitted when novolak-hexamethylene tetramine systems are used (Gardziella et al., 2000). However, international occupational exposure limits are quite different in some cases but exhibit trends toward lower ranges (Gardziella et al., 2000). The occupational exposure limit is the maximum permissible concentration of industrial material in the form of a gas, vapor, or suspended matter in the atmosphere of the workplace (Gardziella et al., 2000). However, based on present knowledge, it does not generally impair the workers' health or represent an excessive nuisance to them even in case of repeated and lengthy exposure (Gardziella et al., 2000). Some primary factors, like toxicity, flammability, hazardous incompatibility, and equipment safety, could be considered imperative in adhesive bonding procedures (Frihart and Hunt, 2010). The environmental impacts of adhesives are the issues with human health and the nearby community's problems because of the discharge of volatile organic compounds (VOC) and other waste (Yang and Rosentrater, 2015). For instance, toluene, one of the solvents, is volatile organic compounds (VOCs) used as carrier fluids in conventional heat-melt adhesives. This solvent is environmentally damaging and causes safety concerns (Yang and Rosentrater, 2015).

2.3.3 Impact due to disposal of the waste wood products

Considering the future downstream uses and final disposal of wood products, this creates various environmental impacts, especially in the urban area. The primary sources of municipal wood wastes are commercial and industrial wastes, construction and demolition activities, pallets and packaging, and utilities (Adhikari and Ozarska, 2018). All these are mostly disposed of in landfills. However, when the waste products are disposed of instead of being reused, recycled, or refurbished, they will create outside pollution and greenhouse gas emissions in many ways. Such as transfer from the source to a landfill site, discarding of synthetic materials adds to poisonous waste, which can leach from landfill, and finally, such materials take up a large volume of space in landfill locations and create the need for new waste disposal sites (Adhikari and Ozarska, 2018, Kharazipour and Kües, 2007). Besides, if the disposal is carried out by burning of used products, it also releases smokes, contamination, and emissions in the

environment. Table 2.1 below shows the damage and impact categories of synthetic adhesives on human health (Disability-adjusted life years-DALYs), ecosystem quality (potentially disappeared of affected fraction-PDF) and depletion of resources (surplus energy for future extraction-MJ) while figure 2. 2 is showing the comparison of life cycle value of environmental impact using Eco-indicator '99 points (McDevitt and Grigsby, 2014).

Table 2.1: Damage and impact categories of synthetic adhesives [adapted from (McDevitt and Grigsby, 2014)].

Damage categories	Impact category	Damage unit	Normalization	Weight (%)
Human health	Carcinogens (CA)	DALY	2 9 10^{3}	40
	Respiratory organics (RO)	DALY	6.8 9 10^{-5}	
	Respiratory inorganics (RI)	DALY	1.1 9 10^{2}	
	Climate change (CC)	DALY	2.4 9 10^{3}	
	Ozone (OZ)	DALY	2.2 9 10^{-4}	
	Radiation (RA)	DALY	2.7 9 10^{-5}	
Ecosystem quality	Eco-toxicity (ET)	PDF 9 m^{2}	8.1 9 10^{2}	40
	Acidification/Eutrophication (AE)	PDF 9 m^{2}	3.8 9 10^{2}	
	Land use (LU)	PDF 9 m^{2}	4 9 10^{3}	
Resources	Fossil fuels (FF)	Surplus MJ	8.3 9 10^{3}	20
	Minerals (MI)	Surplus MJ	1.5 9 10^{2}	

DALYs = Disability-adjusted life years-DALYs, PDF = potentially disappeared of affected fraction, MJ= Meter per Joule

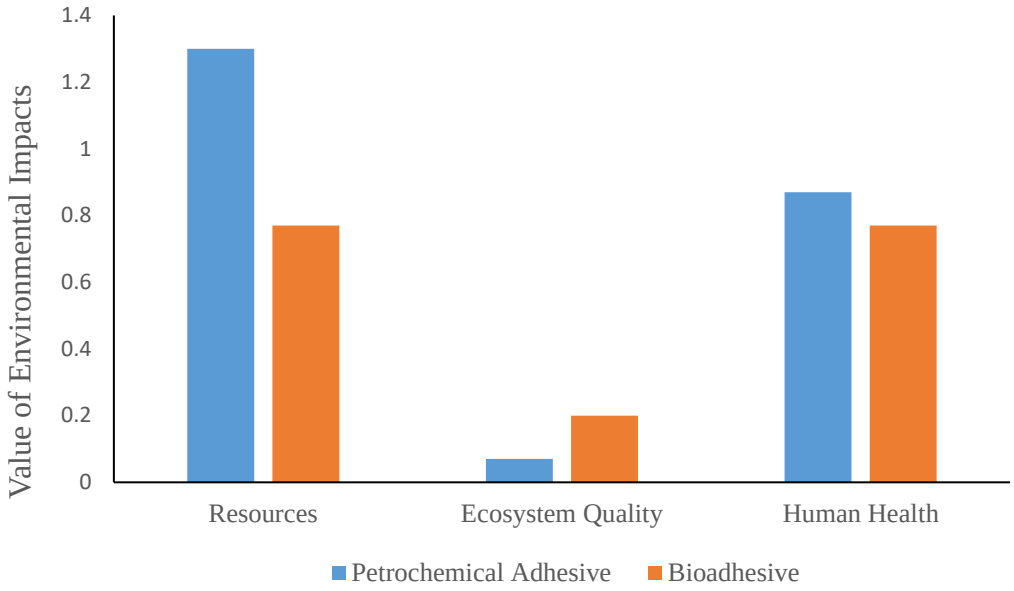

Figure 2.2: Comparing synthetic and bio-adhesive environmental impact using life cycle analysis [adapted from (McDevitt and Grigsby, 2014)].

2.3.4 Cost implication on the downstream disposal

Knowing full well that adhesives are more expensive than wood, the cost of resin and price of related equipment for the application, labor and downstream disposal must all be considered (Frihart and Hunt, 2010). Literature shows that the cost of organic solvents and the cost of recovering volatiles to prevent air pollution have increased in recent years (Frihart and Hunt, 2010). The cost of adhesives accounts for about 20% of the total wood-based panel production (Kharazipour and Kües, 2007). This condition has led to rise in wood panels' price and continued interest in alternative and natural sources of wood adhesives worldwide. For environmental and economic reasons, renewable raw materials are presently the object of increasing attention (Gardziella et al., 2000).

2.4 Natural-based wood adhesives

Adhesive from natural polymers, also called bio-adhesives, include those materials of natural and non-mineral origin used directly or after a little modifications to reproduce characteristics of synthetic resins' behavior and performance (Pizzi, 2006). Some of the natural raw materials are tannins, lignins, carbohydrates, unsaturated oils, proteins, blood, and collagen, which have been in use to some extent for a very long time (Pizzi, 2006) as many of the early civilizations learned how to make and use adhesives from plant and animal sources (Frihart and Satori, 2013). Some of the natural materials used in the formulation of bio-adhesives, their challenges,

and possible modification are discussed below, including materials, which have not been fully exploited as bio-binder applications in the wood products industry.

2.5 Possible renewable materials for bio-based adhesives and their challenges

Some of the renewable biomass that has been investigated for bio-adhesive application and their problems are reviewed below.

2.5.1 Lignin

Lignin is the most abundant and essential polymeric organic substance, next to cellulose in lignocellulosic materials (Bowyer et al., 2007). Besides, it is a complex and high molecular weight polymer built upon phenylpropane units (Bowyer et al., 2007). They are abundant and of low cost and are generally produced in high quantities as waste from pulp and paper mills (Pizzi, 2003). More than 50 million tons of lignin accumulate each year as a by-product of pulp manufactures, such as lignosulfonate from sulfite pulping processes and kraft lignin from sulfate pulping processes (Müller et al., 2007).

In the critical area of potential applications such as wood adhesives, the industrial use has been lagging as they have lower reactivity towards formaldehyde, or other aldehydes, than phenol (Pizzi, 2003). However, technical lignins from different pulping methods do not behave the same way. Kraft lignins and soda-anthraquinone lignins appear to have better reaction properties than lignosulphonate, organosolv and ethanol process lignins (Müller et al., 2007). Varieties of useful lignin adhesive formulations exist, and some of these have been in use for a while, especially in particleboard or plywood mills (Pizzi, 2003). Not all these formulations are used today. They frequently exhibit two general problems: corrosiveness of the formulation or noticeably increase in panel pressing time, which affects productivity at mills (Pizzi, 2003). Industrially, none of the adhesive systems based on pure lignin resins performed very well without the addition of synthetic resins or lignin modification (Hamarneh, 2010). Thermal conversion methods such as fast and vacuum pyrolysis, pressure liquefaction, and phenolysis have been used to produce pyrolytic lignins that are easy to incorporate into phenol-formaldehyde resin formulations (Hamarneh, 2010). Li and Geng's study showed that the treatment of brown-rot-fungus modified lignin with sodium borohydride ($NaBH_4$) followed by mixing with polyethylenimine (PEI) resulted in a formaldehyde-free, durable, and water-resistant wood adhesive. These studies show that modification of lignin is necessary before they can be used successfully as wood adhesives (Hamarneh, 2010, Li and Geng, 2005).

2.5.2 Tannins

In all plants, tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins but exist in high concentrations in only a few species from which they can be isolated (Devendran and Balasubramanian, 2011). Two types of tannins can be extracted from the bark of trees: condensed tannins and hydrolyzable tannins. Condensed tannins are non-hydrolyzable oligomeric and polymeric proanthocyanidins that are formed by the condensation of flavans. They do not contain sugar residues, while hydrolyzable tannin is a type of tannin that can be fractionated hydrolytically into their components by treatment with hot water or on heating with acids, yields gallic or ellagic acids (Khanbabaee and Van Ree, 2001). Hydrolyzable tannins have been successfully used as partial replacements for phenol in phenol-formaldehyde resin adhesives (Pizzi, 2003). Condensed tannins are abundant and have generated considerable interest as precursors for adhesive production (Pizzi, 2003). Some of the chemical compounds found in condensed tannin can be reacted with formaldehyde or phenolic-formaldehyde prepolymers to manufacture suitable thermosetting resins for use in exterior conditions, also for making cold setting, waterproof adhesives (Pizzi, 2006, Müller et al., 2007).

The chemistry of phenol and tannin's reactions has been studied using catechin as a model (Peng et al., 1997). According to Peng et al., the study results show that phenol and tannin's reactions are very complex. However, the lack of macromolecular structure in the natural tannin state, phenol substitution low level, low nucleophilicity, limited worldwide production, and higher price decrease their chemical and economic interest and their use as an adhesive precursor (Pizzi, 2006, Pizzi, 2003). Synthesis of tannin based wood adhesives from condensed tannins in the presence of polyethylenimine (PEI) was successful; the adhesives showed high shear strength and high water resistance, whereas tannin-PEI adhesives showed exhibited a significant loss in the bond strength after long term storage (Li et al., 2004, Hamarneh, 2010). Spruce tannin was used in the fabrication of medium-density fibreboard (MDF) up to about 60%. The boards met moisture resistance specification (according to EN 319 and EN 321). The authors noted that a 100 percent tannin could be used to produce indoor MDF only (Roffael et al., 2000). Some studies reported that tannin-based adhesives are highly viscous, and the addition of water to reduce the viscosity before application always leads to the generation of extra steam during the curing process in the hot press, and this results in deformation in the wood elements (Roffael et al., 2000, Hamarneh, 2010). Moreover, the resins have relatively short gelation time because of their high reactivity and tendency to harden before pressing off

the mat (Roffael et al., 2000). Additionally, they are not readily available, and their supply is limited (Li et al., 2004, Hamarneh, 2010).

2.5.3 Carbohydrates

Carbohydrates in the polysaccharides type are abundant and readily available because they are present in all plant biomass (Baumann and Conner, 1994, Pizzi, 2006). There are three major carbohydrate polymers obtained from plant biomass: polysaccharides consisting of cellulose, starch, and gums (Pizzi, 2006). Cellulose is a high molecular weight polymer made up of glucose monomers linked by β1-4 glycosidic bonds. Starch is a glucose polymer where alpha-linkages bond the glucopyranose units (Izydorczyk et al., 2005). The natural gums are polymers composed of long chains of sugars in a plant that are either soluble in water or can absorb water. They can be used directly as wood adhesives or after modifications used in three main ways, as modifiers of existing phenol-formaldehyde and urea-formaldehyde adhesives, by forming degradation compounds, which can then be used as adhesive building blocks, and directly as wood adhesives (Pizzi, 2006, Hemmilä et al., 2013). Cellulose is one of the major chemical components of the wood cell wall and contributes about 40-45% of the total wood dry mass. It comprises cellobiose units, building blocks of two β(1-4)-glycosidic linked glucose molecules (Müller et al., 2007). It is responsible for the wood fibre strength because of its high degree of polymerization and linear orientation (Klemm et al., 1998). Because of the large number of hydroxyl groups, cellulose molecules readily form hydrogen bonds with other cellulose molecules to give crystalline structures that are not easily disrupted by acid hydrolysis (Baumann and Conner, 1994).

Consequently, cellulose does not dissolve in most common solvents. Thus, it is not useful as an adhesive; therefore, it needs conversion to various derivatives such as cellulose nanocrystals that can be used in the adhesive formulations (Baumann and Conner, 1994). However, the hydrogen bonds in both cellulose and starch are much weaker than chemical bonds; they are not strong enough to glue wood (Hemmilä et al., 2013). The hydrogen groups also simply form hydrogen bonds with water molecules, which leads to low water resistance. The problems associated with cellulose as bio-binders can be solved by modifying cellulose's chemical or physical nature (Hemmilä et al., 2013).

Modification of cellulose can be done through esterification and etherification reactions, which can be carried out at the hydroxyl groups of cellulose (Baumann and Conner, 1994). The esterification can be done using Schönbein’s method, with a mixture of sulphuric and nitric

acids (Hemmilä et al., 2013). Cellulose esters can also be prepared with organic substituents like acetate to form cellulose esters such as cellulose propionate, cellulose butyrate, cellulose acetate propionate (CAP) and cellulose acetate butyrate (CAB) (Hemmilä et al., 2013). If an alkyl group replaces the hydrogen on the cellulose's hydroxyl groups, the result is etherification. Important cellulose ethers are methyl -, ethyl -, carboxymethyl – (CMC), hydroxyethyl – (HEC), hydroxypropyl – (HPC), and benzyl cellulose, while hydroxyethyl cellulose (HEC) is used in the wood product industry (Hemmilä et al., 2013).

However, commonly used carbohydrate-based wood adhesives from both cellulose and starch have some challenges in terms of low water resistance and poor bonding rate, which affects the strength properties of the resultant panels, and exhibit slow curing rates that lead to long-press times during panel production (Hemmilä et al., 2013, Pizzi, 2006).

Recently, there have been several studies that investigated the use of cellulose nanofibers, namely cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC), in combination with polymeric binders for reinforcement in the production of composites(Amini et al., 2017, Veigel et al., 2012). CNF is a material comprise of nanosized cellulose fibrils with a high length to width ratio. The fibrils are isolated from any cellulose containing source, including wood-based fibers through high-pressure, high temperature and high-velocity impact homogenization, grinding or micro-fluidization. CNFs contain amorphous cellulose but not as highly crystalline as CNCs. CNCs, on the other hand, are rod-like nanoparticles most commonly extracted from native cellulose fibers through a controlled acid hydrolysis process (Dastjerdi et al., 2018).

Some studies investigated their use as a replacement for formaldehyde-based adhesives in wood composites production (Kojima et al., 2013). As with other natural binders, property improvement is usually toward changing the molecular structure by modifying them with various hardeners and cross-linkers. Cellulose nanomaterials are reinforcing agents that enhance strength properties, and it is well-documented that their incorporation improves adhesive properties. For instance, Veigel et al. (2012) studied the effect of reinforcing urea-formaldehyde adhesive with cellulose nanofibers on mechanical properties of particleboard and oriented strand board. Adhesive mixtures were prepared by mixing an aqueous CNF suspension with urea-formaldehyde (UF) and melamine urea-formaldehyde (MUF) adhesives (Veigel et al., 2012). The results showed that particleboards prepared with UF containing 1 wt% CNF displayed a minimised thickness swelling and better internal bond and static strength than boards fabricated with pure UF. Also, the reinforcing effect of CNF was even more evident for the oriented strand board (OSB), where it showed a significant improvement in the gluing strength properties (Veigel et al., 2012). Furthermore, Kojima et al. (2013) investigated the use

of cellulose nanofibers (CNF) as reinforcement in wood flour (WF) board to replace chemical adhesives; and the three-dimensional binding effects of the CNF improved the wood flour board's physical and mechanical properties (Kojima et al., 2013). Similarly, Dastjerdi et al. (2018) reported improved mechanical strength and enhanced shear strength with CNC employed as a pressure-sensitive adhesive modifier (Dastjerdi et al., 2018).

2.5.4 Unsaturated oils

Vegetable oils occur as monodisperse low molecular weight substances, being a mixture of triglycerides with a small quantity of free fatty acids (Hemmilä et al., 2013). Triglycerides consist of a glycerol backbone and three fatty acid chains. The fatty acids may be saturated, with no double bonds; alternatively, they may be unsaturated, with one like oleic acid, two or three double bonds such as linolenic acid (Van Erp and Rogers, 2003). The proportion of free fatty acid depends on both the plant species and the extraction conditions. To date, many resin research reports have focused on oils that contain at least one double bond (Pizzi, 2006). For example, double bonds of an oil such as castor or linseed oil can be epoxidated and further cross-linked to produce high molecular weight polyols usable for adhesive purposes. The chemical modification of fatty acids, which facilitates subsequent polymerization, can produce thermosetting resins (Hemmilä et al., 2013). The modified triglycerides produce polymer networks with heterogeneous molecular structures resulting from the reactive groups' reactivity and location in the molecule. This characteristic and availability of dangling chains cause the glass rubber transition to extend over a wide range of temperatures (Mosiewicki and Aranguren, 2016). However, if the reactivity is low, it slows the reactions or leads to an incomplete reaction (Mosiewicki and Aranguren, 2016). Resins from vegetable oils have two major deficiencies, which are long hot-press time and high cost (Pizzi, 2016), making their commercial applications not feasible.

2.5.5 Proteinaceous bio-adhesives

Proteins are abundant organic compounds made of amino acids arranged in linear chains joined together by peptide bonds between the carboxyl and amino acid groups of adjacent amino acid residues (Kumar, 2013). The proteins have a compound three-dimensional structure of extremely coiled chains which depends on amino acid types in the polypeptide chain, their sequence, and the hydrogen bonding and disulphide cross-links between individual amino acid side groups (Frihart and Satori, 2013).

The protein structure can be divided into primary, secondary, tertiary and quaternary structures. The primary form of a protein involves a polyamide backbone made from the condensation of amino acids. The secondary and tertiary structures are based on internal cross-links interactions: hydrogen bonds, disulfide linkages, or coordination around metallic sites (Frihart and Satori, 2013). The quaternary structure is the interaction of two or more folded polypeptides, as shown in figure 2. 3. Proteins offer many advantages that qualify them as possible adhesives for industrial applications, not only for the wood-based panel industry also for other industrial applications such as bioplastics (Müller et al., 2007). They are renewable resources obtained in large quantities from animals such as meat and poultry slaughterhouses and plants by husbandry and fishery, often also in the form of waste products (Müller et al., 2007). Proteins are available all-year-round. Compared to conventional bonding agents, their price is low, and their production does not depend on non-renewable resources such as crude oil (Müller et al., 2007). Noteworthy disadvantages of proteinous adhesives, compared to thermosetting adhesives, are that they are quickly attacked by microorganisms, exhibit low mechanical strength properties of wood composites, and have poor water absorptivity (Frihart and Satori, 2013). Solutions to these problems have focused on the addition of preservation materials that inhibit microbial degradation to some level (Frihart and Satori, 2013, Vnučec et al., 2017). Besides, various crosslinking agents have been used to increase water resistance to some extent (Frihart and Satori, 2013, Vnučec et al., 2017).

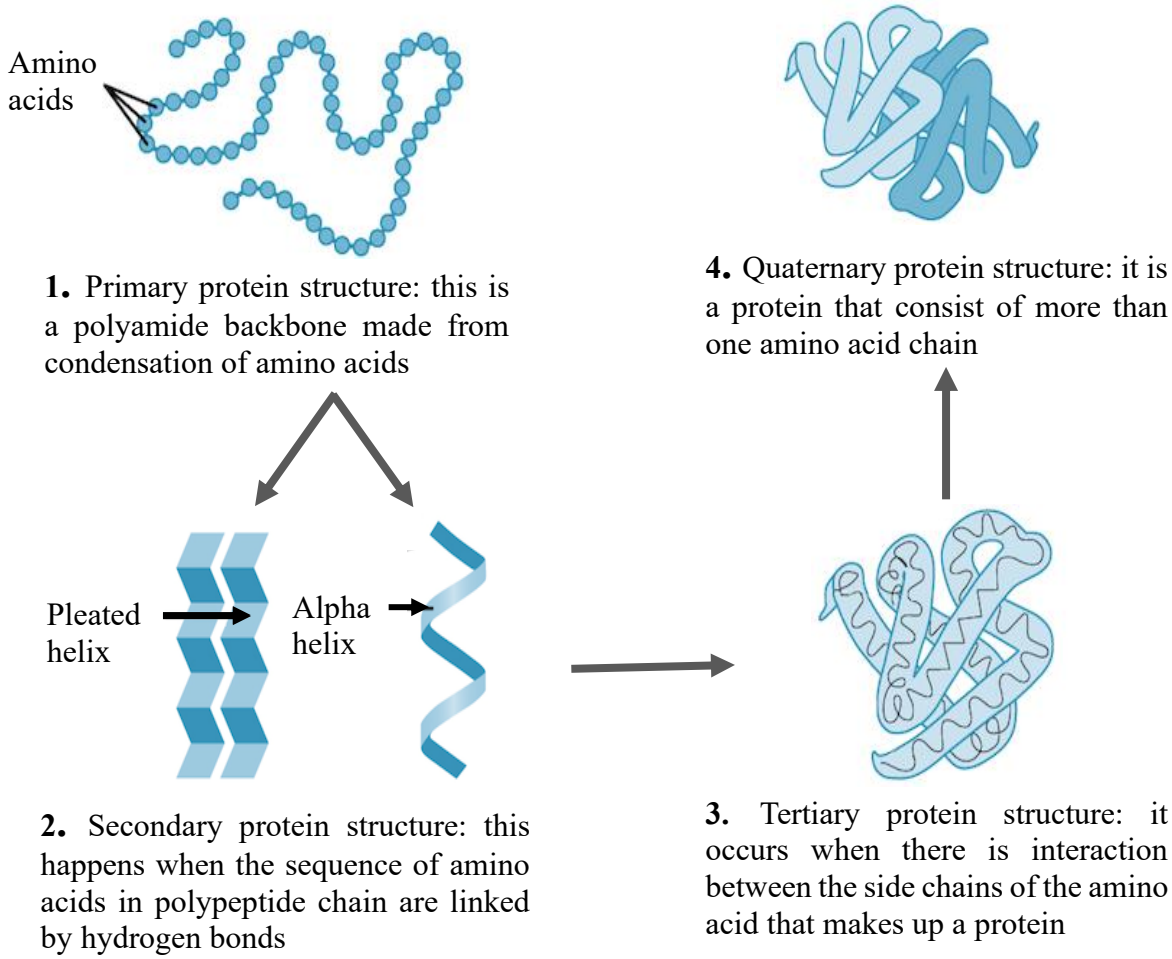

Figure 2.3. The primary, secondary, tertiary and quaternary structure of the protein [Adapted from (Rye et al., 2013)]

The process for using the proteins as adhesives varies and the properties of the binder vary as the source varies due to the variation of their chemical composition. Below are some protein-based adhesives that have been investigated and explored.

Soy protein

Soybeans are an abundant agricultural crop with high oil and protein contents that are complex macromolecules (Vnučec et al., 2017). They are comprised of 20 different amino acids with a common backbone and different side-chain (R). There are two main components in soy protein, which are β-conglycinin, and glycinin. These constitute about 50–90% of total soybean seed protein (Mo and Sun, 2013). Soy meal ground into flour is a feedstock for making adhesives

for wood bonding. The traditional method of dispersing the soy flour in water used sodium hydroxide had poor dispersion stability and weak water resistance (Frihart and Hunt, 2010). Recently researchers have devoted to improving the bond strength of soy-based adhesives while modifying soy protein using different denaturation reagents like urea, guanidine hydrochloride, and sodium dodecyl sulphate, and this has improved the soy-based adhesive bond strength (Mo and Sun, 2013). Besides, modification with enzymes such as trypsin, papain, and urease, cross-linking reagent glutaraldehyde, or cationic detergents also showed improved bond strength of proteins (Mo and Sun, 2013). In a study by Amaral-Labal et al. (2008), soy protein was modified with glyoxal for particleboard production. It was reported that glyoxalated soy flour adhesives for wood particleboard with small amounts of glyoxalated lignin or tannin also, without any addition of either formaldehyde or formaldehyde-based resin yielded that results satisfied the relevant standard specifications for interior wood boards (Amaral-Labat et al., 2008). Polyamide epichlorohydrin (PAE), a wet-strength resin widely used in the paper and pulp industry, has been used to modify soy protein, which showed improved bond strength and water resistance (Mo and Sun, 2013, Gui et al., 2013). The effect of crosslinking soy protein with bio-based PAE was studied by Gui et al. (2013), and the results showed improved water-resistance of the panels (Gui et al., 2013). However, there is a need for more work on improving the strength properties of the adhesives.

Gluten protein

Gluten is a protein derived from wheat (Lei et al., 2010). Gluten is a mixture of several proteins in which gliadin, glutenin, globulin, and albumin predominate. It is the most abundant protein fraction in wheat and represents about 8–15% of the grain's dry weight (Kim, 2008). Gliadin and glutenin are the two significant fractions in gluten, consisting of numerous, almost closely related protein components and characterized by high glutamine and proline contents (Wieser, 2007). According to literature, the amide groups of the peptide links in gluten proteins can react with aldehydes in the same way as other amides, like urea (Lei et al., 2010). Gluten protein is also rich in lysine and arginine amine, and they are even more reactive with aldehyde similar to those present in melamine and phenols (Lei et al., 2010).

Gluten protein-based adhesive has been produced and applied in powdered form but not suitable for particleboard application. However, the liquid form of hydrolyzed gluten modified with formaldehyde and glyoxal, with the addition or without any synthetic resins, were experimented with by Lei at al. and gives the result, which satisfies the relevant standard

specifications for interior wood boards (Lei et al., 2010). Besides, the 30/70 pMDI/hydroxymethylated lignin adhesive formulation was tried at progressively shorter pressing times and yielding internal bond strength with acceptable results (Lei et al., 2010). This study shows that gluten adhesive cannot be used without modification with synthetic resins that are formaldehyde-based.

Casein

Casein is the main protein derived from milk, which is a family of related phosphoproteins. It is found in mammalian milk, comprising about 80% of the cow's milk proteins at 3% concentration and ranges between 20% and 45% of the proteins in human milk (Kunz and Lönnerdal, 1990). Casein protein is recovered from skim milk by acid precipitation to pH 4.5 (Lambuth, 2003). The process is done using mineral acids or cultured the milk with bacteria that convert lactose to lactic acid and precipitates the casein after (Lambuth, 2003). The precipitated casein protein curd can be washed with water to remove the acid, dried, and ground (Lambuth, 2003). Casein's commercial designation often includes its method of acid precipitation like lactic acid or sulfuric acid casein (Lambuth, 2003). Casein contains many proline (10.6%) residues, which do not interact (Bye, 1990). There are also no disulphide bridges because of the low percentage of cysteine (0.34%) (Bye, 1990).

Casein-based glues, formulated from casein powder, water, hydrated lime, and sodium hydroxide, were famous for woodworking (Müller et al., 2007, Lambuth, 2003). While primarily replaced with synthetic resins, casein-based glues are still used for specific applications, such as laminating fireproof doors and bottle labels (Lambuth, 2003). Though it is a traditional glue with a good record, it is not moisture resistance, also vulnerable to mold and fungal attack (Müller et al., 2007, Lambuth, 2003). Because of this, formaldehyde, urea-formaldehyde resin, lime, glyoxal, zinc salts, and oxide also alum have been used to increase moisture resistance of casein-based adhesives (Bye, 1990). These formulations still involve formaldehyde, which poses a threat to the environment.

2.6 Slaughterhouse waste as renewable biomass for wood adhesives

Worldwide, animal slaughterhouses produce lots of animal by-products, including inedible tissues, blood, fat, bones, and other materials found associated during meat processing like chicken feathers (Adhikari et al., 2018). Millions of livestock such as cattle, sheep, pigs, and chickens are slaughtered in abattoirs or slaughterhouses in African countries, including South

Africa, which generates a lot of waste annually (DAFF, 2018). However, animal by-products that originate from abattoirs and slaughterhouses are an essential industrial protein resource that could be utilized in various value-added applications. Still, they are currently underutilized either in high-value applications or in being used to produce relatively low-value products such as animal feed (Adhikari et al., 2018). These proteinaceous waste generated by slaughterhouses can be valorized to extract proteins, which can be incorporated into industrial processes to produce value-added bio-based products such as bio-adhesives (Adhikari et al., 2018). The utilization of some of these wastes in the wood adhesive application is review below.

2.6.1 Animal bone, meat, and blood

Animal glues are resultant from the hydrolysis of the protein constituent collagen of animal hides and bones (Pearson, 2003). It can be found that animal bone based glue has an outstanding relevance in the industry because these glues were used from ancient times and were known for their excellent properties like solid joints when dried and the ability to cure fast (Konnerth et al., 2009). It does not seem prone to creep and does not discolor wood because it is pH-neutral; nonetheless, animal glue has some disadvantages, which includes low moisture resistance and tends to hydrolyze in the presence of water (Konnerth et al., 2009). Animal blood is used for adhesives in fresh liquid form, and these glues performed well on wood. However, the very rapid spoilage rate of liquid blood imposed real limitations on this adhesive's general availability and commercial use (Lambuth, 2003).

Park et al. (2000) studied the adhesiveness of protein concentrate extracted from meat and bone meal at various pH values ranging from 5.0 to 9.0 (Park et al., 2000). It was reported that at pH 6.0–8.0, adhesiveness of meat and bone meal protein concentrate (MBMPC) showed the highest value (78.2 kg). Adhesiveness increased linearly as the MBMPC concentration increased by up to 20% (Park et al., 2000). The study also observed that, concerning temperature for MBMPC adhesiveness, the greatest adhesiveness was in the range of 70^{o}C to 90°C (Park et al., 2000). Besides, modification with 0.05% glutaraldehyde improved adhesiveness and water-resistance of the MBM protein concentrate adhesives (Park et al., 2000).

2.6.2 Waste chicken feathers

Chicken feathers are one of the significant waste generated from poultry slaughterhouses. According to the USA Foreign Agricultural Service, the total domestic per capita consumption

of chickens in the USA and some selected countries, including South Africa in 2019, is about 96,464 x 10^3 metric tons (USDA;, 2019). As a result of an increase in chicken meat consumption worldwide, the poultry meat processing industry generates large amounts of feather by-products that result in about 40 × 10^9 kg as a waste stream annually, with South Africa contributing about 258 million tons (Tesfaye et al., 2017). Currently, they are considered waste, and their uses are economically marginal. At present, the feathers from both small and large-scale poultry industries in most countries are disposed of in landfills or burned, creating environmental problems, except some that are processed to make low-grade animal feedstock. However, feathers are bio-resources with high protein content. They consist of about 91% keratin protein, a hard, fibrous protein also found in hair, skin, hooves, and nails, a good source of a natural adhesive. Feathers might be the most abundant keratinous material in nature (Khosa and Ullah, 2013), that are yet to be adequately utilized in the wood adhesive formulation.

2.6.3 General and specific properties of waste chicken feathers for bio-adhesive production

Chicken feather keratins possess some general and specific features that make them suitable for bio-binder applications. It was shown that about 18–20 amino acids are found in keratin protein, bonded together via peptide bonds (Ayutthaya et al., 2015). Several functional groups are present in keratin proteins, especially peptide backbone, such as disulphide (-S-S), amine ($-NH_2$) and carboxylic acid (–COOH), also hydroxyl (-OH), thiol and an aromatic group, which make it chemically reactive under conducive reaction conditions (Khosa and Ullah, 2013).

Feather keratins are small proteins, uniform in size, with a molecular weight of around 10 kDa (Khosa and Ullah, 2013). However, the approximate chemical composition of chicken feathers is carbon 64.47%, nitrogen 10.41%, oxygen 22.34%, and sulphur 2.64% (Tesfaye et al., 2017). According to literature, the amino acids in keratin chains are intramolecular and intermolecular interacted with disulphide bonds, ionic bonds, hydrogen bonds, or hydrophobic bonds (Khosa and Ullah, 2013). The distribution of amino acids is non-uniform, with the basic and acidic residues and the cysteine residues concerted in the N- and C-terminal regions (Saravanan and Dhurai, 2012). The dominant portion is rich in hydrophobic residues, which is of great advantage for wood adhesive application, also has a crystalline β-sheet conformation (Saravanan and Dhurai, 2012). Its hydrophobicity in nature gives a better water resistance to the final product after a little modification. It is a fibrin, which is less likely to flocculate and is much easier to hydrolyze than globular proteins such as soy, blood, and casein protein. Besides,

some feather protein components are naturally anti-mildew, which prevents a bacterial attack on wood products (Jiang et al., 2008).

2.6.4 Chicken feathers solubilization before use as bio-adhesives

Feathers are very high in keratin protein, which after solubilization, can be chemically synthesized to produce a protein-based adhesive for use in wood composites industries. Keratin protein is insoluble in water, but its solubility in water can be achieved at low or acidic pH, in the presence of heat and some reducing agent (Khosa and Ullah, 2013). Feather keratin is a unique protein with a high content of cysteine (7-13%) in the amino acid sequence, and cysteine has -SH groups that cause the sulphur–sulphur (disulphide) bonding (Shavandi et al., 2017). The high content cysteine makes the keratin stable by forming a network structure by joining adjacent polypeptides by disulphide cross-links (Saravanan and Dhurai, 2012). During the reduction process, disulphide cross-links are broken into free thiol (-SH) besides protonation of some $–NH_2$ and other groups in keratin, making the positive surface solubilization happen (Khosa and Ullah, 2013), as shown in figure 2. 4. After protonation, unfolded and exposed functional groups carry positive surface charges with high reactivity, therefore on chemical modification, keratinous protein becomes a pseudo-natural cationic biopolymer (Khosa and Ullah, 2013).

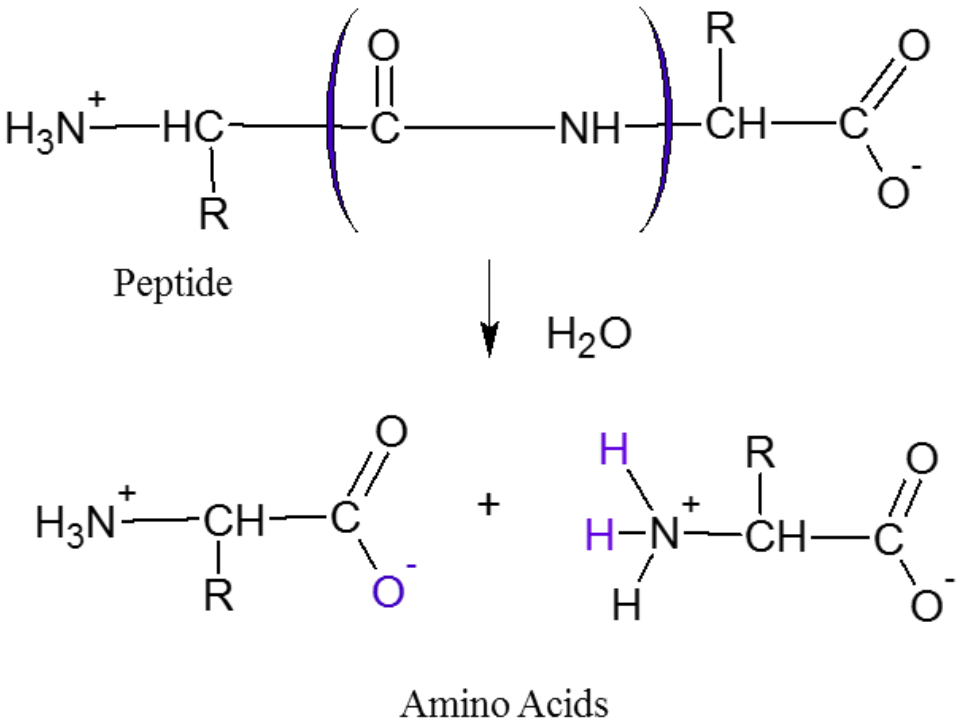

Figure 2.4: Reduction and oxidation process of the peptide during hydrolysis

Several methods have been used to solubilised and remove feather keratin, such as reduction method, alkaline hydrolysis, sulfitolysis, and ionic liquids (Shavandi et al., 2017). In addition, oxidation, supercritical water, steam explosion, extraction aided by microwaves and microbial and enzymatic techniques were also used (Shavandi et al., 2017). However, the alkaline hydrolysis method has been employed in the literature to generate protein hydrolysate for wood adhesive applications (Adhikari et al., 2018). It is also possible to reduce and oxidize agents in alkaline solution to break the disulphide bonds (Jiang et al., 2008).

2.6.5 The utilization of feather keratins in bio-adhesive production

The usage of waste chicken feathers as a source of protein-based adhesives was patented a long time ago in a US patent (Patent No. 2399161; 1946) by Brother and Binkley. In this patent, the alkaline hydrolysis method for producing chicken feather hydrolysate for making keratin-based bio-binder in the bonding of wood composites was described (Brother and Binkley, 1946). The method includes hydrolyzing chicken body feathers in a 1% NaOH solution and a 0.5% sodium sulphide with an autoclave at the temperature of between 80–90◦C and a moderate pressure for 20 minutes solubilize the protein molecules (Brother and Binkley, 1946). The adhesive produced with the feather protein hydrolysate without further modification was used to bond wood veneers for plywood production. The plywood panels had strength comparable to that of commercial plywood under dry conditions, but it exhibits weak water resistance; therefore, it did not satisfy the standards of the Bureau of Standards (test CS 45–40; 1940) for moisture-resistant plywood (Brother and Binkley, 1946). The alkaline hydrolysis method unfolded the protein molecules. It exposed polar functional groups, which could interact with the functional groups of wood veneers and lead to significant adhesion under dry conditions (Adhikari et al., 2018). Nevertheless, the exposed hydrophilic functional groups' water absorption negatively affects the interfacial interactions; hence, the plywood panels could not give sufficient water resistance (Adhikari et al., 2018).

Jiang et al. also carried out a preliminary study on chicken feather protein-based adhesives. In work, chicken feathers were hydrolyzed in a mixture containing 6% sodium hydroxide and 2% sodium bisulfite with and without phenol in the solution during hydrolysis, followed by heating of the mixture at the 90^{o}C for 2.5 h (Jiang et al., 2008). In synthesizing the resin, the partially hydrolyzed protein fragments are co-polymerized and irreversible trapped in the partially condensed polymer network of the phenol-formaldehyde resin (Jiang et al., 2008). The

adhesive formulations' performance was examined using them to produce fibreboard, and the strength properties were assessed by evaluating mechanical properties such as bending strength, bending stiffness, internal bonding strength, and percent thickness swelling of the resulted fibreboards. The formulation consisted of one-part hydrolyzed feather protein and two parts. A phenol and formaldehyde mixture with a mole ratio of 1:2 at pH 10.5 performed similarly to that of commercial phenol-formaldehyde resins (Jiang et al., 2008, Zhao, 2017). The report shows that this formulation resulted in a 30% replacement of phenol with feather protein and compared well to commercial phenol-formaldehyde resins. These results indicate that hydrolyzed feather protein co-polymerizes and blends effectively with the components of phenol-formaldehyde resin and that chicken feather protein has potential as a cost-effective supplement in the manufacturing of phenol formaldehyde wood adhesive resins (Jiang et al., 2008, Zhao, 2017). Both the US patent and the previous work show that partial chemical modification and crosslinking of chicken feathers protein will enhance its utilization as a wood composite adhesive since feather proteins have many advantages over other protein sources for wood adhesive.

2.7 Chemical modification and crosslinking of proteins for enhancing strength and moisture resistance properties

Generally, for the protein to function as an adhesive, modification of the native protein structure is very important; this will expose the polar groups for solubilization and bonding (Adhikari et al., 2017). The protein in its native form, the molecules are highly folded (figure 3). Some of the polar functional groups that can potentially interact with the functional groups of wood are buried inside the folded structure (Adhikari et al., 2017).

The modification is done by denaturing the native protein in water in the presence of low or acidic pH, with heat and some reducing agent (figure 4). During the denaturalization process, the hydrogen bonds will break, while breaking the secondary and tertiary bonds depends on the denaturalization methods and conditions (Adhikari et al., 2017). Once the protein has been denatured, it can flow onto and into the wood substrate and form hydrogen bonds with the wood structure (Frihart, 2011, Adhikari et al., 2017).

However, it is possible to manipulate the hydrophilic and hydrophobic side-chains of chicken feathers protein to modify surface reactivity and accessibility; this is done through chemical modification (Adhikari et al., 2018). The chemical modification method is divided into four

categories: the denaturation of proteins by breaking their internal structure (Adhikari et al., 2017). Besides, molecular protein modification focuses on grafting reactive groups of chemical reagents onto protein molecules. These groups can react with protein's polar groups and form a cross-linked network after curing (Adhikari et al., 2017). Another category is the mixing of protein products with other natural materials such as lignin and tannin. The last type is the mixing of protein products with synthetic resins, such as phenol-formaldehyde (PF), melamine-formaldehyde (MF), melamine urea-formaldehyde (MUF) and epoxy resin (EPR) (Vnučec et al., 2017) but this method post a threat to the environment as formaldehyde is involved, therefore, it is not encouraged.

According to literature, aldehyde, such as glyoxal and glutaraldehyde, have been used as typical polypeptide crosslinking agents (Adhikari et al., 2017). The compounds possessing two or more reactive groups can react with the functional groups of polypeptide chains and are used as polypeptide crosslinking agents. In this method, the polypeptide chains of protein are cross-linked with a cross-linking reagent resulting in the formation of three-dimensional networks of polypeptide chains via covalent linkages (Adhikari et al., 2017). The creation of a rigid structure of polypeptide chains prevents individual polymers' movement, which helps to maintain structural integrity; also, crosslinking of polypeptide chains decreases water's ability to permeate through the polypeptide network (Adhikari et al., 2017). The occurrence of crosslinking enhances the mechanical strength and water resistance of protein adhesives. It is imperative that, for adhesive applications, protein dispersions and solutions must have excellent storage stability, flowability, wetting ability and stickiness, and the resulting adhesive bonding should be strong and have resistant to deterioration under various environmental conditions (Frihart and Hunt, 2010, Adhikari et al., 2017).

Polyamidoamine-epichlorohydrin (PAE) resin was used to chemically cross-linked protein hydrolysate from specified risk material, which are proteinaceous wastes representing a significant proportion of slaughterhouse by-products. The impact of time of crosslinking, ratio of peptides/crosslinking agent, and temperature of hot pressing of plywood specimens on the strength of formulated adhesives were investigated. The author reported that formulations containing as much as 78% (wt/wt) peptides met the ASTM (American Society for Testing and Materials) specifications of minimum dry and soaked shear strength requirement for UF resin type adhesives (Adhikari et al., 2016). Besides, under the optimum conditions verified, the peptides–PAE resin-based preparations lead to plywood specimens with similar shear strength when dry as well as soaked to that of marketable PF resin (Adhikari et al., 2016). Substantial research has been conducted focusing on improving the strength properties and water resistance

of protein-based adhesive using chemical modification. It is done by reacting proteins with compounds with functional groups capable of reacting with active groups of proteins (Adhikari et al., 2017). It shows that more environmentally chemical and natural materials can be used to improve the strength and water resistance properties of protein adhesives (Vnučec et al., 2017, Adhikari et al., 2017).

2.7.1 Wood Adhesives General Requirements

A novel adhesive must meet certain minimum qualities to be desirable and worthwhile on a large-scale production. This adhesive must be relatively inexpensive and readily available, as well as possess consistent properties. In addition, given the high cost of modifying process designs and equipment capacities in the wood products industry, a new adhesive should ideally be compatible with existing equipment and conditions (Frihart, 2011). Moreover, the adhesive's fluid properties are some of the most critical aspects of these qualities. Adhesives are usually pumped into pipes and added to wood using atomization, shear mixing, and roll or curtain coating (Thoemen, 2010). Any new adhesive materials must be manufactured with the appropriate viscosity and solids content to flow through existing equipment for a particular application process (Horie, 2010).

Furthermore, moisture content is another critical parameter in wood adhesive products manufacturing (García et al., 2019). During processing or in the finished product, adhesives must not substantially increase the moisture content of the wood product. Excess moisture content in the manufactured wood product could result in issues with manufacturing speed or consistency in today's composite products, which are heat-cured (Sandberg et al., 2013). Changes in moisture content in the final product could affect the panel's working properties, such as machining, or dimensional stability.

The final new adhesive product is that which meets industrial requirements for strength and stiffness. Product of such industrial qualities are generally classified into three types: internal non-structural, exterior non-structural, and structural (Stark and Cai, 2021). Although, each class has its own sets of criteria and testing specifications, but all three require a certain degree of dry and wet strength. Dry and wet strength, stiffness properties and dimensional stability are used to assess the consistency qualities of interior wood materials. Water resistance qualities are required for exterior adhesives, while structural adhesives must be resistant to water, extreme heat (fire), and creep. Adhesive that attracts mildew, mold, or insect infestations are less desirable for industrial application (Frihart et al., 2014).

2.7.2 Bio-adhesives in particleboard production

Particleboard is a wood composite panel usually produced from particles of wood held together by binder under heat and pressure (Prasittisopin and Li, 2010). It is considered a sustainable wood product because it uses wood residues from other manufacturing processes regarded as a waste. It is a composite panel with broad applications; the extensive use of particleboard is in the furniture industry as a substrate for thin overlays, for the manufacture of home and office furniture, kitchen and TV/stereo cabinets, mobile home decks and underlayment for carpeted floors (Haygreen and Bowyer, 1996). One major disadvantage of particleboard is the formaldehyde-based resin used in its fabrication and the subsequent emission of formaldehyde, a well-known carcinogen (Salem et al., 2012). Currently, urea-formaldehyde continues to be the significant resin used in the manufacture of particleboard, and the issue of formaldehyde emission continue to be tenacious. Therefore, efforts have been made to lower formaldehyde emission from particleboard using the newly developed soy protein resins (Prasittisopin and Li, 2010, Amazio et al., 2011).

Moreover, the utilization of waste animal protein extracts as bio-adhesive for both oriented strand board and plywood has been studied (Mekonnen et al., 2014). Meanwhile, waste products from slaughterhouses such as chicken feathers are underutilized protein biomass that can play an essential role in developing eco-friendly wood adhesives. Prospects for binder development from this waste material are high since they comprise lots of keratin and a fibrous protein that could be converted into valuable products (Sharma and Kumar, 2019).

2.8 Conclusions

Renewable biomass is an alternative material to petrochemicals for wood adhesive production because of their environmental acceptability. However, they face low moisture resistance, reactivity, mechanical strength, and adhesive properties challenges. Nonetheless, there is continuous research on solving these problems through a suitable modification to make them economically and technically viable. Literature reveals that soy protein modified and cross-linked with polyamide-epichlorohydrin shows improved bond and water resistance properties. Feathers protein is another waste biomass of interest that can be utilized for wood adhesive production after chemical modification and cross-linking process. A few research studies have been done on this renewable raw material for thermosetting wood adhesive production. The in-depth research and the understanding of this biomass's chemical composition are significant for their modification and further application for wood adhesives. A stern research study is

currently going on at the council of scientific and industrial development. A bio-refinery industrial development center on developing bio-binder from this abundant proteinaceous waste material. The author suggests using cellulose nanomaterials as a reinforcing agent or additive in protein-based adhesives, as cellulose nanocrystals possess high mechanical strength, excellent rheological and thermal properties. Incorporating this nanomaterial will help enhance the mechanical strength and thermal properties of protein-based adhesives. There is not much research work on the use of this material for protein modification.

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CHAPTER 3

PAPER 2: A JOURNAL ARTICLE PUBLISHED ONLINE

OPTIMIZATION OF KERATIN PROTEIN EXTRACTION FROM WASTE CHICKEN FEATHERS USING HYBRID PRE-TREATMENT TECHNIQUES

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Abstract

This study modeled and optimized the operational conditions for the extraction of keratin from waste chicken feathers via the alkaline hydrolysis method. Response Surface methodology using Box-Behnken design was used to investigate the effect of time, temperature and the concentration of both sodium hydroxide and sodium bisulphite as an extraction solvent on the keratin hydrolysate and protein yield. The coefficients of determination (R^2) of 0.77 and 0.74 were obtained for keratin hydrolysate and protein yield models, respectively, demonstrating the fitness of the models to navigate the optimization space. The optimum parameters were temperature (87 °C), time (111 minutes), sodium hydroxide (1.78 %) and sodium bisulphite (0.5 %). The yields of keratin hydrolysate and protein after validation were 68.3 % and 65.2 %, respectively. Protein analysis via the CHNS analyzer showed the elemental composition of 13.85 % N, 47.25 % C, 6.90 % H and 2.8 % S. The molecular weight of the extracted keratin ranged between 10-15 kDa and 3-10 kDa. Ultimate analysis by FTIR and NMR confirmed the presence of amide, carboxylic groups and alkyl side chains of amino acids. The results of this study proved that chicken feathers, typically disposed of by the poultry slaughterhouses, would be able to serve as a great sustainable source of keratin protein for the manufacture of value-added products like bio-adhesives.

Keywords: waste chicken feathers, alkaline hydrolysis, extraction, optimisation, keratin, bio-adhesives

3.1 Introduction

Chicken meat is one of the significant sources of animal protein mostly consumed worldwide. At the same time, its intake has resulted in the creation of a substantial number of feather waste by-products from poultry slaughterhouses as near seven percent of chicken bodyweight is feathers. It was reported that the estimated number of chicken feathers generated each year globally is about 15 billion tons, with South Africa contributing about 258 million tons (Tesfaye et al., 2017). Currently, small amounts of feathers are beneficiated as animal feedstuff. Still, the majority of them produced from either small or large poultry operations in many countries ends up in landfills, creating environmental problems, except some that are incorporated in low-quality animal feeds (Chinta et al., 2013). However, these are renewable resources that are very rich in keratin protein: a rigid, fibrous type of proteins also found in hair, skin, hooves and nails (Sharma and Gupta, 2016). Since the disposal of waste feathers is not environmentally sustainable, research into their beneficiation is a necessity to produce cheap, biodegradable, sustainable, renewable, and abundantly available green materials (Tesfaye et al., 2018). Keratin protein obtained from other biomass such as nails, hair, horns and wool have been commercially incorporated in hair care, and cosmetic applications, but globally chicken feather waste are the most abundant and sustainable keratinous material in nature that are yet to be adequately utilized, especially in bio-adhesive (Aluigi et al., 2007, Villa et al., 2013, Sharma and Gupta, 2016). Therefore, the drive of waste chicken feathers for the current study. Chicken feathers possess some excellent qualities such as its high keratinous protein and its hydrophobic structure, which give a better water resistance nature on the final product. Besides, the inherent anti-mildew property and fibrin units of feather protein promote its hydrolysation. These distinctive properties, as well as its abundant availability, sustainability, and low cost, make chicken feathers a choice for many applications that require good tensile strength and elasticity (Nuutinen, 2017). Thus keratin protein can be extracted from feathers and used as natural raw material for the production of films, fibres, hydrogels, binders, micro and nanoparticles for cosmetic, medical, textile, composite and other industrial uses (Khosa and Ullah, 2013).

The keratin in feathers is a unique protein that has high cysteine content of about (7-13%) in the amino acid sequence (Shavandi et al., 2017), and cysteine that contains -SH groups which

are responsible for the sulphur–sulphur (disulphide) bonding in the keratin. The high cysteine content stabilises the keratin by forming a network structure joined by adjacent polypeptides and disulphide cross-links (Saravanan and Dhurai, 2012). The chemical composition of keratin exhibits β-helix or β-pleated twisted sheets of small uniform size proteins with a low molecular weight of about 10 kDa (Khosa and Ullah, 2013, Sharma and Gupta, 2016). The cross-linked cystine bond between peptide chains components in keratin imparts high stability and xenobiotic nature. Thus, several methods are used to dissolve and extract keratin from chicken feathers. These methods include reduction hydrolysis, alkaline hydrolysis, sulphitolysis, and extraction with ionic liquids also, oxidation, supercritical water extraction and steam explosion; besides, microwave-assisted extraction, microbial and enzymatic methods have also been used for keratin extraction (Shavandi et al., 2017). A lot of studies have revealed that using these methods that keratin protein from chicken feathers can be obtained by breaking the disulfide bonds in the cystine unit (Kamarudin et al., 2017). For instance, sodium sulphide, 2-mercaptoethanol, and sodium dodecyl sulfate were used to obtain a good yield of keratin protein from various animal sources (Kamarudin et al., 2017). Another previous study reported the addition of urea and sodium dodecyl sulfate in solutions containing 10 g/L Na_2S, 9 M urea, and 10 g/L sodium dodecyl sulfate. The results indicated that the presence of urea enhanced both the process rate and the product yield (Poole et al., 2011). Sinkiewicz et al. (2017) reported the result obtained from the keratin extraction process using various reducing agents. It was shown that 84 and 82 % yield of soluble keratin were obtained respectively after 2 hours of reaction time with the use of mercaptoethanol combined with sodium bisulphite as the reducing agents. It also reported that the use of sodium bisulphite reduced the time of extraction to about 1 hour with the same result (Sinkiewicz et al., 2017). Besides, the author reveals that the chemical treatment of the feathers with 2.5 percentage concentration sodium hydroxide enhanced the efficiency of the extraction and was able to increase the yield obtained to 94 % (Sinkiewicz et al., 2017). Feather keratins are not soluble in conventional polar and non-polar chemical solvents due to its tough structure: an extensive disulfide crosslinking and tightly packed α-helices and β-sheets in a polypeptide chain. Thus, an effective, low-cost process and scalable process to extract the keratin protein is desirable. Solvent for chicken feather solubilization should be a solution which enables the re-crosslinking process after dissolution and does not cause the deformation of the primary protein chains (Poole et al., 2011). There are important functional groups in the keratinous protein that should be considered during processing, regeneration and modification of feather keratin for further applications. These functional groups include sulfhydryl (SH), amino (NH_2) and carboxylic group (COOH). The

choice of pre-treatment technique and chemical solvent chosen is based on the application to which keratin protein is to be utilized and to retain many of the protein attributes present in the native structure (Nuutinen, 2017, Barone and Schmidt, 2006). In some cases, mechanical treatment can be enough (Nuutinen, 2017). For instance, Poole *et al.* (2011) reported high exposure to the extremely alkaline conditions appears to be unfavourable to final product strength. Moreover, shorter exposure times could produce stronger regenerated products, since serious alkalinity chain damage can be significantly reduced (Poole et al., 2011). Sinkiewicz et al. (2017) stated that using acid hydrolysis for keratin extraction is very efficient, but this can harm some amino acid residues, such as tryptophan, while alkaline hydrolysis is less effective, the loss of amino acids residues is minimal (Sinkiewicz et al., 2017). The yield of keratin from chicken feathers using hydrolytic processes depends on some crucial parameters which include, the solid to liquid ratio, the pH of the extraction medium, the level of temperature use and the time of reaction during the extraction process (Sinkiewicz et al., 2017). Besides, the state of the protein hydrolysate, whether it will be stable or soluble, is determined by the level of protein degradation during the extraction process (Sinkiewicz et al., 2017). However, thermochemical hydrolysis is used for feather keratin extraction to achieve a maximum hydrolysate yield. Nevertheless, it was reported that very high temperature could cause much damage to amino acids residues (Sinkiewicz et al., 2017), by denaturing both primary and secondary structure of the protein. The most common method for extraction of keratin is to use reducing agents in alkaline solution. There is insufficient knowledge in the literature on the use of optimized hybridization processes for the extraction of keratin protein from waste chicken feathers. Knowledge of the dynamics of optimized hybridization processes will ease the determination of optimum extractive conditions. The quality of extracted protein can be controlled via the solvent of extraction, dissolution time, and other physical parameters employed. These will reflect on the variability of the extraction parameters (Poole et al., 2011). Regardless of several studies devoted to valorisation of keratin-based materials such as chicken feather, there are still challenges due to the recalcitrant nature of keratin to various solvents, enzymes and physical conditions. As well as the likely negative effect such as protein deformation and aggregation of chosen methods. Hence, approach preventing detrimental changes of amino acids, leading to keratin of desirable dissolution and ultimately improved protein yield are needed. Optimised extraction conditions of such method, especially by the combination of two reducing agents: sodium hydroxide and sodium bisulphite in a solution with moderate concentrations have not been reported. The combined and interactive effect of sodium hydroxide and sodium bisulphite

extraction solvents, dissolution time and temperature have thus far to be studied in keratin dissolution from waste chicken feathers.

Therefore, this research work aimed at optimizing the keratin extraction process from waste chicken feather biomass using a combination of two reducing agents at concentration friendly to keratin protein structure. The extractive effectiveness of sodium hydroxide and sodium bisulphite combined were investigated and optimized using Response Surface Methodology. The extracted and purified keratin hydrolysate were then characterised for their structural and biochemical properties.

3.2 Materials and Methods

Fresh chicken feathers were collected from a slaughterhouse in the province of KwaZulu-Natal, South Africa. Sodium hydroxide pellet, sodium bisulphite with (40%) concentration, sodium dodecyl sulfate (SDS), β-mercaptoethanol, Tris(hydroxymethyl) and hydrochloric acid were purchased from Sigma-Aldrich (South Africa).

Molecular weights of the keratin extracts were estimated by SDS-page Electrophoresis whereby protein ladder Kaleidoscope (BioRad) with molecular weight ranges between 10 - 180 kDa and protein ladder Spectra™ Multicolor Low Range Protein Ladder (molecular weight range 1.7 - 40 kDa from ThermoFisher Scientific, South Africa) were used separately as molecular weight markers. Coomassie Brilliant Blue R-250 (Bio-Rad was used as the staining solution for the protein separation gels.

The principal instruments used in the characterization of the keratin samples were FTIR spectroscopy (Frontier Universal ATR-FTIR, by PerkinElmer), Nuclear Magnetic Resonance (NMR), PerkinElmer Simultaneous PerkinElmer CHNS/O analyzer series II, and Willey mill by Thomas Scientific; besides, small steel containers with tight seal were used in the extraction process.

3.2.1 Feathers pre-treatment

The fresh waste feathers collected from the poultry slaughterhouse were cleaned by separating the heads, intestines and blood from the feathers. After that, the feathers were washed with warm water. They were decontaminated with a 50:50 mixture of hydrogen peroxide and sodium hypochlorite and then oven-dried at 60℃ for 24 h. The dried feathers were defatted by soaking in ethanol for 24 h, washed with water and liquid soap; it was oven-dried for another 24 h and afterward ground with Willey mill into a smaller particulate matter of about 1.5 mm. The ground feather biomass was stored in a cold room at 6℃ until further use.

3.2.2 The experimental design and optimization of the keratin protein extraction process

The design of the experiment was done with the use of design expert software, and the Box-Behnken design model was used for the optimization of the alkaline hydrolysis keratin extraction process. The four independent variables were between the ranges of 90 – 120 minutes for time, 80℃ – 90℃ for temperature, 1.5 – 2 % for sodium hydroxide, and 0 - 1 % concentration for sodium bisulphite, as shown in Table 3. 1. The keratin hydrolysate and the yield of protein were the dependent variables. The extraction parameters and the ranges used were selected according to the method used in the previous work that was reported in the literature (Brother and Binkley, 1946, Jiang et al., 2008, Kamarudin et al., 2017).

Twenty-nine experimental runs were generated for the preliminary experiment and performed (Table 3). The experimental data were fitted into two polynomial quadratic model equations, and this is done to be able to link the keratin extraction process variables with the response variables, which include the percentage yield of keratin hydrolysate and protein, according to Equation (1).

$$Y = \alpha_0 + \alpha_1X_1 + \alpha_2X_2 + \alpha_3X_3 + \alpha_{11}X_1^2 + \alpha_{22}X_2^2 + \alpha_{33}X^2_3 + \alpha_{12}X_1X_2 + \alpha_{13}X_1X_3 + \alpha_{23}X_2X_3 \quad (1)$$

When Y represents the response output, α_0 is the intercept, α_1X_1 to α_3X_3 are the linear coefficients, $\alpha_{11}X^2_1$ to $\alpha_{33}X^2_3$ are the quadratic coefficients, and $\alpha_{12}X_1X_2$ to $\alpha_{23}X_2X_3$ represents the interaction of coefficients. The use of the analysis of variance was employed to authenticate if the model is significant.

Table 3.1: Variables used for designing the experimental

Factors (independent variables)	**Factors level**		
	-1	0	1
Time (Minutes)	90	105	120
Temperature (ºC)	80	85	90
NaOH (%)	1.5	1.75	2
$NaHSO_3$ (%)	0	0.5	1

3.2.3 Keratin extraction and purification process

20 g of the treated and milled feathers was weighed and added to a 100 ml of alkaline solution, which represent (1:5) in ratio containing sodium hydroxide (NaOH) and sodium bisulfite ($NaHSO_3$) in varying concentration according to the experimental design in (Table 2). The pH of the solution ranged between 12 to13. Chicken feather samples were placed in small steel containers with tight seals that were sealed with a cap and immersed in a hot oil bath set at the

appropriate temperature. The temperature and reaction times were inputted and varied according to the parameters generated by the experimental design (Table 3. 2). The turbid solution was then filtered after cooling and then neutralised by adding about 5 ml of HCl (2 M) dropwise. The filtrate was then dialysed using regenerated cellulose tubes (MWCO 3500-500 Da) in distilled water for 72 hours, with changing of the outer water three times a day. The filtrate was removed after dialysis and freeze-dried to collect the keratin powder for storage in the cold-room.

Table 3.2: The different process parameters affecting keratin hydrolysate and the protein yield

Run	A: Time (Mins)	B: Temperature (℃)	C: NaOH (%)	D: $NaHSO_3$ (%)	Response 1 Hydrolysate (%)	Response 2 Protein yield (%)
1	90	80	1.75	0.5	71.51	49.91
2	105	90	1.5	0.5	63.00	62.61
3	90	85	1.5	0.5	60.50	51.48
4	105	85	1.5	0	60.50	51.09
5	105	85	1.75	0.5	69.50	64.04
6	105	80	1.75	1	62.00	53.52
7	105	80	1.5	0.5	53.00	46.81
8	120	85	1.75	0	65.00	59.59
9	105	85	1.75	0.5	69.50	64.04
10	105	85	2	1	71.00	57.39
11	90	85	2	0.5	61.00	54.65
12	120	85	1.5	0.5	64.60	55.61
13	120	85	1.75	1	58.40	50.09
14	120	80	1.75	0.5	62.00	55.66
15	105	85	1.75	0.5	69.50	64.04
16	90	85	1.75	0	60.00	52.43
17	105	80	2	0.5	54.00	49.28
18	105	85	1.5	1	34.00	29.39
19	120	90	1.75	0.5	59.00	60.47
20	105	85	1.75	0.5	69.50	64.04
21	105	85	2	0	41.00	35.07
22	105	90	1.75	1	53.00	62.79
23	120	85	2	0.5	61.00	60.21
24	105	80	1.75	0	54.50	50.77
25	105	85	1.75	0.5	69.50	64.04
26	105	90	2	0.5	60.80	60.22
27	90	85	1.75	1	50.00	47.62
28	105	90	1.75	0	53.00	53.46
29	90	90	1.75	0.5	38.70	39.61

3.2.4 Bradford assay

Bradford assay was used for the detection and the quantification of protein concentration in the keratin hydrolysate; this was done using the Bradford (1976) procedure. The test works based on the capability of the protein solution to absorb the light on the wavelength from 595 nanometres (Bradford, 1976). Bovine Serum Albumin (Sigma-Aldrich, South Africa) was used to generate a linear calibration curve, which was used as a standard. The sample used was prepared by weighing 35 mg of keratin hydrolysate powder and dissolved in 1 ml of deionised water. After that, about 5μL of the prepared sample was mixed in a small tube with 200 μL of the Bradford reagent (Sigma-Aldrich, South Africa). The keratin hydrolysate samples and the blank that contain deionised water and the Bradford assay reagent were allowed to stay for about 10 minutes before the absorbency was measured with a spectrophotometer (Promega GloMax Microplate Multimode Reader). After that, the amount of protein in the solution was determined by comparing the absorbance value obtained of the keratin samples to the calibration standard curve of BSA.

3.2.5 CHNS analysis

The protein content of the feather keratin powder was determined with the use of a CHNS analyser (PerkinElmer, series II CHNS elemental analyser). The equipment results show the percentage of each element in the keratin hydrolysate, which are the content of carbon, hydrogen, nitrogen, and sulphur in the keratin powder. After that, the content of nitrogen was used to quantify the percentage protein content in the keratin hydrolysate by using a factor that is commonly used to multiply the percentage of nitrogen that was given by the equipment, and this factor is 6.25 since most protein contains 16% nitrogen.

3.2.6 SDS-page Electrophoresis

Electrophoretic separations of keratin protein molecular weight were done with the use of the SDS-page gel electrophoresis technique. The analysis was carried out on 12% weight per volume polyacrylamide separating gel and 5% weight per volume polyacrylamide stacking gel for medium molecular weight (10- 180 kDa). Low molecular weight (1.7- 40kDa) estimations were performed on 16% (w/v) tricine-sodium dodecyl sulphate polyacrylamide separating gel, and 5% (w/v) polyacrylamide stacking gel was also used (Haider et al., 2012). The sample preparation was done, according to Wang et al. (2016) and Haider et al. (2012). 35 mg of the keratin extracts were dissolved with 1 ml of deionised water, and about 15 µL of protein sample mixed with 5µL of 5μL of loading buffer containing about 0.25μL β-mercaptoethanol and 5 µL glycerinum. The protein's denaturalisation was done by boiling the blend solution for about 6

min with loading buffer in a hot bath. After that, about 7 µL of protein marker and 10 µL of denatured solution were loaded into gel well, respectively. The separation was performed at 80 V for 30 min, followed by 120 V for 60 min. After that, the gels were rinsed twice with distilled water before staining with Coomassie Brilliant Blue solution. The staining process was for about 30 min; after that, the gel was destained overnight using a 40% glacial acetic acid solution with shaking. Finally, the gel image was taken with an imaging instrument (Wang et al., 2016).

3.2.7 The chemical functional analysis using Fourier transform infrared spectroscopy (FTIR)

The chemical functional groups of keratin powder were analysed using Fourier transform infrared spectroscopy (FTIR) spectroscopy. Universal attenuated total reflectance module was used for the spectra of the analysed samples in a wavenumber range between 3500 cm^{-1} and 550 cm^{-1}.

3.3 Results and discussions

3.3.1 Quantitative analysis of keratin hydrolysate

The quantitative analysis of keratin hydrolysate was determined by Bradford's assay method of protein estimation (Bradford, 1976). The process entails the dissolution of keratin powder in 1 ml of deionised water, then 6µL of the sample dilution was mixed with 200 µL of Bradford reagent. The assay was conducted in four replicates. The protein concentration was determined by comparing the absorbance value of the keratin samples to the calibration standard curve of bovine serum albumin. The nitrogen content of the optimised sample was determined using a CHNS analyser (PerkinElmer, series II CHNS elemental analyser). The average protein concentration obtained with Bradford assay was 0.1μg/ml. However, the average elemental composition of samples of keratin hydrolysate, as shown by CHNS analysis, was 13.85 % of nitrogen, 47.25 % of carbon, 6.90 % hydrogen and 2.8 % of sulphur, respectively (Figure 3. 1). The CHNS results are comparable to those reported in the literature (Tesfaye et al., 2017).

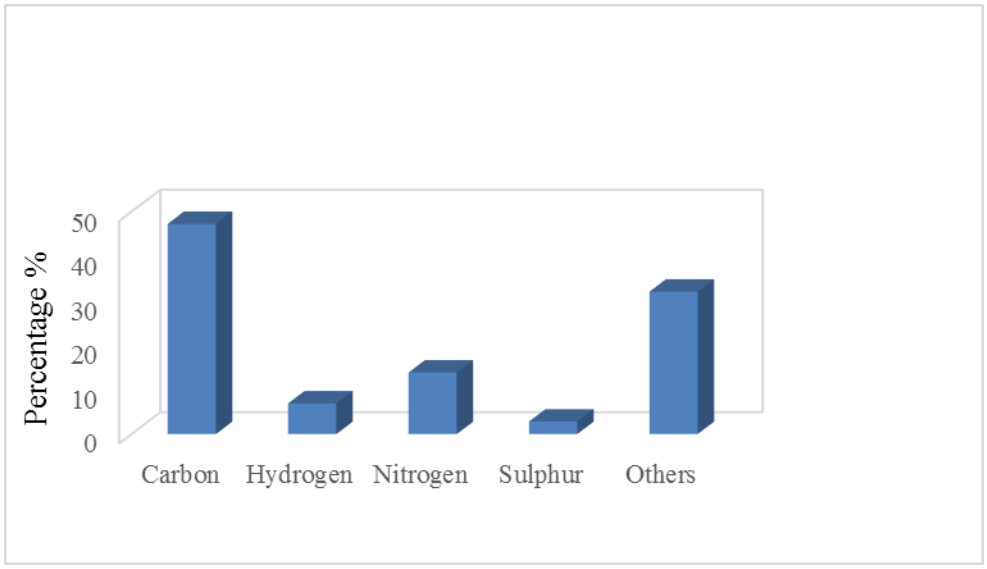
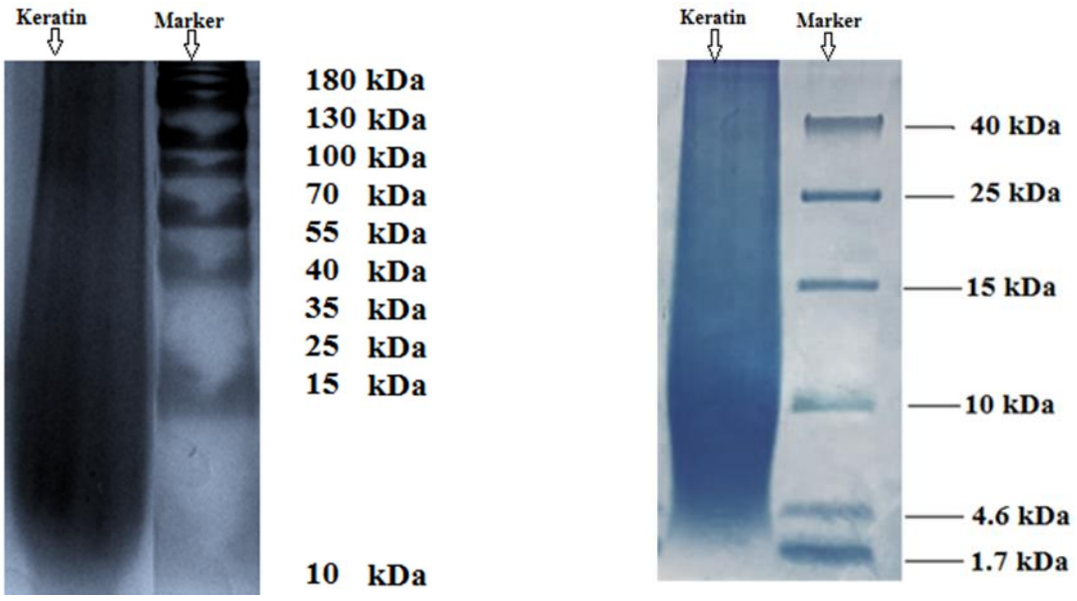


Figure 3.1: Elemental analysis of keratin hydrolysate

3.3.2 Molecular weight analysis using SDS-page

The molecular weight of the keratin hydrolysate was estimated by SDS-page using 12% polyacrylamide and 16 % tricine-sodium dodecyl sulphate-polyacrylamide gel, respectively. The result showed a big band of protein fractions between 10-15 kDa for the medium molecular weight and 3 -10 kDa for the low molecular weight, as shown in Figures 3. 2a & 2b, respectively. The result of this study showed keratin with low molecular weight, which is comparable to the result obtained by Kamarudin et al. (2017), for the low molecular weight as reducing agents, was used in the study. This result also corroborates with the statement made by Floris and Slangen (2005), which stated that keratin protein mostly has a molecular weight of between 1 and 11 kDa (Floris and Slangen, 2007, Kamarudin et al., 2017).



A: Medium molecular weight

B: Low molecular weight

Figure 3.2a & b: SDS-PAGE of keratin protein hydrolysate.

3.3.3 Functional group analysis

FTIR spectra of the keratin protein hydrolysate are compared with feather meal, as shown in Fig. 3. 3. The graph obtained from the keratin hydrolysate sample matched the standard graph of feather meal protein. The wavelength derived from the infrared spectroscopy also confirmed that C-N bond, N-H bond, C=O bond and disulphide bond are in the sample; thus, the peaks showed the presence of carboxyl group and amino groups, the two common groups present in amino acids residues like cystine, threonine and glutamine were observed (Gupta et al., 2011). Therefore, the peaks in the spectra show the characteristics of amide A, I, II and III conformation; besides, it was confirmed that the protein is present in the sample and comparable to what was obtained by other authors (Gupta et al., 2011, Wang et al., 2016, Tesfaye et al., 2017). The solid-state ^{13}C NMR spectrum revealed some chemical shift at 173-190 ppm, which is due to the carbonyl carbon of the amino acids. In addition, the aromatic carbons are seen at around 120 – 150 ppm, while the α-carbon is seen at 60 ppm and the β-carbon at 42 ppm (Figure 3. 4). The alkyl side chains of the amino acids are shown between 10 – 30 ppm. The spectrum and the chemical shift observed were similar to what was seen by (Ghosh et al., 2019), from regenerated feather keratin, which used dimethyl sulfoxide (DMSO) to extract the keratin.

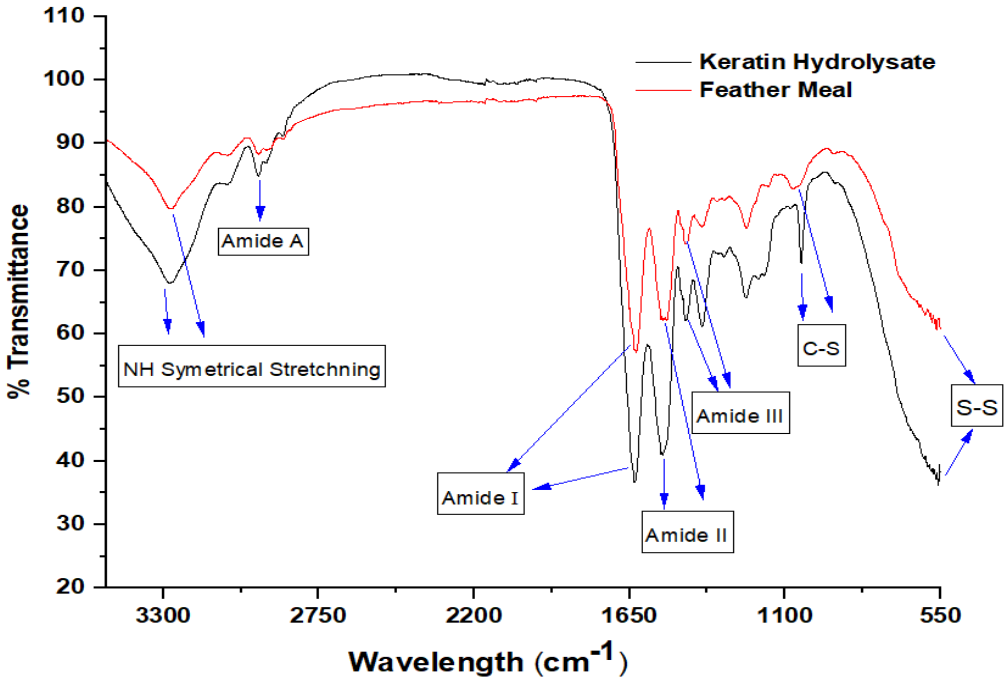


Figure 3.3: FTIR spectra of keratin hydrolysate comparing with feather meal

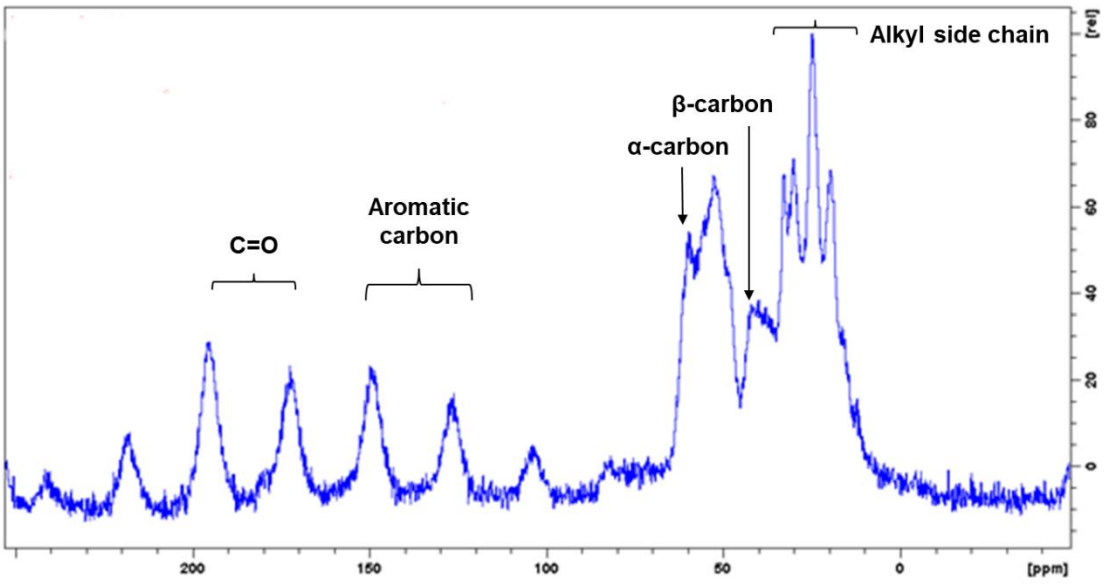


Figure 3.4: The solid-state NMR spectrum showing the solubility of feather keratin hydrolysate

3.3.4 The development of the response surface models

The input data for the experiment and the output responses, including the percentage yield of keratin hydrolysate and the protein yield, are shown in Table 3. 2. The data were used to form two quadratic polynomial model equations, which were developed to relate the percentage keratin hydrolysate and protein yield with the input variables, as shown in both Equations 2 and 3 below:

$$\text{Hydrolysate (\%)} = +69.50 + 2.36A - 2.46B + 1.10C - 0.47D + 7.45AB - 1.02AC + 0.85AD - 0.80BC - 1.88BD + 14.13CD - 2.93A2 - 6.33B2 - 6.35C2 - 9.10D2 \quad (2)$$

$$\text{Protein yield (\%)} = +64.04 + 3.87A + 2.73B + 1.65C - 0.13D + 3.89AB + 0.36AC - 1.17AD - 1.22BC + 1.64BD + 11.01CD - 4.46A2 - 3.49B2 - 7.35C2 - 8.67D2 \quad (3)$$

Where Y represents keratin hydrolysate yield and the percentage of protein in the keratin hydrolysate for two equations, the letters A, B, C and D stand for the reaction time and temperature, percentage concentration of sodium hydroxide and sodium bisulphite, respectively, for Equations (2) and (3) respectively.

The fitted models' authenticity was assessed with the use of the analysis of variance (ANOVA), as seen in Tables 3. 3 and 3. 4. The keratin hydrolysate and the percentage yield of protein models showed high F-values, that is, 4.74 and 4.46, with relatively low p-values of 0.0125 and 0.0156, respectively. While, the coefficient of determination (R^2) values for the two models were 0.77 (percentage yield of keratin hydrolysate) and 0.74 (percentage yield of protein), indicating that the models could account for about 77% and 74% of the variations in the experimental data. The p-values (< 0.05) that are a bit low and the slightly high F calculated values show that the polynomial models used were significance as shown in Table 3. 4.

Table 3.3: Analysis of variance (ANOVA) for hydrolysate and protein yield models

Source	Sum of squares	Df	Mean square	F-value	P-value	R^2
Hydrolysate model	784.32	4.00	196.08	4.74	0.0125	0.77
Protein yield model	735.43	4.00	183.86	4.46	0.0156	0.74

Df: the degree of freedom, F-value: Fisher-snedecor distribution value, P-value: probability value, R^2: coefficient of determination

Table 3.4: Models coefficient of estimates with standard errors

Factor	Hydrolysate CE	Hydrolysate SE	Protein yield CE	Protein yield SE
Intercept	69.50	2.88	64.04	2.87
A	2.36	1.86	3.87	1.85
B	-2.46	1.86	2.73	1.85
C	1.10	1.86	1.65	1.85
D	-0.47	1.86	-0.13	1.85
AB	7.45	3.21	3.89	3.21
AC	-1.02	3.21	0.36	3.21
AD	0.85	3.21	-0.17	3.21
BC	-0.80	3.21	-1.22	3.21
BD	-1.88	3.21	1.64	3.21
CD	14.13	3.21	11.01	3.21
A^2	-2.93	2.52	-4.46	2.52
B^2	-6.33	2.52	-3.49	2.52
C^2	-6.35	2.52	-7.35	2.52
D^2	-9.10	2.52	-8.67	2.52

CE-coefficient estimate, SE-standard error

3.3.5 The linear effect of extraction variables on the hydrolysate and keratin protein yields

The yield of keratin hydrolysate and protein ranges between 34 % to 71.51 % and from 29.39 % to 64.04 %, respectively, signifying how sensitive the keratin hydrolysate's recovery to the input parameters.

Time and temperature

The extraction process carried out at the lowest reaction time (90 minutes) and temperature (80℃) gave a high keratin hydrolysate yield (71.51%) and slightly low protein yield (49.91 %), respectively (Table 3. 3). The process with the highest time (120 minutes) and temperature (90℃) gave keratin hydrolysate yield of (59%) and protein yield (60.47%), respectively. The run with time (120 minutes), temperature (85℃) and sodium hydroxide (1.75%) without sodium bisulphite (0 %) produced a slightly high yield of keratin hydrolysate (65%) and protein yield (59.59%), respectively. Compare to the previous studies, Kamarudin et al. (2017) obtained 43.8% and 41.5% keratin hydrolysate yield by treating feathers with sodium hydroxide and sodium sulphide respectively, with the temperature of 60℃ for 2 hours (Kamarudin et al., 2017); however, these results were lower than the yields obtained in this

current study. Similarly, Poole et al. reported about 55 % keratin yield using sodium sulphide at the temperature of 30℃ for about 24 hours incubating under nitrogen gas (Poole et al., 2011), this yield is lower compared to the result in this present study. The variation in these studies with the current work could be due to differences in operation parameters and reducing agent employed. Because the structural nature of chicken feather biomass makes it difficult for smooth degradation, thus a suitable pre-treatment for extraction is required to release the locked-up protein. Physical pre-treatment and extraction techniques, such as thermal treatment, inert atmosphere, and chemical methods requiring acids or bases to break through the feather protective structures for keratinous protein release have been reported (Kamarudin et al., 2017). There are indications that the various extraction parameters and medium impact differently on the primary and secondary feather structure, which results to variations in the keratin hydrolysate released and, consequently, protein yield (Poole et al., 2011).

For instance, in the current study, the extraction process carried out at 90 minutes reaction time and 80℃ process temperature gave a high keratin hydrolysate yield (71.51 %) and lower protein yield (49.91 %), compared to 120 minutes and 90℃ temperature process conditions, lower keratin hydrolysate yield of 59 % and higher protein yield of 60.47% were obtained. When the physical parameters interacted with the chemical parameters, a different set of results were observed. Process time of 120 minutes, temperature 85℃, sodium hydroxide of 1.75 %, and sodium bisulphite (0 %) produced higher keratin hydrolysate yield (65 %) and protein yield (59.59 %). In comparison to previous studies, Kamarudin et al. (2017) obtained 43.8 % and 41.5 % keratin hydrolysate yield by treating feathers with a mixture of sodium hydroxide and sodium sulphide, at 60℃ process temperature for 2 h (Kamarudin et al., 2017).

Similarly, the degree of degradation or solubilization of chicken feather is temperature dependant when employed as a physical parameter. Hence, the need for optimum extraction parameters. The chicken feather structure becomes more accessible and solubilizes into desirable monomeric and soluble units when suitable chemical solvent and physical parameters such as heat are combined.

The concentration of NaOH and $NaHSO_3$

As shown in Table 3. 2, the extraction carried out at 1.5 % and 1 % concentration of sodium hydroxide and sodium bisulphite, respectively, showed a low yield of keratin hydrolysate (34 %) and protein (29.39 %); this might be because of high solid to liquid ratio and feather aggregation during the extraction process. However, (Kamarudin et al., 2017) have reported a

similar effect of these reducing agents concentration on keratin hydrolysate and protein yield from chicken feathers biomass. The highest keratin hydrolysate (71.51 %) was observed in the mixture with sodium hydroxide (1.75 %) and sodium bisulphite (0.5 %) with a low temperature and time while the mixtures that contained the same concentration but with a relatively high temperature, (85℃) and time (105 mins) gave the highest protein yields (64.04). This result is higher than the result obtained by Poole et al. (2011), which is 55 % using sodium sulphide (Poole et al., 2011). Similarly, high keratin hydrolysate and protein yield were observed in experimental runs with sodium hydroxide (1.5 % and 1.75 %) without the addition of sodium bisulphite (0 %) (Table 3. 2).

The interaction of the experimental factors on the keratin hydrolysate and protein yield

The effects of the interaction of the extraction process variables were assessed using the 3D response surface graphs. The assessment shows that the concentration of sodium hydroxide and the reaction time had a direct interaction on the yield of keratin hydrolysate (Fig. 3. 5(a)) when these parameters were varied from their lower to higher points. The same trend was observed with the protein yield, as shown in Fig. 3. 5(b), sodium bisulphite concentration and the extraction time have a similar linear relationship on the keratin hydrolysate yield.

It was displayed in Fig. 3. 5(c), the graph shows that assuming the temperature of the reaction was maintained at around 86 ℃ and the concentration of sodium hydroxide was approximately 1.88 %, the keratin hydrolysate yield also increased to about 70 %. This pattern is similar to what was seen for sodium bisulphite and the temperature of the reaction (Fig. 3. 5(d))

The interactive effect of reaction time and the percentage yield of protein in the hydrolysate indicate that as the reaction temperature was maintained at the maximum level, as seen in Fig. 3. 5(e). The graph shows that the higher the reaction time and the temperature, the higher the protein yield obtained.

It can be seen in (Fig. 3. (6a-d)) that the optimum condition is at the centre of the graphs; however, the maximum protein yield is observed at the sodium hydroxide and sodium bisulphite concentration of about 1.75 % and 0, 5 %, respectively and the reaction time of approximately 112 minutes. Likewise, the protein yield increases as the reaction temperature increases at sodium hydroxide and sodium bisulphite concentration of 1.88 % and 0.5 %, respectively. The influence of the extraction chemicals on the yield of protein is presented in Fig. 3. 6(e); the graph shows that the highest protein yield is found at the sodium hydroxide concentration of (1.75 % and 1.88 %) and sodium bisulphite of about (0 % and 0.5 %),

respectively. Hence, the strength of the keratin extraction solvent is one of the essential parameters because the higher the intensity, the more the disulfide bonds will decrease, resulting in shorter keratin particles (Kamarudin et al., 2017, Poole et al., 2011). The percentage keratin hydrolysate and the protein yield were higher than what was observed by (Kamarudin et al., 2017) that treated chicken feathers with sodium hydroxide and sodium sulphide, respectively, with the temperature of 60 ℃ for 2 hours. The outcome of these studies indicates that the keratin yield of the hydrolysis depends on process pH, operating temperature and exposure time, and likewise on the nature of acid or base and concentration of solvent used (Sinkiewicz et al., 2017).

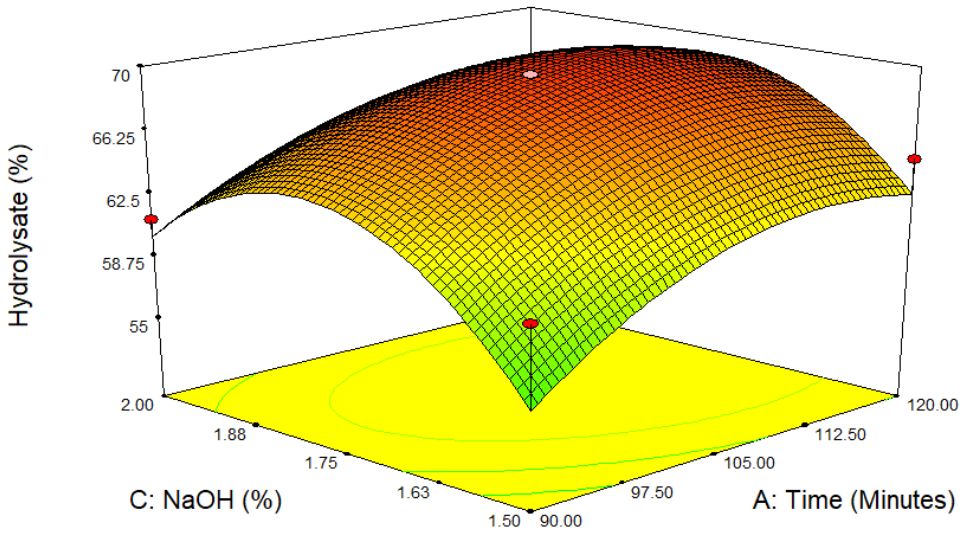


Figure 3.5a: 3-D response surface plot showing the interaction of percentage NaOH and heating time on the keratin hydrolysate.

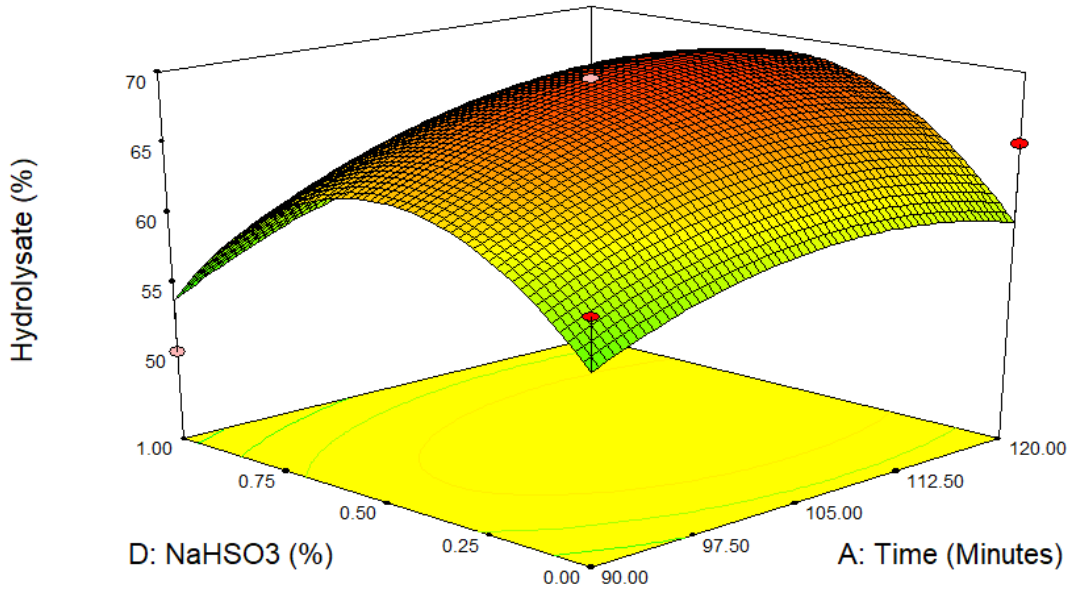


Figure 3.5b: 3-D response surface plot showing the interaction of $NaHSO_3$ and heating time on the keratin hydrolysate.

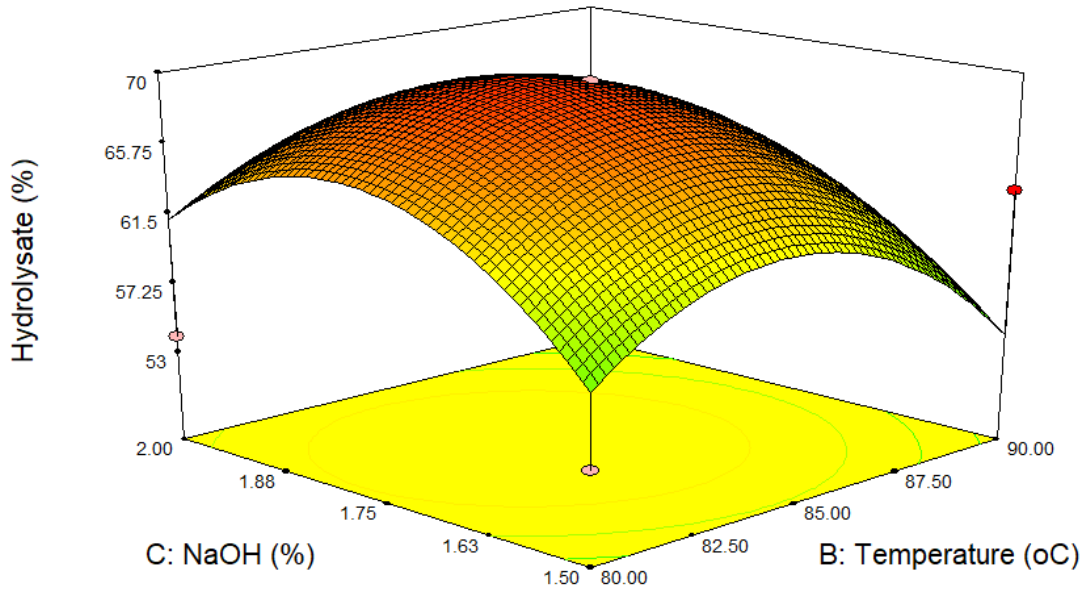


Figure 3.5c: 3-D response surface plot showing the interaction of percentage NaOH and temperature on protein hydrolysate.

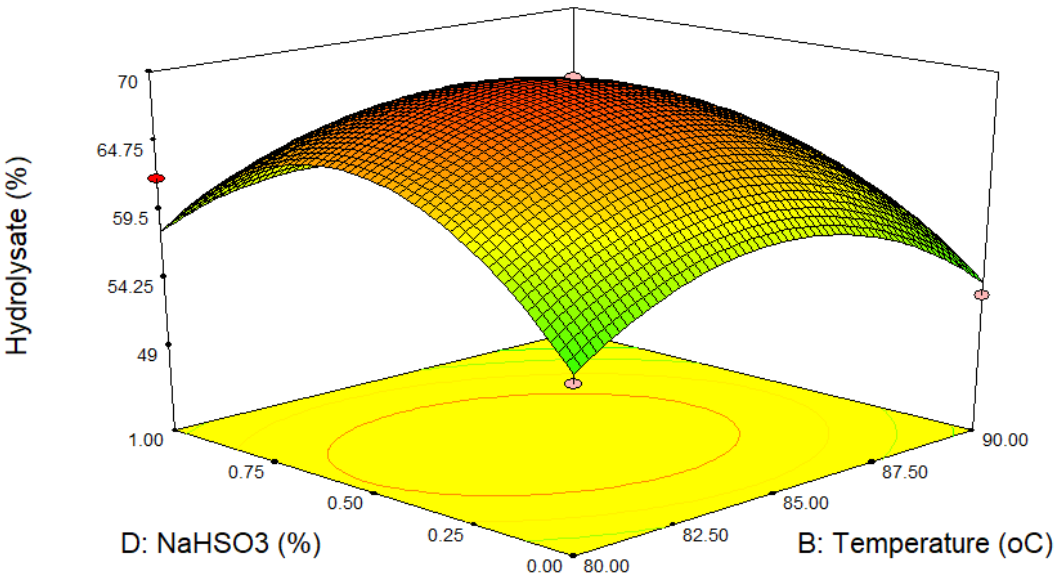


Figure 3.5d: 3-D response surface plot showing the interaction of $NaHSO_3$ and temperature on keratin hydrolysate.

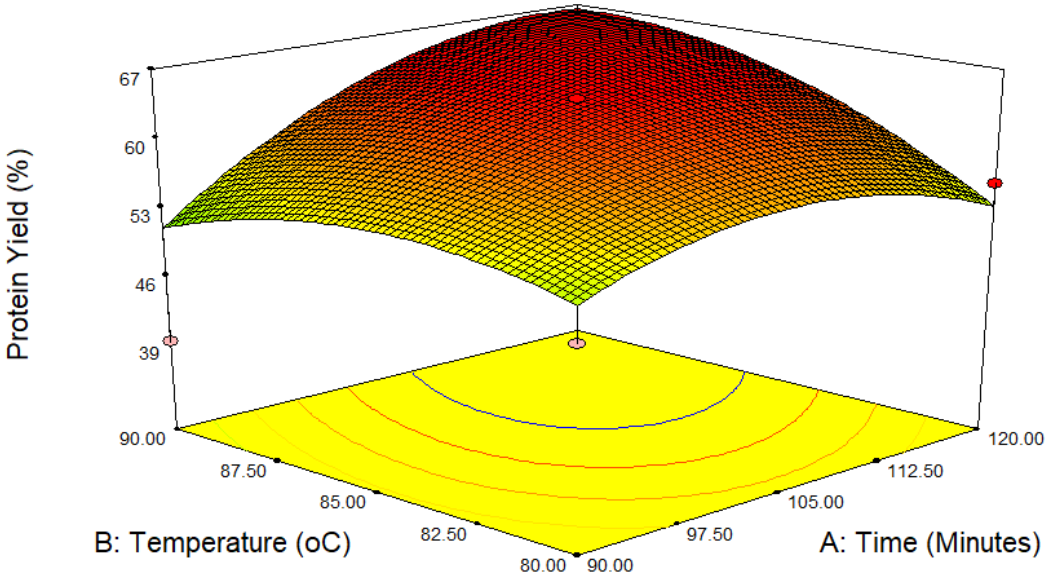


Figure 3.5e: 3-D response surface plot showing the interaction of temperature and heating time on the protein yield.

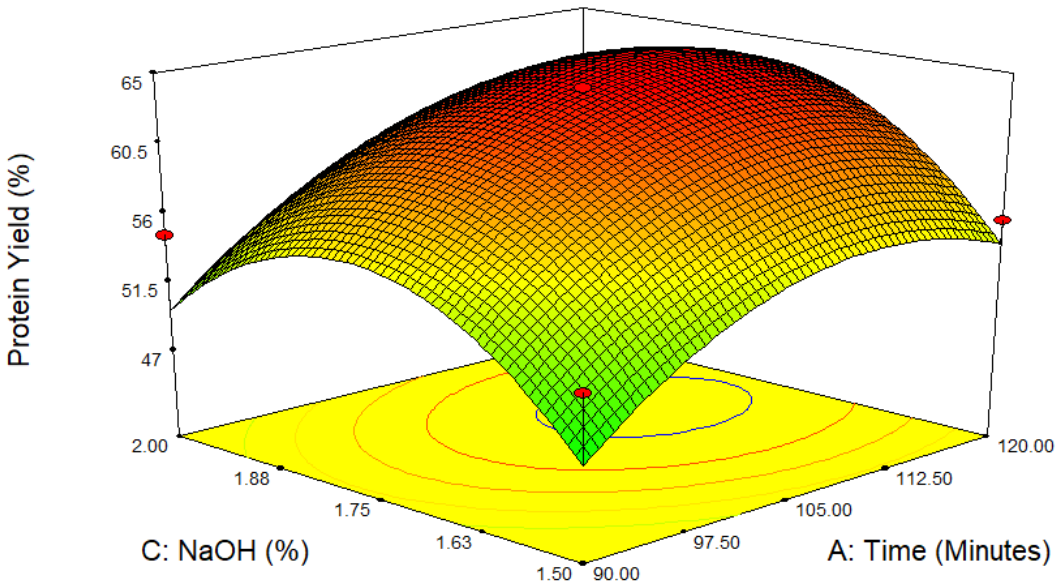


Figure 3.6a: 3-D response surface plot showing the interaction of percentage NaOH and heating time on the protein yield.

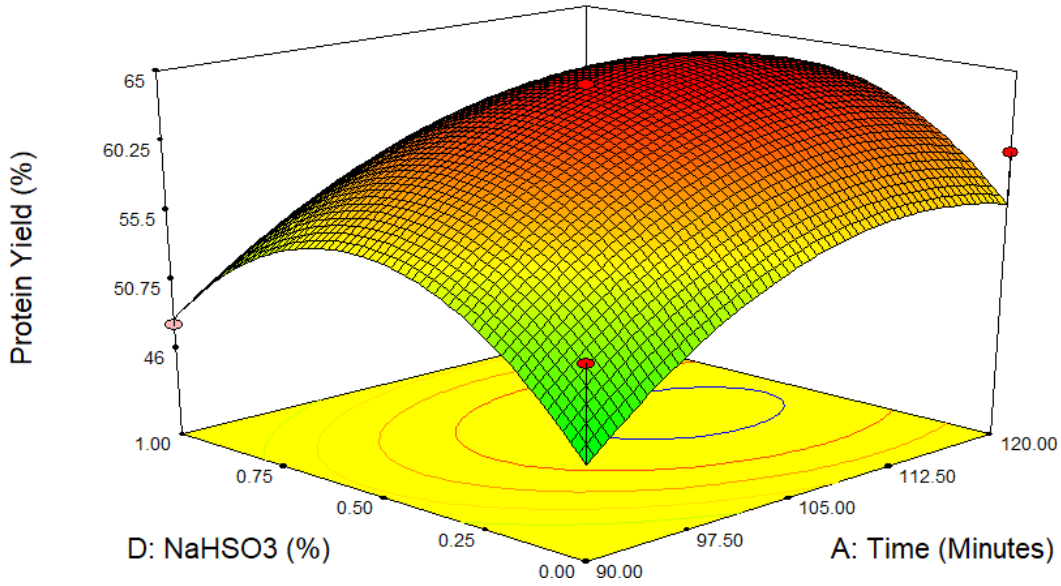


Figure 3.6b: 3-D response surface plot showing the interaction of $NaHSO_3$ and heating time on the protein yield.

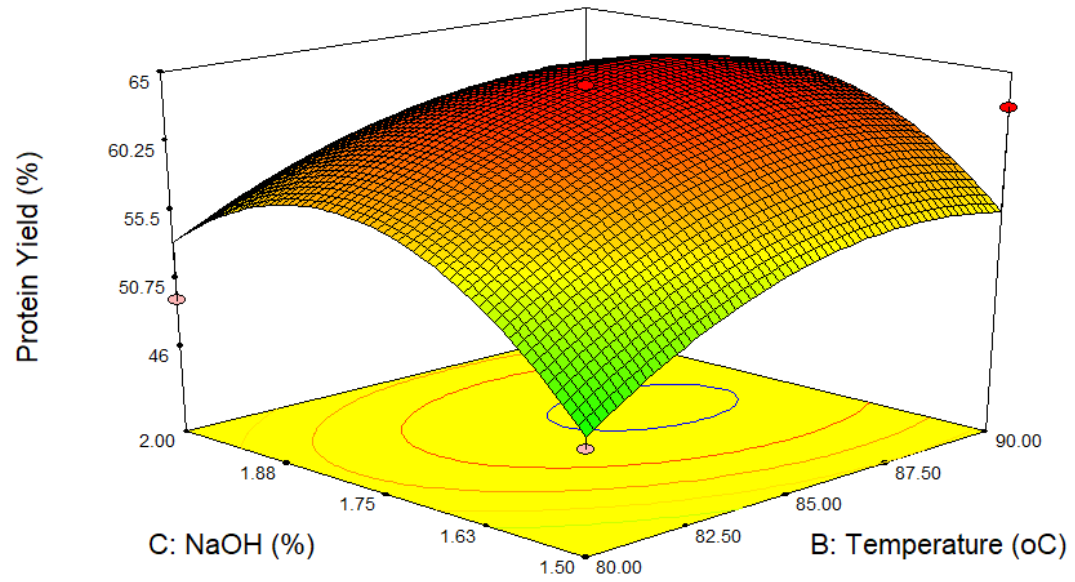


Figure 3.6c: 3-D response surface plot showing the interaction of heating time and NaOH concentration on protein yield.

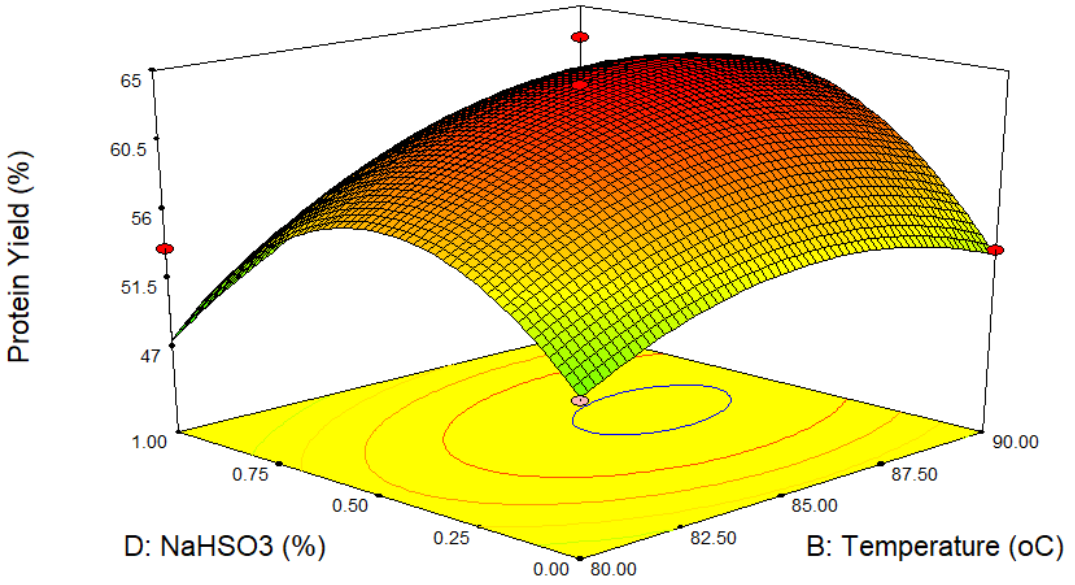


Figure 3.6d: 3-D response surface plot showing the interaction of $NaHSO_3$ and temperature on protein yield.

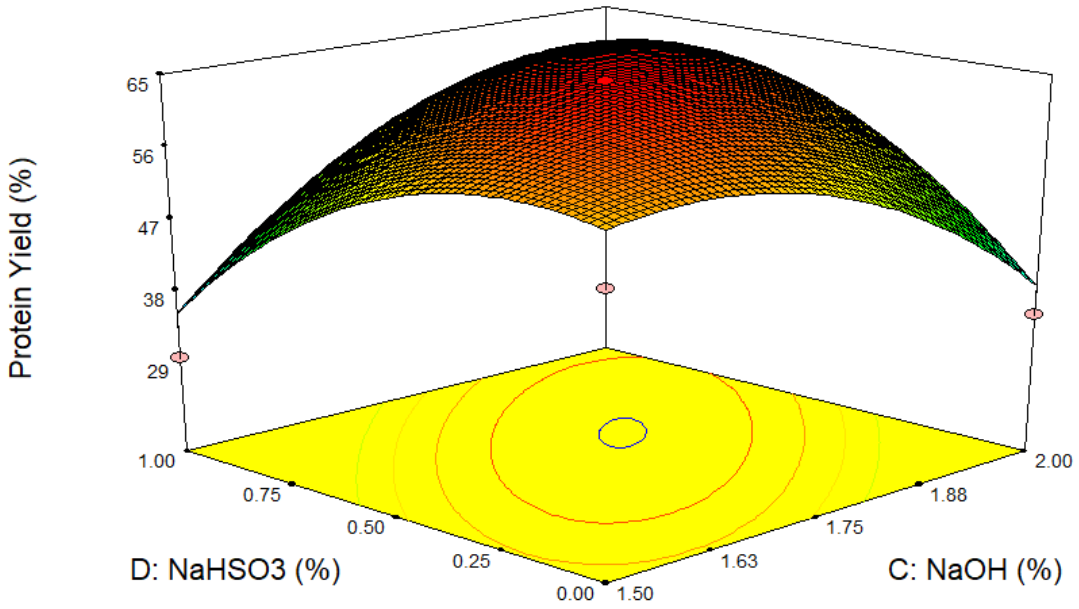


Figure 3.6e: 3-D response surface plot showing the interaction of percentage $NaHSO_3$ and NaOH on protein yield.

Validation of keratin extraction process

The extraction parameters that include reaction time, temperature and concentration of the reducing agents were optimised using design-expert software (Stat-Ease Inc., USA). The optimal set-points based on the developed model were sodium hydroxide (1.78 %), sodium bisulphite (0.5 %) concentration, reaction time (111 minutes) and temperature (87 ℃), respectively (Table 3. 6). The optimised extraction process conditions based on the model predicted keratin hydrolysate yield and protein yield of 70.27 % and 65 %, respectively, based on the optimal extraction parameters. The experiment was carried out in triplicates and gave average keratin hydrolysate and protein yields of 68.29 % and 65.21 %, respectively (Table 3. 6). This result shows a slightly lower keratin hydrolysate and protein yield than what was predicted by the model, which is 70.27 % and 65.50%, respectively. The difference might be because of a higher decrease in disulfide bonds during the extraction process. Simultaneously, most of the smaller keratin particles could have been lost during the dialysis process. This observation agrees with the disulfide bonds spectrum of the FTIR analysis, where the disulfide bonds of the extracted hydrolysate were much lower compared to the feather meal used as control (Fig. 3. 3).

Furthermore, the reduction in amides A, I, II, and III, as depicted by the spectra of this protein on the FTIR figure (Fig. 3. 3), could also have accounted for the obtained lower protein yield compared to the predicted values. These results further underscore the importance of the choice

of suitable extraction technique that reduces the negative impact of extraction conditions. Notwithstanding, the yields observed were higher than what was reported by (Kamarudin et al., 2017), which extracted keratin using sodium hydroxide and bisulphite mixture. Similarly, comparing what was obtained by (Poole et al., 2011), using sodium sulphide at the temperature of 30 ℃ for about 24 hours incubating under nitrogen gas, the yields from this current study were higher than the 55 % yield that was observed by these authors.

Table 3. 5: Optimum levels of variables during the keratin extraction process

Independent variables		**Predicted optimum levels**
Time		111 Mins
Temperature		87 ℃
NaOH		1.78 %
$NaHSO_3$		0.5 %
Response	**Predicted value**	**Observed value**
Hydrolysate	70.27 %	68.29 %
Protein yield	65.50 %	65,21 %

3.4 Conclusions

The thermochemical process models connecting the extraction design of keratin hydrolysate and protein yield from chicken feathers subjected to hybrid pre-treatment of sodium hydroxide, sodium bisulphite and the heat have been developed. The optimum extraction conditions were (1.78 %) sodium hydroxide and (0.5 %) sodium bisulphite concentrations, (111 minutes) reaction time, and (87℃) temperature. The keratin hydrolysate and protein yield obtained from the optimum conditions were 68.29 % and 65.21 %, respectively. The determined optimum physicochemical set points of sodium hydroxide, sodium bisulphite, dissolution time and temperature positively impacted protein yield from the waste chicken feathers. Under these conditions, the extracted protein structure was not denatured. The FTIR, CHNS and SDS-page results confirmed the extraction of the desired protein units, which further underscores the suitability of operation parameters chosen. An additional interest lies in the availability, renewability, and the global challenge in the disposal of the waste chicken feathers, which fundamentally would influence the techno economics and the industrial process scalability.

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CHAPTER 4

PAPER 3: A MANUSCRIPT PAPER SUBMITTED AND UNDER REVIEW

Beneficiation of waste chicken feathers as a formaldehyde-free bio-adhesive for particleboard production

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4.1 Abstract

The present study aimed at investigating the beneficiation of extracted keratin protein hydrolysate from chicken feather waste biomass as bio-adhesive to produce particleboard. Chicken feathers were hydrolysed using hybrid alkaline hydrolysis, and the obtained keratin protein fraction was used for bio-adhesive formulation. The formulated adhesive was employed for particleboard fabrication. The quality of bio-adhesive and the particleboard mechanical strength performance were evaluated. The FTIR spectra confirmed the amine, alkyl side chains and carboxylic groups of the amino acids in the unmodified keratin-based binder. Besides, the spectra revealed the covalent bonding between the azetidinium of the citric acid-based polyamide-epichlorohydrin cross-linking and the hydroxyl groups of the keratin protein hydrolysate. The mechanical strength performance of the fabricated particleboard met the specification for the 1-L-1 grade of the American National Standards Institute (A208.1). 6, 5 and 1184, 34 MPa, were the respective values obtained for modulus of rupture and modulus of elasticity of the panels made with unmodified keratin-based adhesive. The cellulose nanocrystals incorporation as a filler enhanced the static bending and bonding strength properties of the formulated bio-adhesive. These findings, therefore, demonstrate that keratin

hydrolysate protein extracts from chicken feather waste could be considered as one of the potential feedstock for the production of environmentally friendly wood composites bio-binder.

Keywords: C*hicken feather waste, Keratin hydrolysate, Bio-adhesives, Formaldehyde-free, Particleboard*

4.2 Introduction

The manufacture of wood products like particleboard (PB), plywood (PW), oriented strand boards (OSB), medium-density fibreboards (MDF) and hardboard (HB) has continued to increase steadily(Adhikari et al., 2017). This trend has gained popularity because the low quality and small-diameter trees that are not suitable for lumber manufacture can be utilized, as well as waste biomass such as plain shavings and sawdust from sawmills. According to the FAO, worldwide wood-based composite production reached about 408 million m^3 in 2018 (FAO, 2020). This production entails the use of wood binders as bonding agents for wood composites (Adhikari et al., 2016). Currently, most of these binders are synthetic, formaldehyde-based derivatives from petroleum sources. These adhesives emit formaldehyde, which resulted in the pollution of the environment and is harmful to human health (Kim et al., 2011). The formaldehyde is considered carcinogenic by both the Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) (Mao and Kim, 2013).

Furthermore, the prices of synthetic adhesives are typically dependent on the oil market with its price fluctuations. Additionally, the depletion of fossil fuel reserves is a vital concern, which makes accessibility of these synthetic adhesives unpredictable in the future (Alawode et al., 2019). The above problems can be alleviated by replacing the synthetic adhesives with green, environmentally sourced natural resins that can be modified to reproduce properties and performance characteristics of synthetic adhesives (Pizzi, 2006, Norström et al., 2018). Consequently, the interest in the natural and sustainable sources of wood adhesives with similar strength properties to synthetic wood binders commonly used in the wood products industry has since been stimulated (Lambuth, 1989, Dunky, 2020)

Some natural materials such as tannins, lignins, carbohydrates and soy proteins have been investigated for wood adhesive potentials, and some of them are presently in use by the industry (Amaral‐Labat et al., 2008, Adhikari et al., 2019, Mi et al., 2021). The most desirable and conventional natural-based adhesives are protein-based wood adhesives (Müller et al., 2007).

Natural proteins offer many advantages such as renewability, availability and relatively low cost that qualify them as a possible source of adhesives for industrial applications (Müller et al., 2007).

There are two primary sources of protein biomass that could be used to synthesized bio-based wood adhesives; these include plant and animal sources. Substantial research work has been done on the bio-adhesive from plant protein sources, especially soy protein (Amaral‐Labat et al., 2008, Gui et al., 2013, Mo and Sun, 2013). While glues derived from animal hides and bones have been used from ancient times (Pearson, 2003), nevertheless, only scanty research has been carried out on animal sources of bioadhesive from slaughterhouse by-products (such as waste from poultry meat processes). Thus, Park *et al*. (2000) investigated the adhesiveness of protein concentrate extracted from meat and bone meal for plywood production and reported the obtained adhesive showed promising adhesion potential. Besides, the study revealed that the modification with 0.05% glutaraldehyde improved the adhesiveness and the water-resistance of the MBM protein concentrate adhesives (Park et al., 2000). Moreover, utilization of waste animal protein extracts from specified risk material as bio-adhesive for both oriented strand board, and plywood was studied by Mekonnen et al. (2014). The authors reported that the formulated adhesives had desirable resin requirements that make them suitable to be used in a dry environment, and the obtained binder showed less resistance to moisture (Mekonnen et al., 2014).

Some of the disadvantages of the proteinous adhesives identified in the literature were low mechanical strength properties of wood composites, and are much less resistant to moisture compared to the synthetic resins (Frihart and Satori, 2013). However, Adhikari et al. (2016) produced and chemically modified the protein extract with polyamidoamine-epichlorohydrin (PAE). They reported that the plywood specimens produced with the protein–PAE adhesive formulations lead to binders that the shear strength at dry and after soaked in water was similar to that of conventional phenol-formaldehyde adhesive (Adhikari et al., 2016).

This research work explores the utilization of waste chicken feathers, which is one of the slaughterhouse waste by-products that are yet to be fully utilized as bio-adhesives for particleboard production. According to the USA Foreign Agricultural Service, the total domestic per capita consumption of chickens in the USA and some selected countries, including South Africa in 2019, is about 96,464 x 10^3 metric tons (USDA;, 2019).

Chicken meat is widely eaten as food globally being part of the principal animal protein sources. Its global consumption has resulted in significant feather waste from poultry

slaughterhouses. The yearly estimate of chicken feathers generated worldwide is around fifteen billion tons (Tesfaye et al., 2017). The Republic of South Africa currently contributes two hundred and fifty-eight million tons to the global generation of chicken feather waste from poultry slaughterhouses (Tesfaye et al., 2017). The bulk of the generated chicken feather waste in many countries is disposed of in landfills or burnt, and this action creates further environmental problems such as air pollution (Chinta et al., 2013). On the other hand, research into their beneficiation has gained global attention and has necessitated the search for the best application of chicken feathers. Waste chicken feathers biomass could be utilized industrially because of its biodegradability, renewability, sustainability and ready availability (Tesfaye et al., 2018).

Presently, petroleum-based adhesives for plywoods, fibreboard, particleboards are costly; hence, new interest in cheaper, environmentally friendly and renewable materials for wood adhesives are being sought after (Jiang et al., 2008, Ostendorf et al., 2020). One of these preferred renewable materials is waste chicken feathers due to merits like its high keratin protein percentage and hydrophobicity nature, that proffer a more excellent moisture resistance character on the end product, also the intrinsic property that can defend mildew fungi. These distinctive properties and the merits above make chicken feathers a potential choice of bio-adhesive feedstock for particleboard applications that requires properties like tensile strength and elasticity (Nuutinen, 2017). Currently, waste chicken feathers are not exploited for valuable products at commercial scale (Nuutinen, 2017). There are limited research works on the evaluation of potential bonding properties of protein extracts from chicken feather waste, especially for wood composites. Jiang et al. (2008) carried out a preliminary study on chicken feather protein-based adhesives. The adhesive was synthesized in a mixture that contained 6% sodium hydroxide and 2% sodium bisulfite with and without phenol in the solution during hydrolysis (Jiang et al., 2008). The performance of the adhesive formulations was examined by using them for the production of fibreboard. The authors reported that the adhesive comprising of a portion of hydrolyzed feather protein and a double part of mole ratio 1 of phenol to 2 of formaldehyde mixed at pH 10.5 performed similarly to that of conventional phenol-formaldehyde adhesive (Jiang et al., 2008, Zhao, 2017). Earlier, in 1946, the use of waste chicken feathers as bio-binder was patented, and the alkaline hydrolyzed chicken feathers were used for the production of plywood (Brother and Binkley, 1946). It was reported that the plywood samples possessed mechanical strength properties similar to that of conventional plywood used in dry environments, but it exhibits low water resistance (Brother and Binkley, 1946). To improve both mechanical strength and moisture resistance of protein based-adhesive

polyamide-epichlorohydrin (PAE) resin was used to chemically cross-linked protein hydrolysate from soy and the specified risk material, which is proteinaceous waste biomass that shows a more significant percentage of waste from animal slaughterhouse (Gui et al., 2013, Adhikari et al., 2016, Adhikari et al., 2018b) and the results were promising.

There is an insufficient studies in the literature on the use of waste chicken feathers-based adhesives for particleboard production. The knowledge on the use of waste chicken feathers-based adhesive for particleboard fabrication will help in the valorization of waste chicken feathers from slaughterhouses. Hence, beneficiation of waste chicken feathers leading to building valuable products such as particleboard and ultimately removing chicken feathers from the environment are needed. The application of waste chicken feathers adhesive for particleboard production has thus far to be studied in the valorization of waste feather biomass. Consequently, the present study focused on using extracted protein hydrolysate from chicken feather waste through alkaline reduction hydrolysis to synthesize bio-binder for particleboard production. The potential impact of citric acid-based polyamide–epichlorohydrin (PAE) and cellulose nanocrystals as modifiers in the synthesized bio-binder on mechanical strength properties of the panels were also investigated.

4.3 Materials and methods

The waste chicken feathers used in this study were collected from Rainbow chicken slaughterhouse, Hammarsdale, KwaZulu-Natal province, Republic of South Africa. The wood particles of *Pinus pinaster* used for the production of one layer particleboard in this study were supplied by Merensky Timber, a subsidiary of Hans Merensky Holdings, Johannesburg, Republic of South Africa.

Sodium bisulphite ($NaHSO_3$, 40%), Sodium hydroxide (NaOH), ethanol (99%), and diethylenetriamine (DETA, 99%), citric acid (CA), and epichlorohydrin (ECH, 99%) used in this research work were purchased from Sigma-Aldrich (South Africa). Cellulose nanocrystals (0.5%) in suspension were supplied by Biorefinery Industry Development Facility, Council for Scientific and Industrial Research (CSIR) South Africa. FTIR spectroscopy (Frontier Universal ATR-FTIR, by PerkinElmer) was used for the functional groups' characterization of the keratin-based binders. A Willey mill and a laboratory hot press were utilized during the particleboard production process. An Instron testing machine series IX was used for the characterization of the mechanical strength properties of the particleboard products.

4.3.1 Chicken feather hydrolysis

The thermochemical hydrolysis method was used to solubilized waste chicken feathers for keratin extraction followed the process reported by (Fagbemi et al., 2020). Simply, before hydrolysis, the feathers were chemically decontaminated and, after that, dried in the oven at the temperature of 60℃ for about 24 hours. After drying, ethanol was used to remove the feathers fat by soaking for about 24 hours, and it was removed, washed and dried in the oven for 24 hours again before reducing the size with the Willey mill machine into small particles of around 1.5 mm. Milled chicken feathers (20 g) were transferred to a steel pressure vessel, and a 100 ml alkaline solution, consisting of a mixture of 1.78% NaOH and 0.5% $NaHSO_3$ was added to it. The cap tight vessel was immersed in an oil bath with the temperature set at 87°C and a reaction time of 111 minutes. The hydrolysate was filtered after cooling, and about 5 ml of HCl, 2M, was added to neutralize the solution. The filtrate was dialyzed against the water with the use of dialysis tubing cellulose membrane (MWCO 3500-500 Da) for three days while changing the water constantly three times per day. The hydrolysate was finally removed and dried with a freeze dryer to recover the keratin hydrolysate powder, as shown in Figure 4. 1.

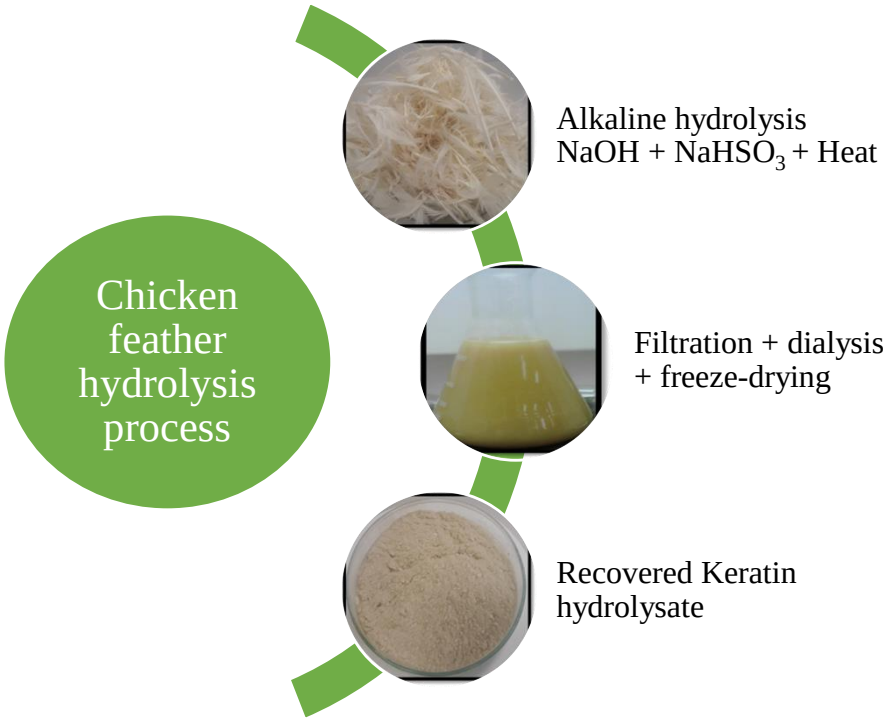


Figure 4.1: Process chart showing the chicken feathers hydrolysis for keratin extraction

4.3.2 Citric acid-based polyamide-epichlorohydrin (CA-PAE) synthesis

The citric acid-based polyamide-epichlorohydrin CA-PAE was synthesized to be used as a cross-linking agent. The synthesizing process comprises two steps, as illustrated by Gui et al. (2013), with a slight modification (Gui et al., 2013). The first step involved the preparation of polyamidoamine by the poly-condensation of diethylenetriamine (DETA) and citric acid (CA). However, the PAE solution was produced by dissolving the obtained polyamidoamine in water, after which it was reacted with epichlorohydrin (ECH) in aqueous solution. The molar ratio of 1/1/1 was used for DETA/CA/ECH, respectively. Furthermore, 31g DETA, 57.6g CA and 20g of water were added to the mixture and placed on a hotplate with a magnetic stirrer, a thermometer, and a condenser attached to it. Citric acid-based polyamidoamine (CA-PADA) molten was obtained after the reaction occurred under 170 ^{o}C for 90 minutes. Then 100ml of water was added to dissolve the citric acid-based polyamidoamine resin. While, at the second step, 27. 8g ECH was added and mixed at room temperature for about 2 hours. After that, the Citric acid-based polyamidoamine was reacted with ECH in aqueous solution under 70 ^{o}C for 1 hour to form citric acid-based polyamide-epichlorohydrin (CA-PAE). The solid content of the resultant CA-PAE solution was around 50wt %, as determined using the freeze-drying process.

4.3.3 keratin-based adhesive formulation without modification

The adhesives based on the hydrolyzed keratin powder were formulated following the method established by Adhikari et al. (2018), with little modification (Adhikari et al., 2018b). Briefly, the hydrolysate powder was dissolved based on a dry weight basis at different concentrations to achieve the solid content of 5, 10, 15, 20, 25 and 30% in a solution containing 0.5 % sodium hydroxide; this is to assess the performance of the Keratin-based adhesive system. The mixture of keratin hydrolysate and sodium hydroxide solution was placed on a magnetic hot plate and stirred for about 15 minutes, at 70 ^{o}C. After that, the solution was left to cool down to a temperature of 25^{o}C before use.

4.3.4 Modification of the adhesive formulation with synthesized citric acid-based PAE

Effect of the citric acid-based PAE resin (cross-linking agent) modified keratin-based adhesive on the static strength properties of the resulting particleboard samples was investigated. The adhesive was formulated, followed the method described by Adhikari et al. (2018), as shown in Table 4. 1. Based on the dry weight of the keratin powder, the formulations were varied from 5% to 30% solid content. The solid content target for the formulated adhesive was confirmed using freeze-drying process, about 10 g of the adhesive solution was weighed into a vial, and

the resultant solid content of the binder was calculated after freeze-drying according to the Equation (2) below.

$$Solute\ output\ weight = Weight\ of\ vial\ with\ dried\ adhesive - Weight\ of\ empty\ vial \quad (1)$$

$$Solid\ content\ (\%) = \frac{Initial\ weight - output}{Intial\ weight}\ x\ 100 \quad (2)$$

Table 4.1: Formulations of keratin–PAE adhesive developed by varying the solid content of the formulation

NF	PAE resin (g)	Keratin (g)	TAS (g)	DS PAE resin (g)	TS (g)	SC (%)
1	8	5	180	4	9	5
2	8	10	140	4	14	10
3	8	15	126.7	4	19	15
4	8	20	120	4	24	20
5	8	25	116	4	29	25
6	8	30	113	4	34	30

NF=Number of formulation, PAE=polyamide–epichlorohydrin, TAS=Total amount of the solution, DS=Dry solid PAE resin, TS=Total solid, SC=Solid content

4.3.5 Adhesive modification with cellulose nanocrystals and synthesized PAE resin

Keratin-based adhesive modified with cellulose nanocrystals (CNC) and PAE by varying the solid from both CNC and the PAE to have a binder with a solids content of about 20 % (Table 4. 2). The formulation was done to evaluate the effect of the addition of cellulose nanocrystals on the mechanical properties of the particleboard. The cellulose nanocrystals were pre-treated through the solvent exchange method by replacing the water with acetone before incorporating it into the bio-based adhesive. The solvent exchange process involved the addition of acetone (99 %) to the CNC suspension gradually for about 5 times, followed by centrifugation until the water was replaced by acetone. The solid content of the keratin-based binder used in this formulation was 15%.

Table 4.2: Formulations of keratin–PAE adhesive developed by varying the mixing ratio of CA-PAE and CNC

NF	PAE(g)	CNC(g)	Keratin(g)	TAS(g)	DS PAE(g)	TS(g)	SC(%)
1	5,5	0,1	4,5	35,6	2,75	7,35	20,6
2	5	0,2	4,5	35,2	2,5	7,2	20,5
3	4,5	0,3	4,5	34,8	2,25	7,05	20,3
4	4	0,4	4,5	34,4	2	6,9	20,06
5	3,5	0,5	4,5	34	1,75	6,75	19,85
6	3	0,6	4,5	33,6	1,5	6,6	19,64

NF=Number formulation, PAE=polyamide–epichlorohydrin, CNC=cellulose nanocrystals, TAS=Total amount of the solution, DS=Dry solid PAE resin, TS=Total solid, SC=Solid content

4.3.6 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) was used to study the nature of the hydrolyzed keratin protein, the citric acid-based polyamide-epichlorohydrin (CA-PAE) and the bio-based adhesive formulations. The spectra were gotten in attenuated total reflection (ATR) transmission mode over a spectral range of between 3500 cm^{-1} and 550 cm^{-1}.

4.3.7 Preparation of particleboard

The pine wood chips were milled using a Willey mill to achieve a maximum wood particle size that is between 1mm and 1.25mm. The resultant particles were oven-dried at the temperature of 60^{o}C for 24 hours to about 6% - 7 % moisture content. Each panel of 700 kg/m^3 target density was prepared; the required wood sawdust was calculated using the formula in Equation (3). Weight of the materials was calculated based on the target density of the panels after that, the required adhesive for bonding was added to the wood particles based on the 15% of the wood particles that were dried in the oven initially and then mixed by hand. After the particles have been prepared, they were laid into an even and consistent mat in a steel mould with the size 218 x 75 x 40 mm, while steel block bars of about 28mm in thickness were placed on top of the moulds for pressing into a panel thickness of approximately 10±1mm. After mat formation, the mat was pre-pressed before hot-pressing, in other to reduce the height of the board mat and to consolidate the mat for hot-pressing. After pre-pressing, the mats were hot-pressed into panels using a laboratory hot press. The temperature of the hot-press was regulated

to 180ºC at the 200 kPa pressure and press for about 15 minutes. Six types of one layer particleboard panels in triplicate were produced with the different adhesive formulation. The production process is shown in Figure 4. 2. During the manufacturing process, the hot-press temperature, pressure and time were set manually and monitored. The only parameter varied in this study was the adhesive formulation.

$$\boldsymbol{Density}\left(\frac{g}{cm}^{3}\right) = \frac{\boldsymbol{mass(g)}}{\boldsymbol{volume(cm^3)}} \qquad \textbf{(3)}$$

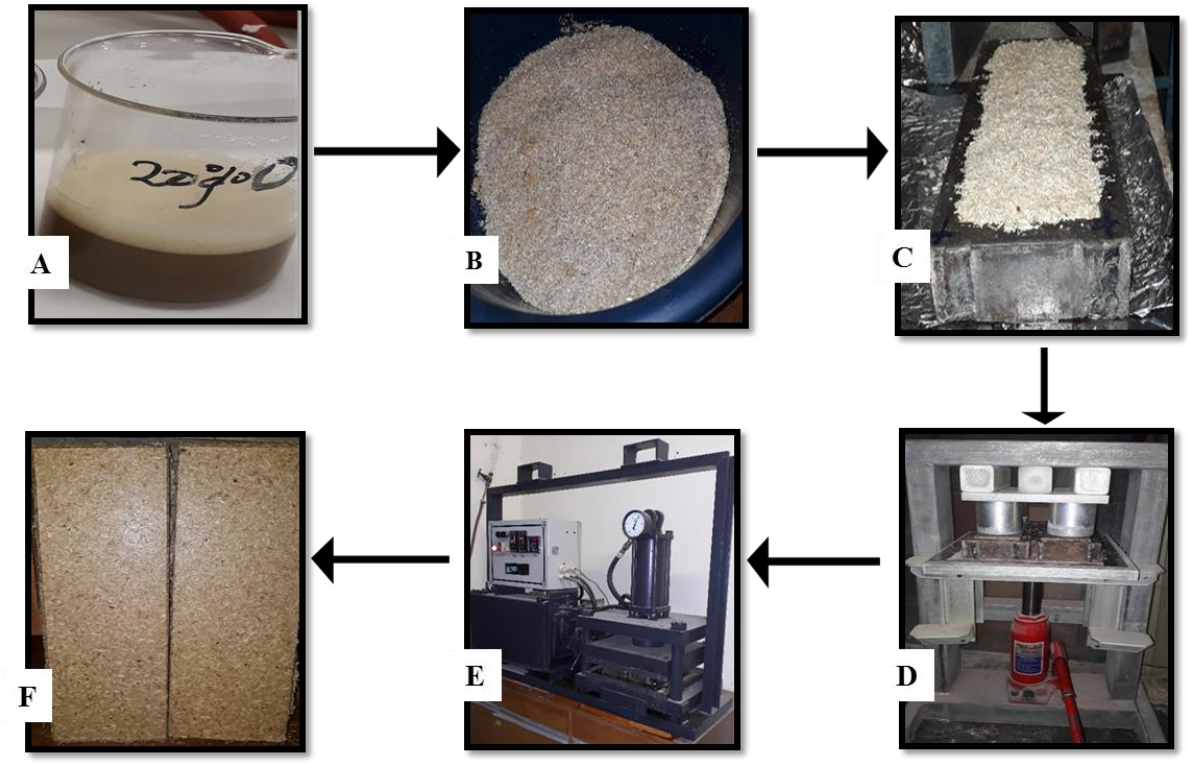


Figure 4.2 : Flow diagram of particleboard samples production procedure: (A) keratin-based binder; (B) wood sawdust; (C) board forming mould; (D) mat pre-press; (E) mat hot-press; (F) final board

4.3.8 Particleboard characterization

The mechanical strength properties of the fabricated boards were assessed to determine the suitability of the keratin-based adhesive for particleboard application and the effect of the adhesive modification on the static strength and the stability in the dimension of the experimental panels. The procedures used in these tests are based on those described in ASTM D1037 (Standard Methods for Evaluating the properties of the wood base, fiber and particle panel materials) using an Instron testing machine fitted with a 5KN load cell, operated at a rate of 5mm/min. The specimens were tested to failure: the modulus of rupture (MOR) and the

modulus of elasticity (MOE) was determined according to the formula stated in the standard (ASTM, 2013). The particleboard specimens cut into the dimensions of 75mm x 50mm were used to assess the dimensional stability of the boards. The sample thickness used for this analysis was about 9.6±1mm, which is corresponding to the design of the mould that was used. 3 samples from each of the board test series were submerged in water vertically for 2 hours. Before submersion, the weight and the thickness of the samples were measured. After 2 hours, the samples were removed, drained, and the same measurement was repeated. The specimen thickness was measured using a veneer caliper, and the thickness of the boards was calculated as the mean of three measurements. The average of the data was obtained, and the percentage thickness swelling was calculated according to Equation (4). In contrast, the percentage of water absorption of the experimental samples was derived from the weight gain after soaking in water and computed mathematically based on the percentage of the initial weight of the samples.

The influence of some factors on the formulated adhesives was assessed on the performance of the particleboard panels. The effects of the following were evaluated (i) total solid content of the unmodified keratin-based adhesive formulation, (ii) Keratin and CA-PAE resin cross-linking, and (iii) of varying cross-linker and CNC incorporation on the properties of the particleboard panels.

$$\boldsymbol{Gt = \frac{t2 - t1}{t1} \times 100} \qquad \textbf{(4)}$$

Where: Gt = Percentage of thickness swelling;

t2 = thickness of the sample before immersion (mm);

t1 = thickness of the sample after immersion (mm)

4.3.9 Statistical evaluation

The statistical analysis was carried out using a one-way analysis of variance (ANOVA) with Statistica software (Statsoft v13), and the mean was compared with the use of the post-hoc Fisher LSD test to determine the significance of formulation parameters on the measured properties of the particleboard panels.

4.4 Results and discussions

4.4.1 Pre-treatment of waste chicken feathers

The pre-treatment of waste chicken feathers was carried out to expose the polar functional groups that are buried within the folded protein structure for easy solubilization, as these desirable active functional groups will eventually interact with the wood functional groups during the bonding process (Adhikari et al., 2017).

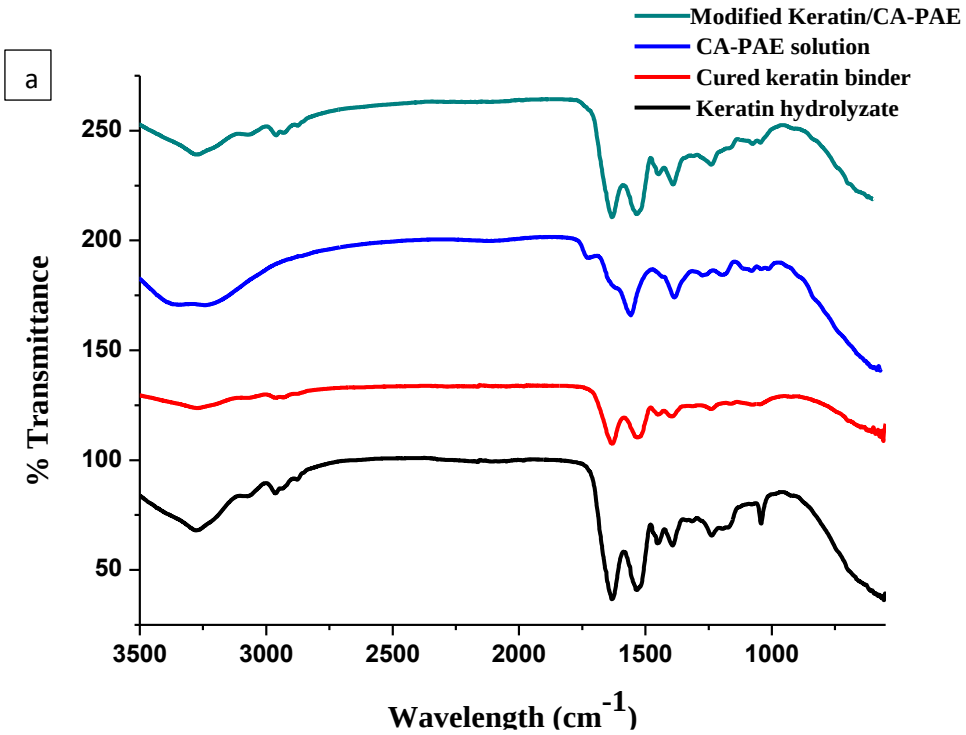
The thermochemical pre-treatment of waste chicken feathers in this study resulted in keratin hydrolyzate that afterward served as feedstock in the synthesis of keratin-based bio-adhesives. The functional group composition to illustrate the effectiveness of the pre-treatment process employed is represented in Figure 4. 3a. Furthermore, the resultant keratin protein showed a molecular weight of between 3-15 kDa using both low and medium protein molecular weight markers through the SDS-page gel electrophoresis method; this is a common molecular weight characteristic for keratin protein hydrolysate from chicken feathers (Fagbemi et al., 2020). The hydrolyzate recovered from the waste chicken feathers through the alkaline hydrolysis pre-treatment had sufficient protein functionality, as confirmed by the FTIR analysis (Fig 4. 3a), which is essential for both bio-adhesive formulation and further modification. The presence of these protein functionalities leads to the intended reaction of protein hydrolyzates with the citric acid-based polyamide-epichlorohydrin as the cross-linker and, eventually, the reaction and adhesion by the wood particles. Additionally, the extracted keratin hydrolyzate was able to dissolve well in mild alkaline solution with less viscosity in resin systems. This property is desirable for the fabrication of quality wood bio-adhesives (Mekonnen et al., 2014).

4.4.2 The reaction of hydrolyzed keratin protein with CA-PAE

The FTIR spectra (Figure 4. 3 a&b) display different absorption bands of functional protein groups in this analysis, with various stretching vibrations of -CH2, = C-H, -C-H, -CONH-, -OH and NH groups. The citric acid-based polyaminoamide (CA-PAE) cross-linker shows typical absorption bands of N–H, C=O, -CONH- and C-H at 3307, 1631, 1547 and 1272 cm^{-1} respectively. Functional groups such as –C-H, -CONH-, -OH and NH groups usually present absorption peak above the wavenumber of 1000 nm in the non-fingerprint region (Li et al., 2004). The absorption bands at 2944, 2872, and 1465 cm^{-1} correspond to the asymmetrical, symmetrical stretching vibration and bending vibration of CH_2, respectively (Liu et al., 2004, Li et al., 2004). Likewise, spectra of CA-PAE solution, cured keratin binder and keratin hydrolysate were similar. For instance, the keratin hydrolysate spectrum showed the presence

of carbonyl groups (C=O stretching, absorption in the range 1650-1590 cm^{-1}), amino group (NH stretching above 3000 cm-1 and NH bending in the range 1550-1485 cm^{-1}) with hydroxyl groups (OH stretching above 3000 cm^{-1}). Moreover, the FTIR spectrum for the cured keratin-based binder modified with CA-PAE resin under the temperature of 180℃ showed a similar absorption band with the keratin hydrolysate. A probable justification for the observation might be the reaction of the hydroxyl group, carboxyl, and the amine group of the keratin protein hydrolyzate with the residual amine functional group of the CA-PAE cross-linker (Adhikari et al., 2016).

The absorption bands suggest the incorporated citric acid-based polyaminoamide has not negatively impacted the structure of the synthesized adhesive. Instead, each component retained many of its inherent desirable properties for which they were incorporated. It is known that under very high-temperature many reactions happened in keratin-based adhesive cross-linked with CA-PAE, such as the homo-crosslinking, which occurred between the azetidium group in PAE and the remaining secondary amine within the CA-PAE resin. Besides, the interaction among the azetidinium group of CA-PAE and the active hydrogen groups of protein, like the carboxyl, hydroxyl and amino functional groups, results in co-crosslinking of the resins (Xi et al., 2020).



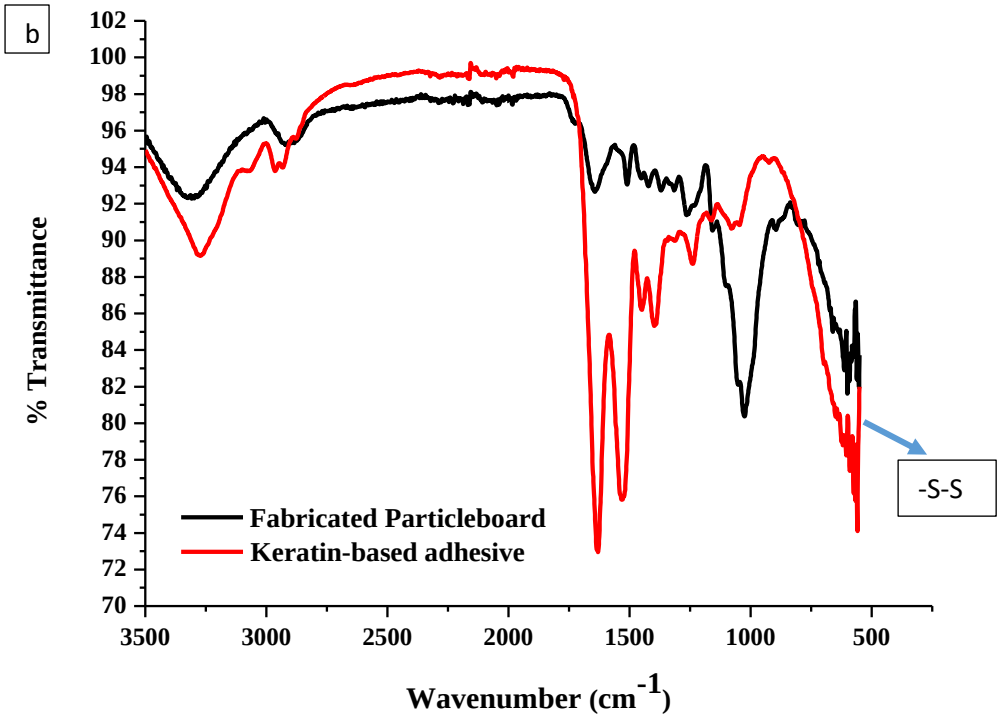


Figure 4. 3a & b (a): FTIR spectra of a keratin protein hydrolysate, cured keratin binder, citric acid-based polyamide-epichlorohydrin (CA-PAE) and keratin binder modified with CA-PAE resin. (b): Spectra showing the presence of disulphide (-S-S) in the particleboard sample

4.4.3 Performance evaluation of particleboard panels

Density

Tables 4. 3 and 4. 4 show the average density of the panel bonded with an unmodified keratin-based binder and modified keratin-based binders. The average density ranges between 690.88 kg/m^3 to 719.24 kg/m^3 for the unmodified keratin-based binder and 699.80 kg/m^3 to 727.76 kg/m^3 for the modified keratin-based binders respectively. The target density for the experimental particleboard in this work is 700 kg/m³. The obtained empirical density varies compared to the target density. The variation in empirical density can be attributed to the fact that the density of mat-formed hot-pressed particleboard is vertically not uniform in the thickness (Zhang et al., 2021). Particleboard density has a significant influence on the performances of the composites and affects almost all the panel properties, including strength properties (Youngquist et al., 1997). As it was stated by the American National Standards Institute (ANSI) (2016), particleboard between 0.60 and 0.8g/cm^3 is classified as medium density panels and density >0.8g/cm^3 as high-density panels (ANSI, 2016). Hence, the density of the panels produced in this work can be classified as medium density particleboard panels.

Table 4.3: Panel density of the unmodified and the modified keratin-based binder

	Density(kg / m³)	
Solid content (%)	**Keratin binder panels**	**Keratin/CA-PAE panels**
5	699,50	699,80
10	698,24	716,18
15	690,88	703,02
20	699,71	705,63
25	702,80	720,60
30	719,24	727,76

Table 4.4: Panel density of the CA-PAE and CNC modified keratin-based binder

Mixing ratio (Panels)	**1**	**2**	**3**	**4**	**5**	**6**
Density (kg / m³)	712,75	710,42	695,92	705,55	705,55	703,22

Impact of unmodified keratin-based binder solid content on static bending properties of the particleboard

The modulus of rupture (MOR) and the modulus of elasticity (MOE) of the fabricated particleboard panels are presented in Figures 4. 4a and 4. 4b. The average values for the MOR of the unmodified keratin-based binder panels range between 3,17 MPa to 6,52 MPa, respectively. Though the boards made with binder consist of 15, 20, 25 and 30%, solid content shows no significant difference. However, there is a significant difference among panels made with 5 and 10% solid content. The highest MOR was recorded with the board made with the adhesive formulation that contains 25% solid content. The literature revealed that the solid content of 20–35% was commonly used for the preparation of protein-based binders for wood product fabrication (Adhikari et al., 2018a, Xu et al., 2020). The bending strength of a material is determined through the modulus of rupture (MOR) and the modulus of elasticity (MOE) of the material (Youngquist et al., 1997). The MOR is the highest bending stress of material in flexure or bending, while MOE is the resistance to deformation or stiffness. Besides, they are used to compare one material to another as well as a fundamental determinant for particleboard application.

The MOE result of the panels fabricated with the unmodified keratin-based binder are shown graphically in figure (4. 4b); the average MOE values range between 644,73 MPa to 1184,34

MPa, respectively. The panels produced with 5 and 15% resin solid content showed a significant difference. In comparison, there is no significant difference with those prepared with 10 and 20 % solid content, respectively. Likewise, those made with 25 and 30% solid content showed no significant differences. The panel made with the formulation that contains 5% resin solid content shows the lowest MOE while the board made with the adhesive formulation that contains 30 % solid content has the highest MOE. The difference in the static bending strength properties of the boards as a function of binder formulation could be ascribed to the extent of the binder curing, the chemical bond that is formed with the wood particles, besides, the ability of a cured resin to spread (Mekonnen et al., 2014). However, according to ANSI (2016), the panels manufactured with 25 and 30% adhesive formulation satisfied the required specification; it could be considered under the grade 1-L-1 panel specification (ANSI, 2016).

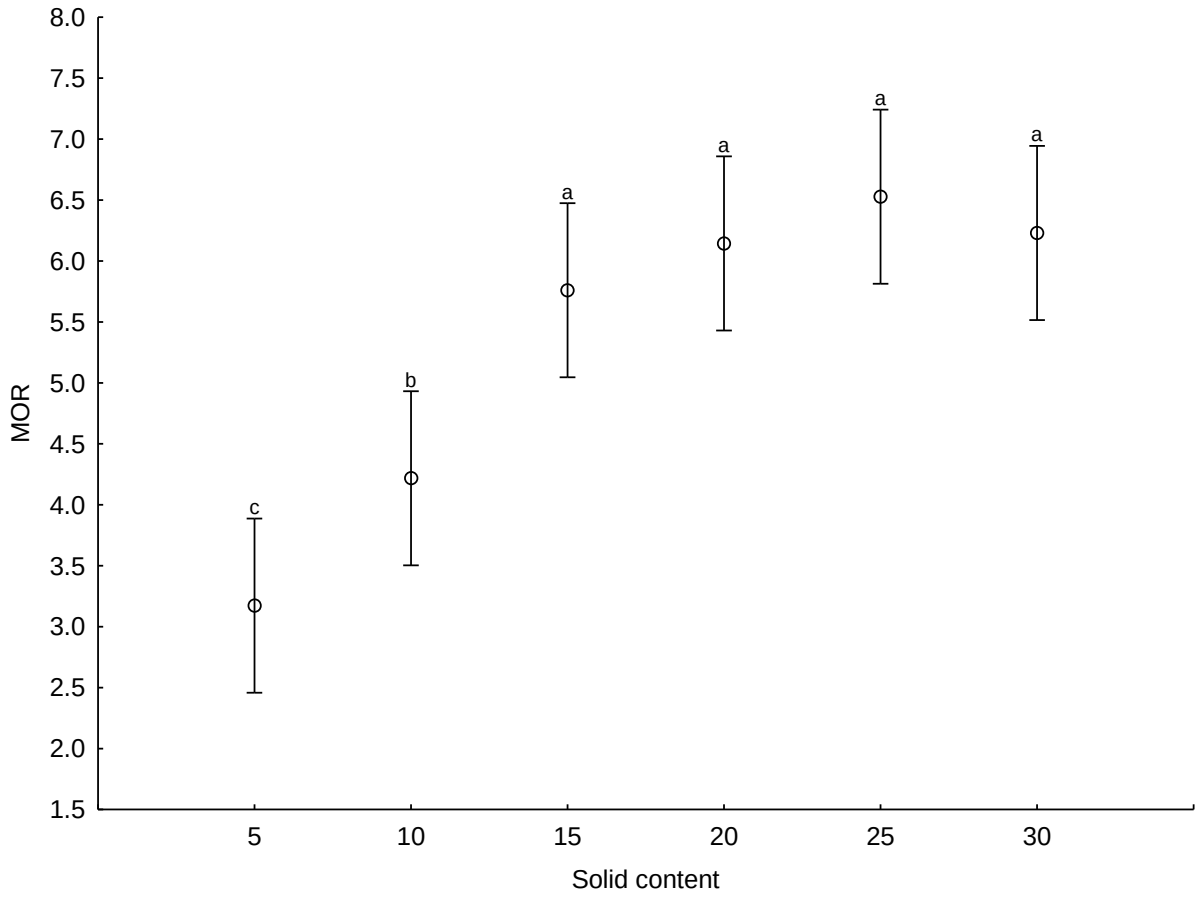


Figure 4.4a: Effect of total solid content on the modulus of rupture of the particleboard samples produced with the unmodified keratin-based binder

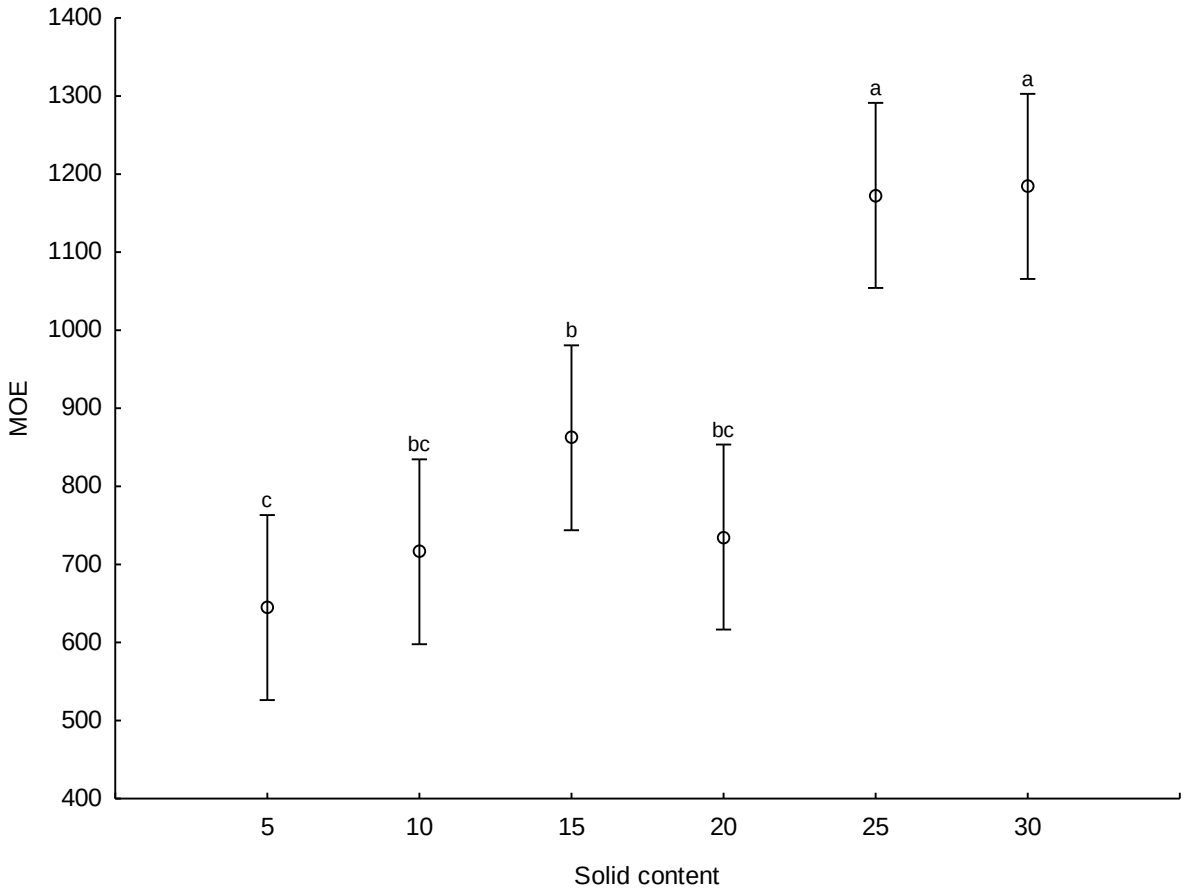


Figure 4.4b: Effect of total solid content on the modulus of elasticity of the particleboard panels produced with the unmodified keratin-based binder

Effect of the cross-linking agent on the particleboard panel performance

The MOR and MOE for citric acid incorporated polyamide-epichlorohydrin keratin-based adhesive are shown in Figures 4. 5a and 4. 5b. The average MOR value ranges from 3.76 - 8.01 MPa. There are not many differences among most of the formulations evaluated, except 5% and 30% adhesive formulation, with 5% solid content adhesive panel having the lowest MOR. In comparison, the panel with adhesive of 30% solid content produced the highest MOR. Expectedly, the keratin–PAE incorporated adhesive panel showed a considerable improvement in the bending strength (MOR) compared with the unmodified keratin-based adhesive board. This improvement in the keratin–PAE adhesive panel can be because of the combined influence of chemical bonding of keratin protein and CA-PAE resin molecules, also the reactions of the cross-linked products with the wood particles functional groups (Zhang et al., 2018). The literature revealed that chemical bonding results in the development of hard and three-D bonds

of polymers that are linked via covalent linkages and do not allow the polymer chains from creeping during mechanical testing (Adhikari et al., 2016, Xi et al., 2020).

The values for the MOE are represented in Figure 4. 5b, and the MOE values range from 472, 14 to 1118,04 MPa. Most of the fabricated panels show no significant difference except the board fabricated with 5% solid content adhesive formulation. A decrease in the MOE performance of the keratin–PAE cross-linked adhesive panel was observed compared to the MOE of the unmodified keratin-based binder board for most of the formulations (5%, 10%, 25% and 30%) evaluated. The cause for this decrease is currently unclear; therefore, further research will be required to ascertain the reason for this observation.

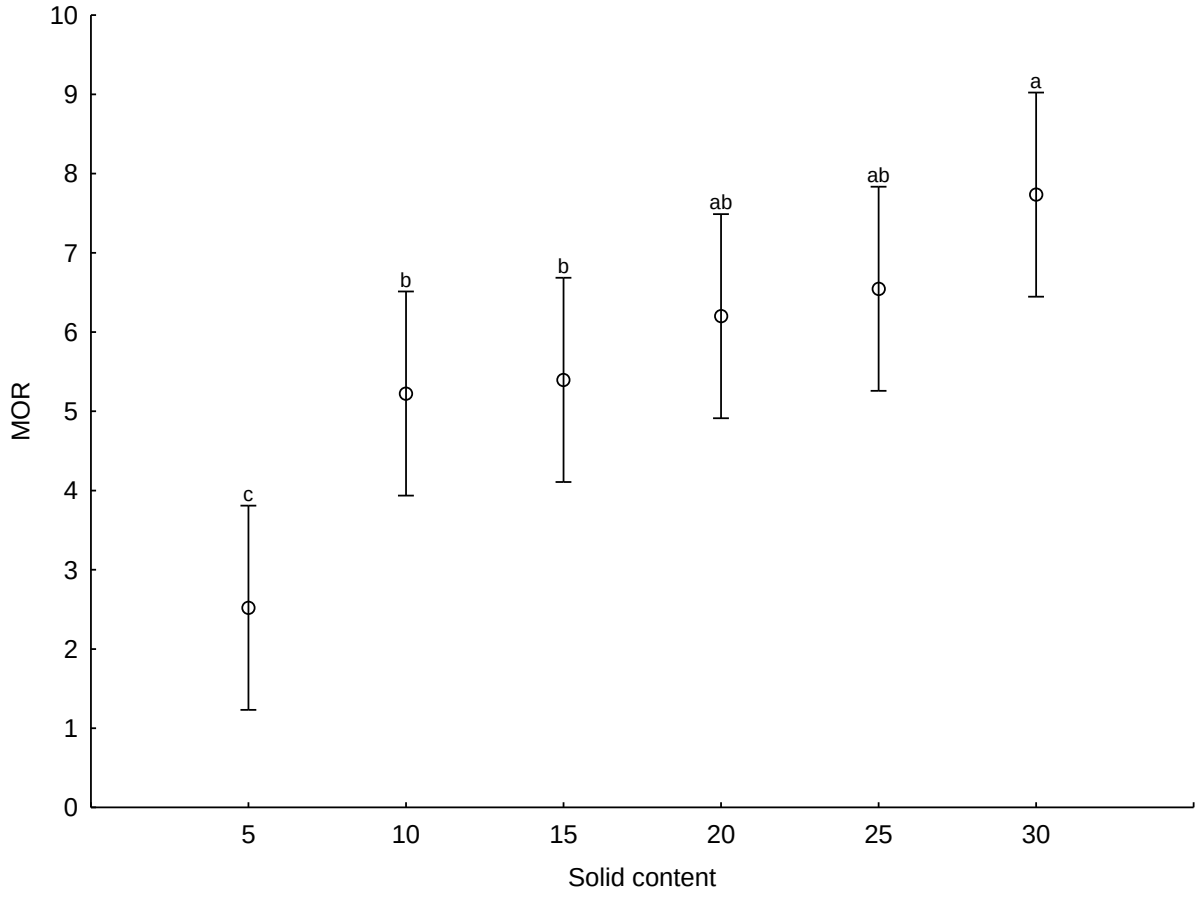


Figure 4.5a: Effect of the cross-linking agent on the modulus of rupture of boards fabricated with CA-PAE cross-linked keratin-based binder

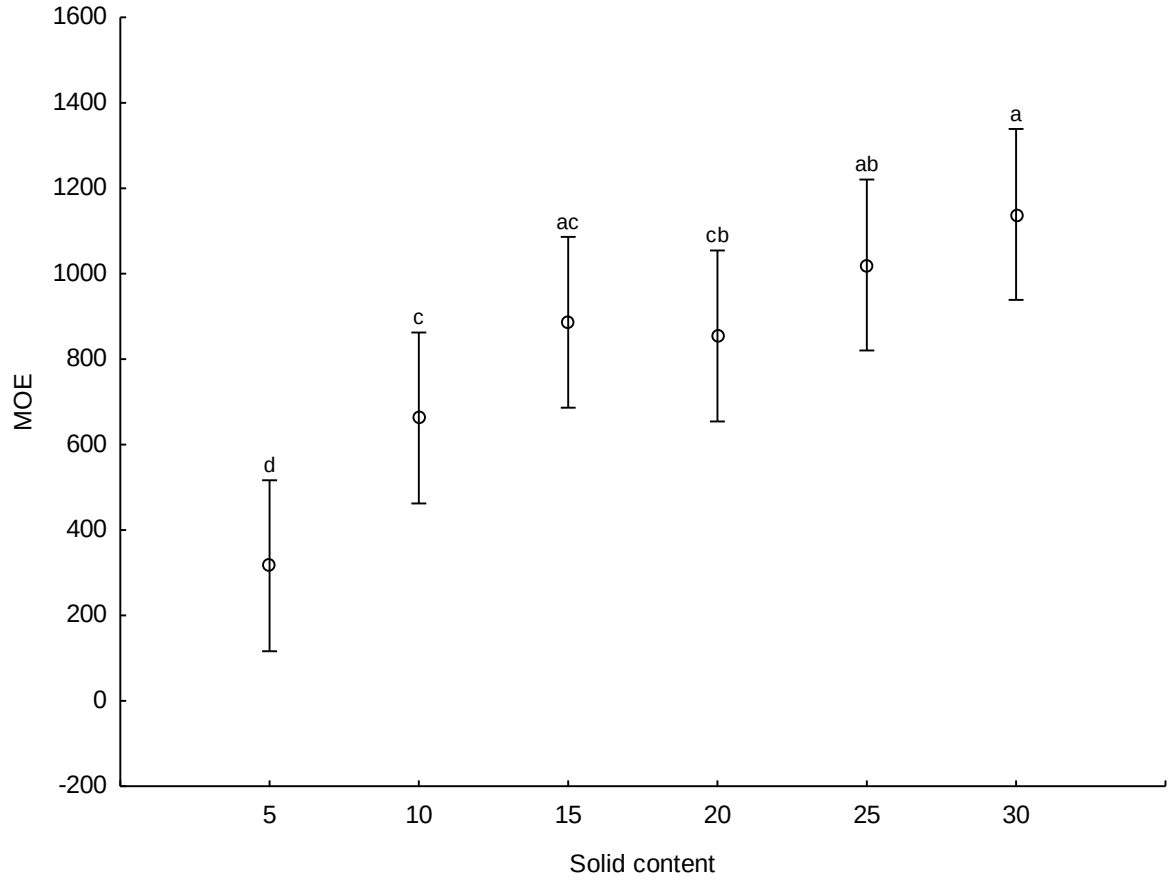


Figure 4.5b: Effect of the cross-linking agent on the modulus of elasticity of boards produced with CA-PAE cross-linked keratin-based binder

Effect of varying cross-linker and addition of CNC on the particleboard panel performance

The values of the MOR and the MOE data are presented in Figures 4. 6a and 4. 6b. Except for the least mixing ratio, the obtained data on the MOR of the cross-linker-CNC incorporated particleboard panel showed there are no substantial differences in the MOR values for most of the adhesive formulations implemented. The values range from 5,10 to 6,76 MPa. The highest MOR of 6,76 MPa was obtained in mixing ratio one formulation, which has 2,75 g and 0.1 g of CA-PAE and CNC, respectively. The result obtained from this formulation is of advantage because adhesive with low solid content have economical benefits over the adhesive with high solid content (Rosseto et al., 2019).

Furthermore, the literature reveals binders1 with very high solid content are highly viscous, leading to weak interaction of chemical and functional components and, consequently, reduction in bond strength due to the lack of effective mechanical interlocking (Adhikari et al., 2018b, Cheng and Sun, 2006). Besides, previous research work shows that the addition of

cellulose nanocrystals to wood adhesives could contribute significantly to their bond performance, thereby improve the strength properties of the panels (Mahrdt et al., 2016, Veigel et al., 2012). In line with the present study, the incorporation of cellulose nanocrystals (CNC) to particleboard adhesives had dual advantages; the inclusion of cellulose nanocrystals at the lowest concentration resulted in the highest modulus of rupture (MOR).

The MOE values of the cross-linker-CNC incorporated particleboard panel performance (Figure 4. 6b) ranges from 790, 01 to 1232,76 MPa. The panel with mixing ratio one shows the highest MOE of 1232,76 MPa. The cross-linker-CNC incorporated particleboard panel adhesive formulations showed an improvement in particleboard strength properties compared to other fabricated panels: the unmodified keratin-based adhesive formulation and the CA-PAE cross-linker without the addition of CNC. This improvement in cross-linker-CNC incorporated particleboard panel strength might be attributed to the fact that the addition of CNC worked as a filler in the adhesive formulation to improved the strain to failure and the toughness of the adhesive and consequently the performance of the adhesive which contributed to the mechanical strength of the fabricated particleboard panel(Wang et al., 2019).

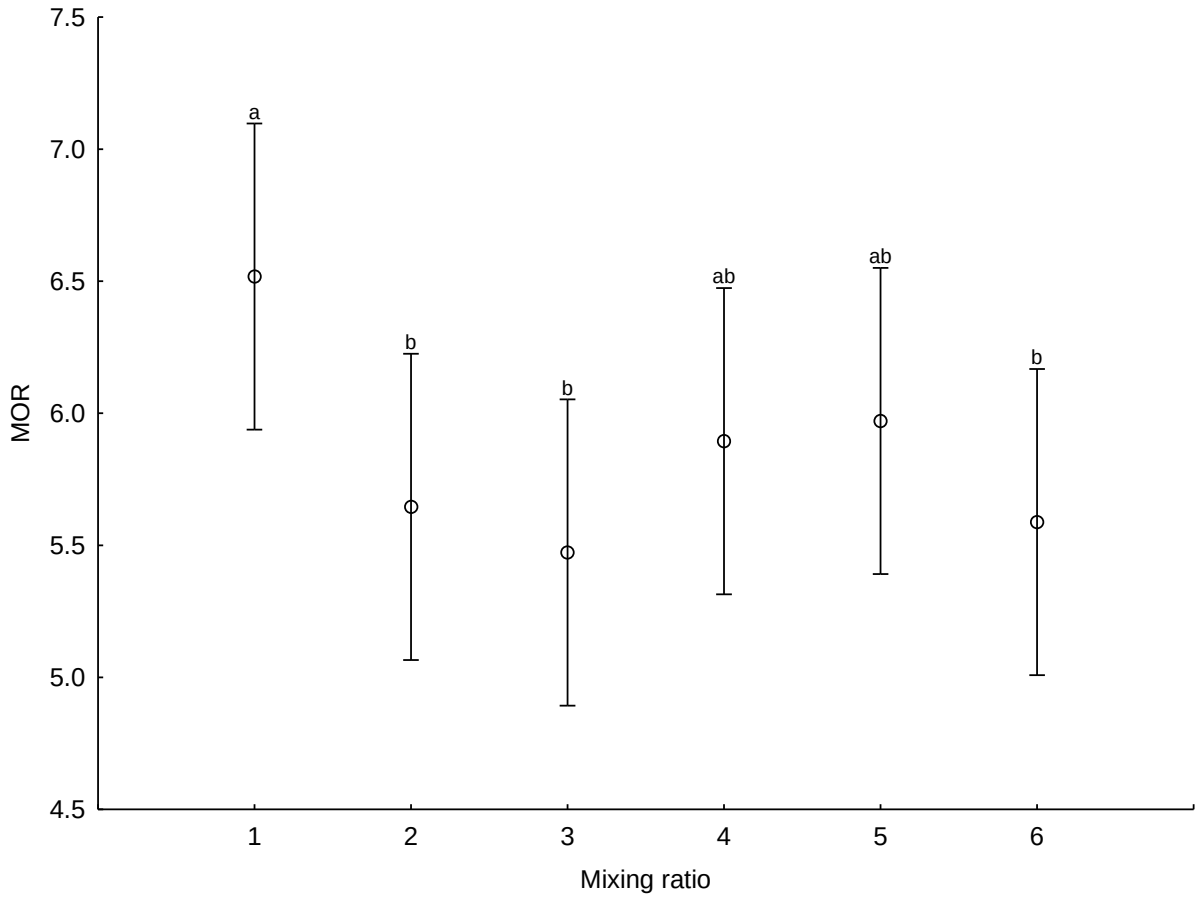


Figure 4.6a: Effect of cellulose nanocrystals inclusion and mixing ratio on the modulus of rupture of panels manufacture with the CA-PAE/CNC cross-linked keratin-based binder

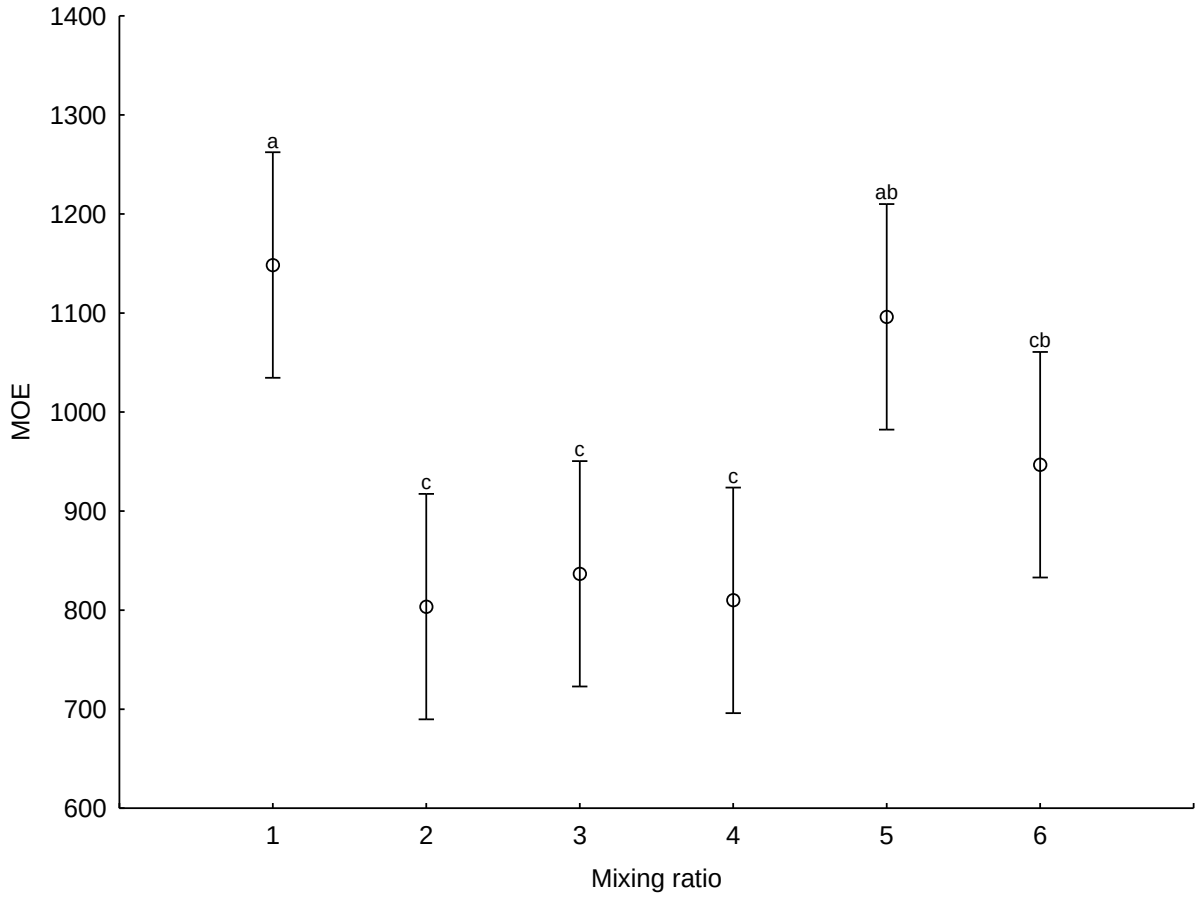


Figure 4.6b: Effect of cellulose nanocrystals inclusion and mixing ratio on the modulus of elasticity of panels produced with the CA-PAE/CNC cross-linked keratin-based binder

Thickness swelling of the particleboard panels

Figures 4. 7a and 4. 7b show the graphical representation of percentage thickness swelling of the particleboard panel obtained from the unmodified keratin-based binder, the CA-PAE cross-linked-keratin binder and the CA-PAE-CNC cross-linked keratin binder. The percentage of thickness swelling obtained after two hours submersion for all the particleboard panels were very high. Thus, it could not meet the ANSI minimum requirement (ANSI, 2016). Therefore, the fabricated particleboard in the present study will mainly be suitable as core material for doors, indoor and dry condition applications (Mahieu et al., 2019). Particleboard is hygroscopic and not dimensionally stable because it is made out of wood particles; therefore, it has hygroscopic properties like wood when exposed to water vapour or liquid water (Balducci et al., 2020). Although the thickness swelling observed in the present study, might be highly corresponded to the moisture-resistance of the adhesives formulated. The poor performance in water resistance could be attributed probably to the non-synergetic interaction of the active functional groups of the hydrolyzed keratin proteins with the molecules of water (Saha et al.,

2019). This reaction is because the hydrogen chemical bonding gives excellent static strength performance when in a dry condition; however, the chemical bonds formed among the adhesive formulated and wood particle substrate were ruptured because of their interaction with the water molecule (Li et al., 2019). The presence of partial protein in CA-PAE cross-linker network chains in the formulated binder could also result in the release of the adhesive from the particleboard into the water. This process would create cavities that will later permit further water circulation in the particleboard (Mekonnen et al., 2014). This reaction would eventually lead to lots of absorption of moisture in the boards and, thus, higher thickness swelling percentage (Mekonnen et al., 2014).

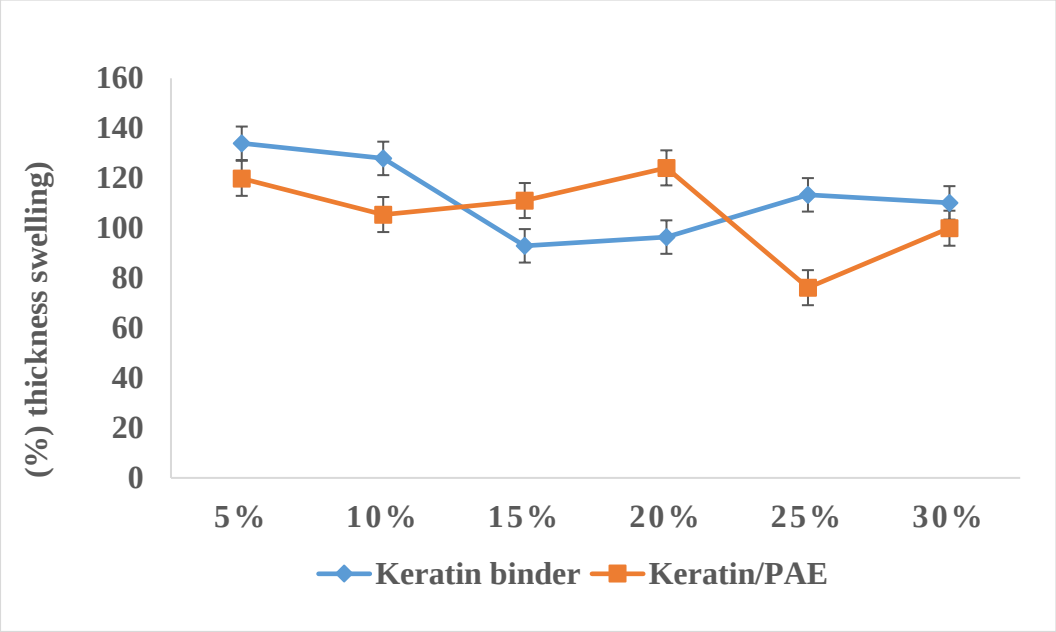


Figure 4. 7a: Comparison of thickness swelling of particleboards made with unmodified and cross-linked adhesive formulations

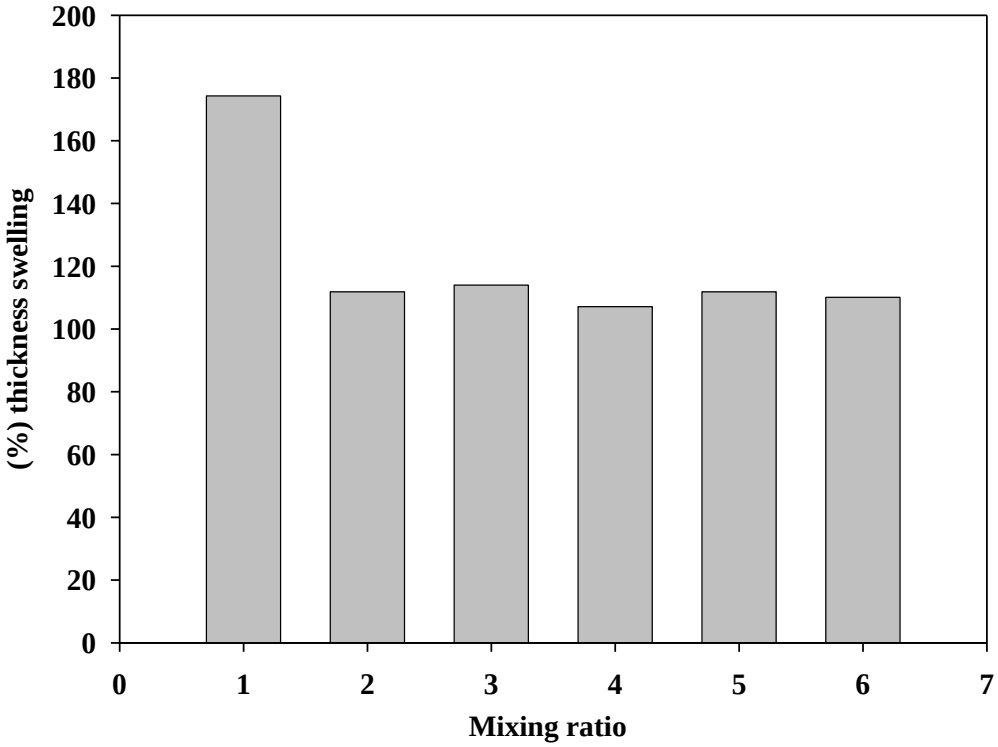


Figure 4. 7b: Effect of cellulose nanocrystals addition on the thickness swelling of the particleboards made with keratin-based binder modified with CA-PAE and CNC adhesive formulations

4.5 Conclusions

In this study, extracted keratin hydrolysate from waste chicken feather biomass was utilized as a raw material in wood adhesives for particleboard production. The FTIR results confirmed the covalent bonding of the citric acid-based polyamide-epichlorohydrin azetidinium functional group and the hydroxyl groups of the keratin protein that resulted in an effective co-crosslinking product. The formulated adhesives with 20, 25, and 30% solid content met the ANSI A208.1 requirements. Similarly, the mechanical strength performance of the fabricated particleboard using the formulated adhesives was promising. The addition of cellulose nanocrystals (CNC) in the adhesive formulation with 20% solid content enhanced the strength performance of the particleboard panel. However, due to the hydrophilic characteristics of some functional groups of hydrolyzed keratin proteins, the resistance to water of the adhesives produced did not satisfy the ANSI A208.1 specifications for structural applications. The particleboard manufacture in this research work is suggested to be utilized as a solid door core, indoor materials and dry environment applications. The valorization of the waste chicken

feather to value-added products such as wood composites bio-adhesives could be expanded to other slaughterhouses proteinaceous waste as well as their commercialization.

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Disclosure statement

The authors state that, no conflicts of interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER 5

PAPER 4: A MANUSCRIPT PAPER SUBMITTED AND UNDER REVIEW

BENEFICIATION OF WOOD SAWDUST INTO CELLULOSE NANOCRYSTALS FOR APPLICATION AS A BIO-BINDER IN THE MANUFACTURE OF PARTICLEBOARD

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Abstract

This study reports on the beneficiation of wood sawdust into cellulose nanocrystals (CNC) for application as a binder in the manufacture of particleboard. The cellulose nanocrystal from wood sawdust was extracted using acid hydrolysis and an oxidizing agent. This was used as it is for particleboard fabrication. Likewise, after cross-linking with several crosslinking agents, viz., CNC-glyoxal, CNC-hexamine, CNC-polyamide-epichlorohydrin, and CNC-polyethylene to make cross-linked binders. The tensile strength performances of the particleboard panels were determined by modulus of rupture (MOR) and elasticity (MOE). Characterization of the CNC by Fourier transform infrared spectroscopy **(**FTIR) confirmed cellulose functional structures in the CNC. X-ray diffraction (XRD) analysis indicated high crystallinity index (78%) of the CNC and typical nano dimensions of 2.1–10 nm for diameter and 150-350 nm for length as revealed by the transmission electron microscope (TEM). Thermogravimetric analysis (TGA) and differential thermogravimetric (DTG) analyze high thermal stability (250

– 400 ℃) of the CNC. Significant mechanical strength performances of the particleboard panels were evident in the modulus of rupture (MOR) and the modulus of elasticity (MOE) values that were determined. The panels met grade 1-L-1 specification of the American National Standards Institute A208.1. The incorporation of cross-linking agents enhanced the static bending and bonding strength properties of the formulated bio-binders. It can be concluded that cellulose nanocrystals extracted from waste wood sawdust could be considered for use as a binder to produce environmentally friendly wood composites bio-adhesives and particleboard panel fabrication.

Keywords: *Sawdust; cellulose nanocrystals; bio-binder; particleboard; organic cross-linkers*

5.1 Introduction

Particleboard is an engineered wood product in which particles of wood are bonded together using bonding agents to form a panel. It is most fabricated using a synthetic formaldehyde-based resin adhesive. Consequently, these manufactured particleboard panels emit formaldehyde during manufacturing and application, resulting in environmental pollution and a negative impact on human health (Mao and Kim, 2013). Organic polymers of either natural or synthetic origin are the primary chemical ingredients in the formulation of all wood adhesives and the crosslinkers used to improve these adhesives (Vick, 1999). The industrial production of most adhesives is derived from petroleum resources. However, the environmental health concerns, increasing oil prices, and limited oil reserves have given rise to research into cheap, biodegradable, sustainable, renewable, and abundantly available green materials such as cellulose nanocrystals (CNC) (Tesfaye et al., 2018) for the production of adhesives. Although several cross-linkers such as polyaminoamide–montmorillonite, 2-hydroxy-3-chloropropane groups and polyamide amine-epichlorohydrin (PAE) resin have been investigated (Liu et al., 2004, Zhao, 2017), there is a lack of information on the application of cross-linkers in general to enhance the properties of CNC formulated adhesives. Cross-linkers such as citric acid-based polyamide-epichlorohydrin, glyoxal, hexamine and polyethylene could improve the functionality of bio-adhesives in their application as wood or particleboard binders (Amini et al., 2017, Tayeb et al., 2018). The use of crosslinking agents to formulate bio-based adhesives for particleboard bonding applications has increased in recent years (Adhikari et al., 2018b). The presence of multifunctional groups or reactive sites in cross-linkers is the basis for their usage in binders to obtain specific or desired properties in particleboard binders (Adhikari et al., 2017a). Some of these cross-linkers properties include their water-resistance improvement, enhanced cationic characteristics, and viscosity of the binder that meet industry requirements. For instance, polyamide amine epichlorohydrin (PAE) are polymeric resins that react with nucleophilic groups such as amines, hydroxyls and carboxyl groups (Obokata et al., 2005). The reactions with such functional groups result in the PAE resins being used in wet strength papers because of their resultant characteristic wet strength addition into the paper (Wågberg and Bjőrklund, 1993, Espy, 1995). Crosslinking can lead to improved molecular weights with three-dimensional rigid networks of polymers connected through covalent linkages (Adhikari et al., 2018b). The need to understand the effects of crosslinking agents on the shear or mechanical strengths of the resultant particleboard has continued to receive attention in recent studies (Amini et al., 2017, Alawode et al., 2020, Solt et al., 2019). Reviews of literature on bio-based adhesives revealed that the approach of

chemical modification with suitable crosslinking agents have resulted in bio-based wood adhesive formulations with enhanced binder strength and moisture resistance (He, 2017, Solt et al., 2019). However, there is little information on crosslinkers such as hexamine, polyethylene, glyoxal and citric acid-based polyamide-epichlorohydrin to enhance the functionality for cellulose nanocrystal as bio-adhesives. Binder properties such as penetration ability of uncured adhesive and bond strength may be influenced by intermolecular interactions and the extent of polymer crosslinking (Adhikari et al., 2018b). Hence, it is imperative to understand the interaction between the cross-linkers and binder components, and consequently, how they can be improved.

An exciting natural biopolymer is cellulose nanocrystals (CNCs), which can be extracted from native cellulose. Cellulose from plant biomass is one of the most abundant and renewable bio-based polymer on the planet (Wohlhauser et al., 2018). It is one of the primary chemical components of plant and wood cell walls and contributes about 40-45% of the total dry mass (Rowell et al., 2005). Hence, cellulose can be found abundantly in waste with woody cell walls such as sawdust. Wood sawdust is a waste product generated from the wood products industry. During the processing of the wood in sawmills, for example, about 10% of sawdust is produced as a waste (Olufemi et al., 2012). Currently, a small amount of the sawdust is utilized for energy, wood compost or valorized as particleboard. However, most of the sawdust is either stockpiled or landfilled, causing additional environmental problems.

Cellulose is widely used in many industries, such as paper making, textile, construction, and packaging. Cellulose comprises cellobiose units, consisting of two β (1-4)-glycosidic linked glucose molecules as the building blocks (Müller et al., 2007). It is responsible for the strength of the wood fiber because of its high degree of polymerization and linear orientation (Klemm et al., 1998). Moreover, cellulose consists of crystalline domains with highly ordered microfibrils, and amorphous domains randomly arranged microfibrils. The amorphous areas are relatively easy to remove by acid hydrolysis and results in highly crystalline cellulose in the nanoscale range that is referred to as cellulose nanocrystal (Gibril et al., 2018). They are rigid, rod-like particles with a width of several nanometres and lengths of up to hundreds of nanometres (Tang et al., 2017).

Cellulose nanocrystals (CNC) are unique nanomaterials with several notable mechanical, optical, chemical, electrical and rheological properties (George and Sabapathi, 2015). Their size, shape and charge may lead to unique behavior in solution. The high chemical reactivity of the CNC surface makes them customizable for various modifications and applications such as adhesive and reinforcement agents. Moreover, CNC is heat stable, which allows them to be

used in high-temperature applications such as wood binders. Hence, they could serve as a sustainable and environmentally friendly wood adhesive to improve the performance of the binder application.

Currently, the studies on the potential of cellulose nanofibrils as a binder have been reviewed in recent literature (Tayeb et al., 2018, Tajvidi et al., 2016). CNF is nano-fibrils isolated from cellulose-containing materials like wood through high-pressure, high temperature and high-velocity impact homogenization, grinding or micro fluidization. CNFs contain amorphous cellulose and are not as highly crystalline as CNCs. Veigel et al. (2012) investigated the influence of reinforcing urea-formaldehyde (UF) adhesive with cellulose nanofiber (CNF) on the mechanical properties of particleboard and oriented strand board (OSB). It was reported that particleboards made with urea-formaldehyde comprising 1 wt% of CNF displayed a lower thickness swelling, better internal bond and bending strength than boards produced with pure UF. The reinforcing effect of CNF was more evident for OSB, showing a significant improvement in the strength properties (Veigel et al., 2012).

Similarly, Kojima et al. (2013) investigated the use of cellulose nanofibers (CNF) as reinforcement in the wood flour (WF) board to replace synthetic adhesives. The authors observed that the three-dimensional binding effects of the CNF improved the physical and mechanical properties of the wood flour board (Kojima et al., 2013). In other studies, Amini et al. (2013) reported the use of CNF as a binder to produce particleboard. They found that particleboard panels with low densities perform well while those with medium density met the standard specified for internal bonding.

Furthermore, the addition of polyamide-epichlorohydrin as a crosslinking agent improved the moisture resistance of the boards (Amini et al., 2017). Nevertheless, many of the current particleboard production technologies using CNF are still faced with several challenges. These include the need to reduce the moisture content in the mat before the hot press, improve the bonding capacity and shear strength of binders, and, overall, improve the water-resistance of fabricated particleboard. There is also a need for economical and environmentally friendly adhesives. Despite the availability of data on the use of CNF in the bio-adhesive formulation, very little is known about particleboard-binder formulation using CNC on its own or reinforced with citric acid-based polyamide-epichlorohydrin, glyoxal, Hexa(methoxymethyl)melamine (Hexamine) and polyethylene cross-linkers for the fabrication of sawdust-particleboards.

The main objective of this study, therefore, was to evaluate CNC produced from wood-sawdust CNC for use in high-performance binders for the fabrication of particleboard. The effect of cross-linking agents on the particleboard mechanical strengths was also assessed.

5.2 Materials and methods

Merensky Timber, a subsidiary of Hans Merensky holdings, Johannesburg, South Africa, supplied the wood sawdust samples of *Pinus patula* to produce one layer particleboard in this study. Cellulose nanocrystals were extracted from *Eucalyptus grandis* wood sawdust using an in-house method developed at the Bio-refinery Industry Development Facility (BIDF). The summary of the methodology is shown in Figure 5. 1. Glyoxal (40 wt.%) and polyethylene were purchased from Sigma Aldrich, South Africa. Hexamine was obtained from United Scientific, South Africa, whereas citric acid-based polyamide-epichlorohydrin was synthesized at the BIDF. Bondtite (PTY) Limited, South Africa, supplied Bondtite, a commercial resin used for the control experiments.

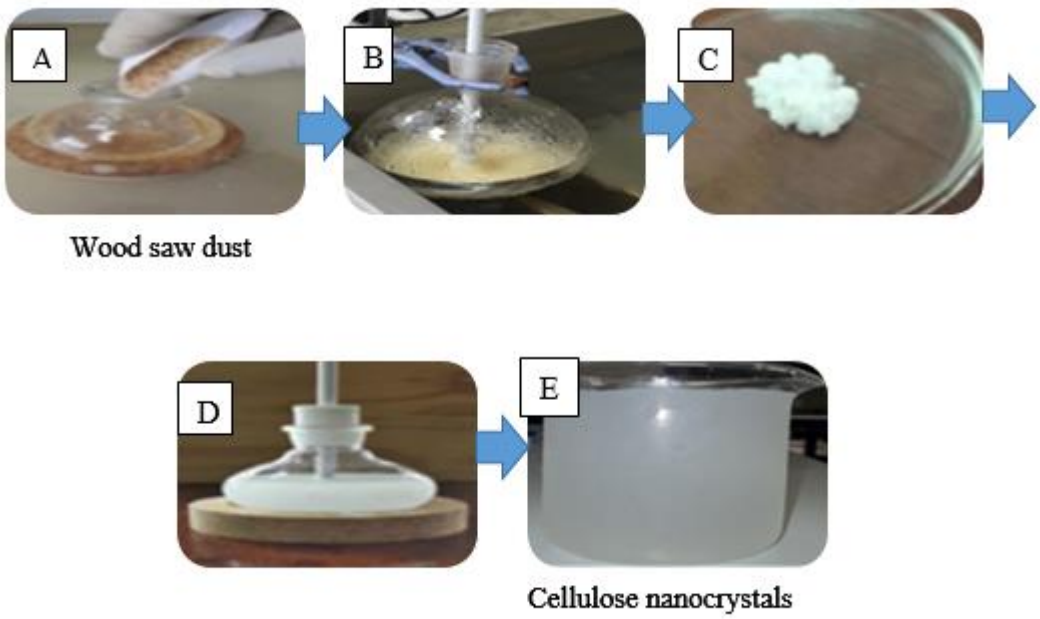

Figure 5.1: Schematic overview of the cellulose nanocrystals extraction process.

5.3 Characterization of cellulose nanocrystals

5.3.1 Fourier transform infrared spectroscopy (FTIR)

Structural and compositional characteristics of CNC were analyzed on a Spectrum 100 Fourier transform infrared (FTIR) spectrophotometer (Perkin Elmer, USA). Attenuated total reflection (ATR) transmission mode over a spectral range of 4000-400 cm^{-1} at a resolution of 2 cm^{-1} was used to obtain the spectra.

5.3.2 Transmission electron microscope (TEM)

The morphology of the cellulose nanocrystals was obtained using a JEOL 1010 transmission electron microscope (TEM, JEOL, USA). Dilute suspensions of CNC approximately 100 µl were placed on formvar-coated grids and stained with a 2% uranyl acetate solution for 10 min. Image capture was performed at 100 kV at varying magnifications (20 000-200 000x). The micrograph was analyzed using Image J software.

5.3.3 X-ray diffraction (XRD) analysis

X-ray diffractograms were obtained using a multi-purpose X-ray diffractometer D8-Advance from Bruker (BRUKER AXS, (Germany)) operated in a continuous $\theta - \theta$ scan in locked coupled mode with Cu Kα x-rays (λ = 1.5406 Å) radiation. The measurements run within a range in 2θ defined by a typical step size of 0.034° in 2θ. A position-sensitive detector, Lyn-Eye, was used to record diffraction data at a speed of 0.5 sec/step, which is equivalent to an active time of 92 sec/step for a scintillation counter.

The crystallinity index (CI) was calculated according to the empirical equation recommended by Segal et al. (1959) (Eq.1), whereas crystalline size was calculated by using Debye-Scherrer's equation (Eq. 2):

$$CI(\%) = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \qquad (1)$$

Where:

$I_{(200)}$ is the maximum intensity of the (200) lattice diffraction peak,

I_{am} is the intensity scattered by the amorphous part of the sample. The peak of the diffraction for the plane (200) is located at a diffraction angle of around $2\theta = 22.5^{0.}$ Simultaneously, the intensity scattered by the amorphous part was measured as the lowest intensity at a diffraction angle of approximately $2\theta = 18.0^{0}$.

$$D = k\lambda/\beta Cos\theta \qquad (2)$$

Where k is the form factor (0.89), λ is the wavelength of the incident X-ray

(0.15418 nm), θ is the diffraction angle, and β is FWHM (Full Width Half Maximum) of maximum intensity (I200) in radian.

5.3.4 Thermogravimetric analysis (TGA)

Thermogravimetric analysis of the CNC was performed using an STA 6000 thermogravimetric analyzer (Perkin Elmer, USA), which allowed thermogravimetric and DSC measurements the synchronous recording of the mass spectra of thermal decomposition products. The heating of the samples was carried out under a constant nitrogen flow of 20 ml min^{-1}. The samples were gradually heated from 25 to 600°C, at a constant heating rate of 10°C min^{-1}.

5.3.5 Preparation of particleboards

The pine wood sawdust was milled using a Willey mill to a particle size range of 1 mm - 1.25 mm. The resultant particles were oven-dried at the temperature of 60^{o}C for 24 hours to about 6% - 7 % moisture content. Each panel of 600 kg/m^3 target density was prepared; the required wood sawdust was calculated using the equation (3). The weight of the materials was calculated based on the target density of the panels. CNC slurry with 0.5 % solids content was added to the sawdust based on a 15% dosage and was thoroughly mixed. The moisture content of the sawdust mixture increased from about 7% to 22% due to the high percentage of water in the CNC slurry. The CNC-sawdust mixture was then poured into a steel mould with dimensions 218 x 75 x 40 mm. A steel bar of about 28 mm thickness was applied to press the wood particles into an even panel thickness of approximately 10±1 mm. After the panel formation, the panel was pre-pressed before hot pressing using a laboratory hot press. The temperature of the hot-press was regulated to 180^{o}C at 200 kPa and pressed for about 30 minutes to allow moisture evaporation and proper board formation. Six types of one-layer particleboard panels in triplicate were produced with the CNC only and combinations of the different cross-linking agents. When used in combination, 10% CNC and 5% of each of the cross-linking agents based on the weight of the sawdust was used to give the required binder dosage for the board binding, and the binder was prepared to follow the procedure adapted from (Amini et al., 2017). The control panels were fabricated with 15% Bondtite resin having the same density as the experimental panels. The illustration of the production process is shown in Figure 5. 2. The influence of some cross-linking agents on the mechanical strength and physical performance of the particleboard panels were then assessed.

$$Density\left(\frac{g}{cm}^{3}\right) = \frac{mass(g)}{volume(cm^3)} \quad (3)$$

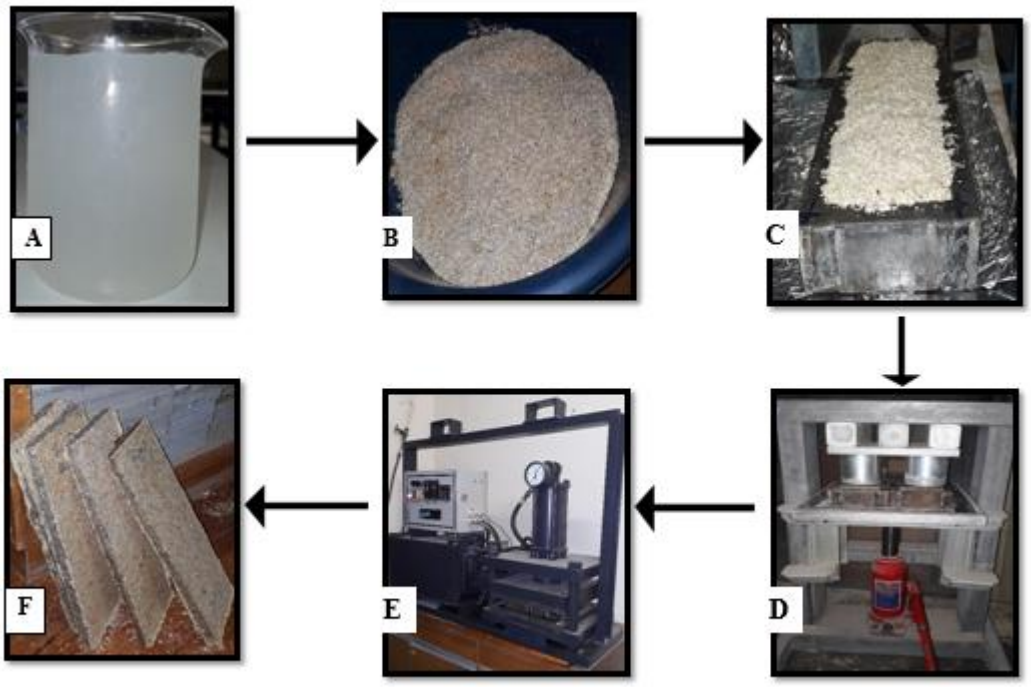

Figure 5.2: Flow diagram of particleboard panel production process: (A) Extracted cellulose nanocrystal gel; (B) wood sawdust; (C) board forming mould; (D) mat pre-press; (E) mat hot-press; (F) final board

5.3.6 Particleboard characterization

The mechanical strengths of the particleboards were assessed to determine if any improvements were achieved when using CNC on its own or in combination with the cross-linkers. The stability in the dimension of the boards was also determined. The procedures used for these tests were based on those described in ASTM D1037 (Standard Methods for Evaluating the properties of the wood base, fiber and particle panel materials) using an Instron testing machine fitted with a 5 kN load cell and operated at a rate of 5 mm/min. The specimens were tested to failure: the modulus of rupture (MOR) and the modulus of elasticity (MOE) were determined according to standard methods (ASTM, 2013). The particleboard specimens were cut into

dimensions of 75 mm x 50 mm and were used to assess the dimensional stability of the boards. The thickness of the samples used for this analysis was 9.6±1 mm, which corresponded to the design of the mould that was used. Three representatives from each of the board test series were submerged vertically in water for 2 hours. Before submersion, the weight and the thickness of the samples were measured. At the end of the 2 hours, the samples were removed, drained, and the same measurements were repeated. The thickness of the board samples was measured using a veneer caliper, and the thickness of the boards was calculated as the mean of three board measurements. The average of the data was obtained, and the percent thickness swelling was calculated according to equation (4). In contrast, the percentage of water absorption was derived from the weight gain after soaking in water.

$$Gt = \frac{t2 - t1}{t1} \times 100 \qquad (4)$$

Where: Gt = Percentage of thickness swelling;

t2 = thickness of the sample before immersion (mm);

t1 = thickness of the sample after immersion (mm)

5.3.7 Statistical evaluation

Statistica software (Statsoft v13) was used to carry out one-way analysis of variance (ANOVA) to evaluate the mechanical strength properties result statistically. The mean result of each test was compared using the post-hoc Fisher LSD test to determine the significance of formulation parameters on the measured properties of the particleboard panels.

5.4 Results and discussions

5.4.1 FTIR characterization

FTIR spectra of the CNC extract in comparison to the wood sawdust are shown in Fig 5. 3. The obtained absorption bands were comparable, indicating that the structural and compositional integrity of cellulose was not compromised during the production of CNC from sawdust using a combination of acid hydrolysis and an oxidising agent the BIDF in-house methodology. The spectra of the wood sawdust and the CNC showed the characteristic peaks of the cellulose backbone, which is the principal chemical composition of wood. The peaks at wavelengths of 3334 cm^{-1}, 2898 cm^{-1} and 1638 cm^{-1} were allotted to O-H symmetrical, aliphatic C-H

stretching and C-O vibration. In addition, peaks were observed at 1315 cm^{-1} for C-H bending vibration, 1031 cm^{-1} for C-O stretching and 830 cm-1 for C-O - C deformation and stretching. These observations are comparable to those obtained by other authors (Shaheen and Emam, 2018, Mahmud et al., 2019).

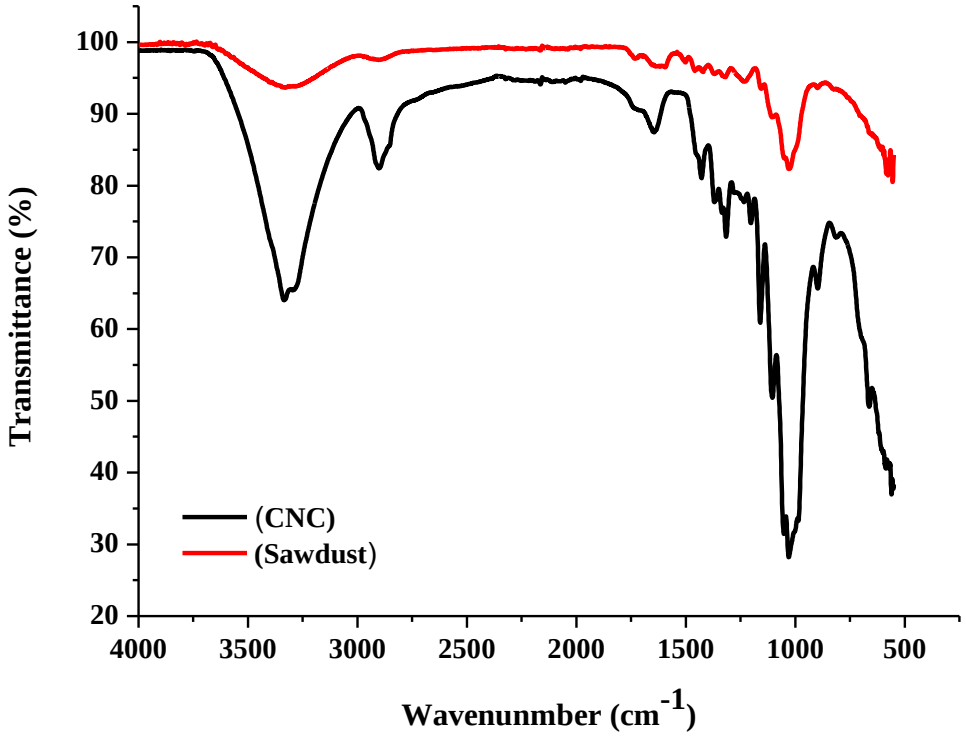

Figure 5.3: FTIR spectra of wood sawdust and extracted cellulose nanocrystals

5.4.2 XRD characterization

The X-ray diffraction of the CNC is shown in Fig 5. 4. The XRD pattern showed two distinctive diffraction peaks of cellulose I at 2θ= 14.8° and 22.56°, respectively. They correspond to the (010) and (002) crystal lattice planes of cellulose crystal. The crystallinity index (CI) and the crystal size of the CNC were 78% and 2.73 nm, respectively. The results indicated a substantial percentage of amorphous was removed from the wood sawdust during CNC production.

The extraction process did not disrupt the crystal structure of the cellulose in the starting material but had a significant impact on the crystallinity of CNC. Cellulose crystallinity is one of the most critical factors determining the mechanical and thermal properties of CNC. The CI and the crystallite size obtained were like those reported by (Landry et al., 2011, Li et al., 2011,

Kargarzadeh et al., 2012) for CNC extracted using acid hydrolysis, from bleached softwood kraft pulp and kenaf bast, respectively. The crystal size observed in this work was relatively small compared to previous studies (Li et al., 2011, Mahmud et al., 2019). This could be owing to the enhancement of the CNC crystallinity when acid treatment is used during the extraction process. The acid treatment enhances CNC crystallinity because of the removal of the amorphous regions of cellulose. The relatively small crystal size could also indicate rearrangement of the molecular chains of cellulose (Gong et al., 2017) during CNC production, which could lead to smaller crystallite sizes.

Nevertheless, various crystallinity values and crystal sizes have been reported in the literature for CNC. Generally, crystallinity varies significantly according to the starting material used and the methods of preparation (Gong et al., 2017). The crystallinity of CNC is essential because it affects its application. A high degree of crystallinity would result in enhanced mechanical, chemical and thermal properties (Gong et al., 2017, Landry et al., 2011).

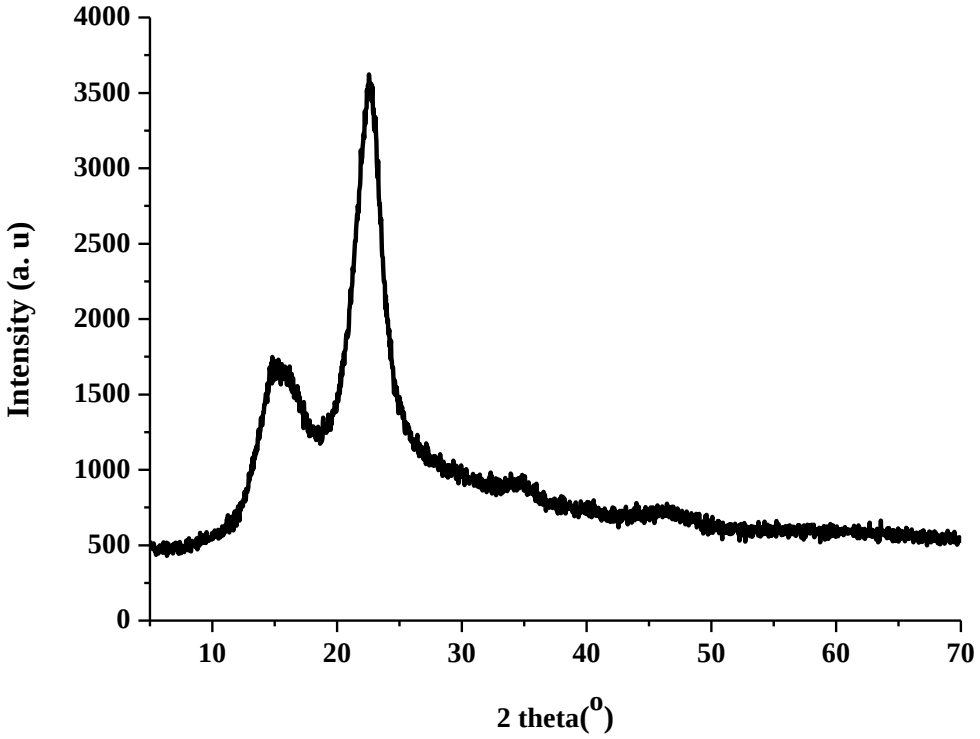

Figure 5.4: XRD pattern of extracted cellulose nanocrystals

5.4.3 Transmission electron microscope (TEM) characterization

The TEM micrograph of CNC is shown in Fig. 5. 5a. The rod-like morphology of the CNC particles is evident with dimensions ranging from 100-200 nm in length and 10-20 nm in width.

The image obtained shows mostly individual nanocrystals and some aggregates. These observations were due to the high density of the hydroxyl groups on the surface of the cellulose chain molecules, resulting in the formation of hydrogen bonds (Fig 5.5a). Fig. 5. 5b represents the histogram of diameter distribution in the range of 2.1–9.53 nm. These CNC dimensional values of length, width and diameter are essential because it provides a better understanding of the binding and reinforcement capacity of the nano-cellulose with both wood particles and the polymers. These results indicate a high geometric aspect ratio. Cellulose nanocrystal with a high aspect ratio is more amenable for reinforcing for mechanical properties improvement of polymers (Landry et al., 2011). The CNC dimensions and morphology are comparable to other studies on CNC derived from wheat and microcrystalline cellulose (Qin et al., 2017, Bondeson et al., 2006).

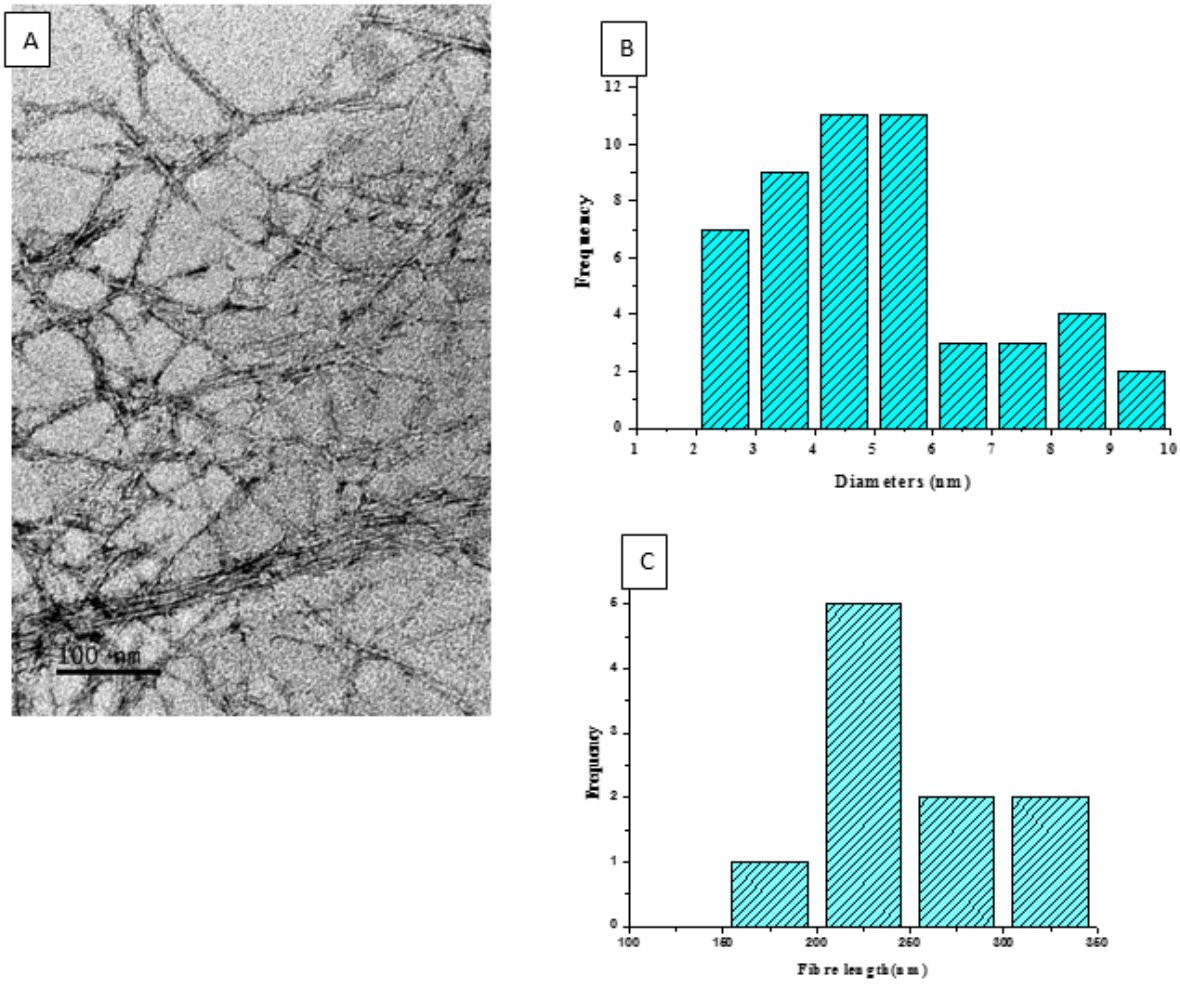

Figure 5.5 (a & b): TEM micrograph of the extracted cellulose nanocrystals and (b) diameter distribution of the CNC calculated from TEM micrographs. (c) average length

5.4.4 Thermal stability of cellulose nanocrystal

The thermal stability of the extracted CNC using thermogravimetric analysis (TGA) and the differential thermogravimetric (DTG) is shown in Figure 5. 6a & b. The thermal analysis revealed that the weight loss occurred in three stages; a slight weight loss was noticed at low temperatures from 25 to 100◦C and was attributed to the evaporation of moisture from the CNC. The amorphous polymer chain in the CNC underwent degradation at the temperature of around 205^{o}C to 249^{o}C. Finally, at a temperature between 250^{o}C and 400^{o}C, depolymerization, dehydration and decomposition of glycosidic units of cellulose crystals occurred (Roman and Winter, 2004). The thermal stability of CNC is usually related to many factors such as dimension, crystallinity, composition, and the extraction conditions employed. In general, CNC with a high crystallinity has high thermal stability due to the high thermal conductivity in the crystalline regions (Chieng et al., 2017). The thermal stability of CNC is vital for binder formulation, polymer reinforcement and thermoplastics applications since the temperature involved in these processes is usually between 180^{o}C to 200^{o}C (Wang and Huang, 2007).

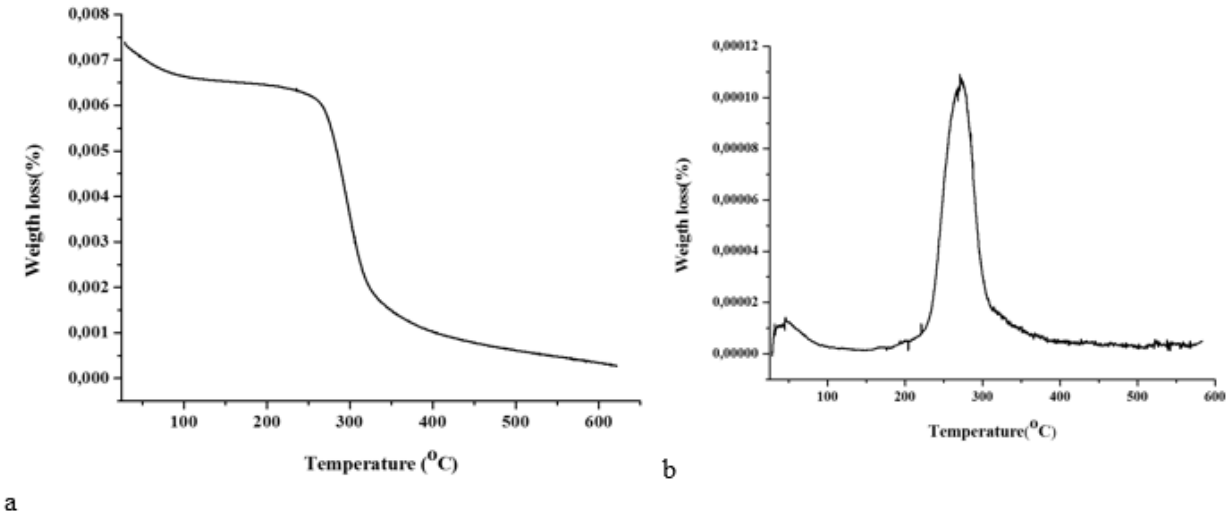

Figure 5. 6 (a & b): TGA and DTGA curve showing the thermal stability of extracted cellulose nanocrystals

5.4.5 Physical and mechanical properties of particleboard panels

Density

Table 5. 1 shows the mean density of the particleboard panels fabricated with CNC, Bondtite (commercial tannin-based adhesive as control) and cross-linked CNC. The average density of the boards ranges between 599, 68 kg/m^3 to 611, 22 kg/m^3. Some were below the target density of 600 kg/m^3. This variation in the density obtained can be ascribed to the fact that the density of the mat-formed hot-pressed particleboard varies through the vertical direction of the board thickness. Besides, the density of a board depends on the particle configuration, moisture distribution in the mat entering the press, rate of press closing, the temperature of the hot press, the resin reactivity, and the compressive strength of the wood particles component (Kelly, 1977). The density of the panels produced in the current work can be classified as medium density particleboard panels using the American National Standards Institute (ANSI) (2016) classification of particleboard between 0.60 and 0.8 g/cm^3 (ANSI, 2016).

Table 5.1: Fabricated particleboard Panel density

Binder	CNC	Bondtite	CNC/GLY	CNC/HEXA	CNC/PAE	CNC/PE
Particleboard density	600,42	605,23	599,68	600,24	611,22	604,23

CNC=Cellulose nanocrystals, GLY= glyoxal, HEXA= hexamine, PAE=polyamide–epichlorohydrin, PE= **polyethylene**

Flexural evaluation and the effect of the crosslinking agents on particleboard panels

The modulus of rupture (MOR) and the modulus of elasticity (MOE) of the different fabricated particleboard panels are presented in Table 5. 2. The statical representation is shown in Figure 5. 7a & b respectively. The MOR for particleboards produced using bondtite was significantly different than the panels bonded with CNC and the CNC-cross-linked incorporated particleboard panels. Also, the results for the cross-liked-CNC particleboard panels showed there are no substantial differences in the MOR values for most of the adhesive formulations but differed significantly from panels fabricated with CNC suspension solely.

The value means and the standard deviation of the MOE result of the panels fabricated with the cellulose nanocrystals solution and cross-linker-CNC are shown in Figure 5. 7b. The average MOE values range between 157,77 MPa to 826,77 MPa, respectively. These values were significantly lower compared to panels produced with bondtite. The boards made with

CNC suspension showed the lowest MOE results but was improved considerably with the addition of cross-linking agents.

The only significant difference was observed with the CNC cross-linked with CA-PAE, comparing the impact of the crosslinking agents. A positive effect was seen in the increase in both MOE and MOR of the panels. The significant difference in the bending strength properties of the boards as a function of binder formulation could be attributed to the degree of the curing of the binder, the chemical bond that occurred with the wood particles, besides the ability of a cured resin to spread well (Mekonnen et al., 2014). Based on ANSI (2016) particleboard panel classifications, the panels with CNC-cross-linked adhesive formulation satisfied the required specification and could be considered under the grade 1-L-1 panel specification (ANSI, 2016). Grade 1-L-1 panel particleboards are used to construct core material for the manufacture of solid doors (Carll, 1986).

This improvement in the strength of the cross-linked-CNC particleboard panels could be ascribed to the function of the CNC in the adhesive formulation. It worked as filler to improve the strain to failure and the toughness of the adhesive and, consequently, the performance of the adhesive, which contributed to the overall mechanical strength of the fabricated particleboard panel (Amini et al., 2017).

Table 5.2: Effect of the cross-linking agents on the modulus of rupture and modulus of elasticity of the particleboard panels

Binder	**CNC**	**Bondtite**	**CNC/GLY**	**CNC/HEXA**	**CNC/PAE**	**CNC/PE**
Modulus of Rupture (MPa)	1,33	21,34	5,28	5,05	6,43	6,29
Modulus of Elasticity (MPa)	157,77	2493,29	659,51	737,04	826,78	804,98

CNC=Cellulose nanocrystals, GLY= glyoxal, HEXA= hexamine, PAE=polyamide–epichlorohydrin, PE= **polyethylene**

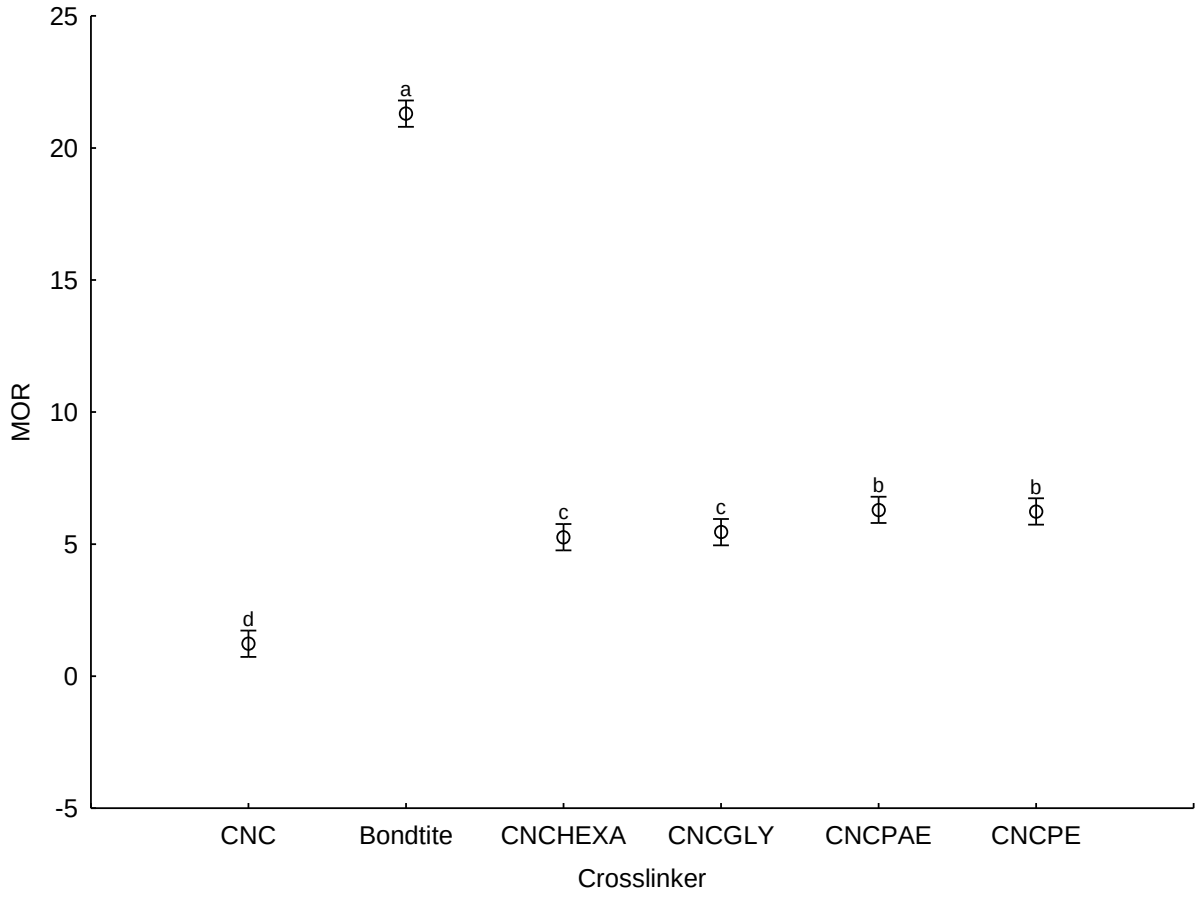

Figure 5.7a: Fisher LSD test showing the significant differences in the modulus of rupture of the particleboard samples produced with CNC and CNC combine with different cross-linking agents

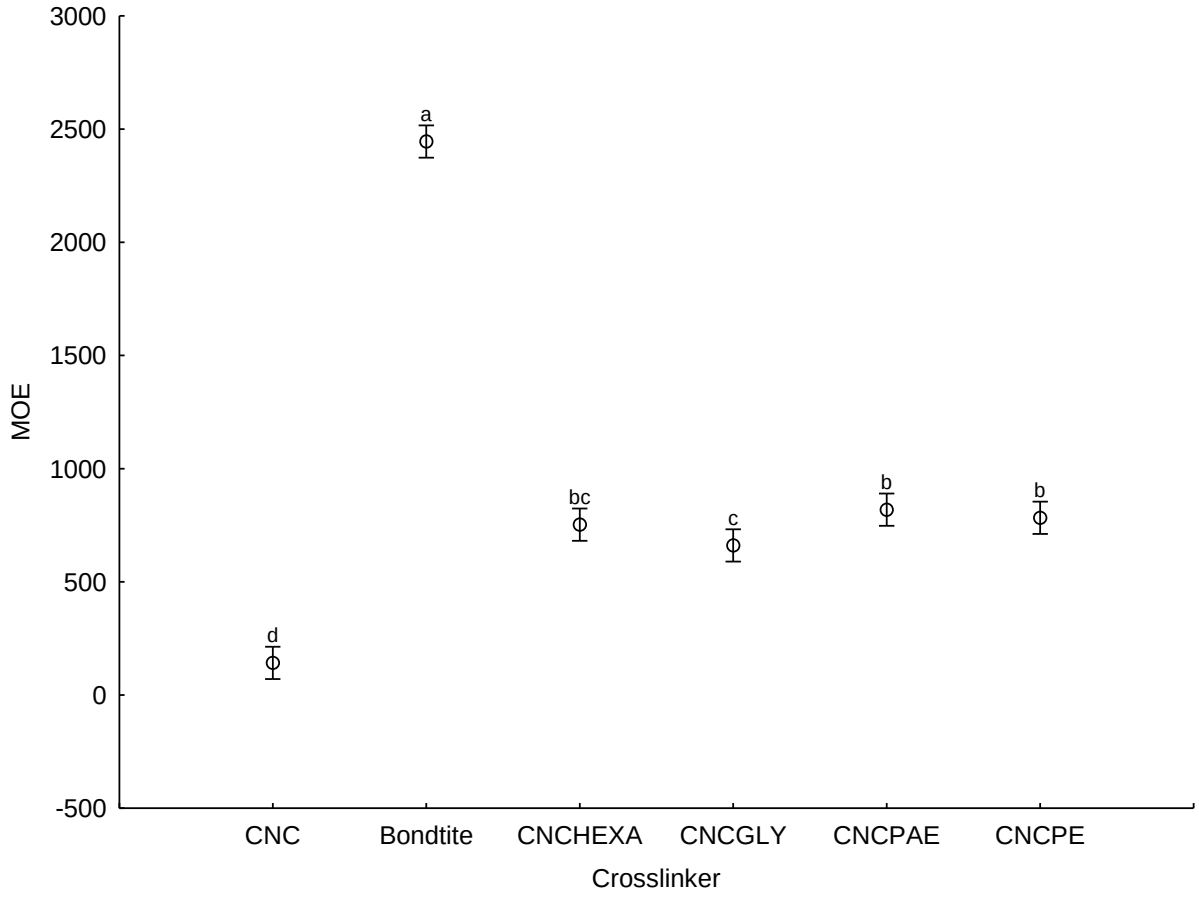

Figure 5.7b: Fisher LSD test graph showing the significant differences in the modulus of elasticity of the particleboard panels produced with CNC and CNC combine with different cross-linking agents

Thickness swelling

Figure 5. 8 depicts the swelling of the particleboard panels when immersed in water. The particleboard fabricated with CNC showed a massive increase in swelling. However, the addition of crosslinking agents reduced the swelling significantly. Nevertheless, the panels could not exceed 2 hours of submersion in water. There were insignificant differences in the thickness swelling of the particleboards when comparing the four crosslinking agents, except for the citric acid-based polyamide-epichlorohydrin that showed the lowest thickness swelling. A likely explanation for this observation might be ascribed to the interaction between the carboxylic groups in the CNC with the azetidinium functional groups of the citric acid-based polyamide-epichlorohydrin crosslinker, which resulted in a low molecular compound (Gui et al., 2013a, Amini et al., 2017) and as a result, a smaller degree of swelling.

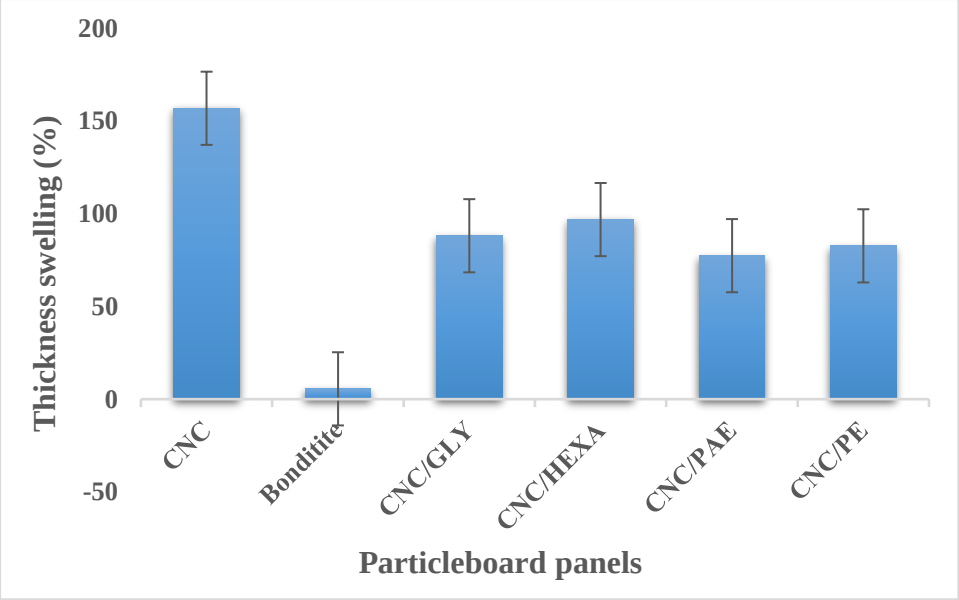

Figure 5.8: Comparison of swelling of particleboards produced with CNC and CNC combine with different cross-linking agents.

5.5 Conclusion

In this study, CNC from waste wood sawdust was successfully used as a bio-binder to produce particleboard panels. Additionally, the impact of different cross-linkers on the performance of the CNC as a bio-binder for particleboard panel production was evaluated by assessing the mechanical strengths of the panels in comparison to the board produced using a commercial binder. FTIR, XRD, TGA and TEM analyses were first used to confirm the undistorted functional composition of cellulose, its crystallinity and thermal stability and dimensions. Impressively, the different cross-linkers in the adhesive formulation improved the strength performance of the particleboard panels significantly, and panels fabricated with CNC containing cross-linkers satisfied the required manufacturing specifications of grade 1-L-1 panels. However, due to the hydrophilic nature of CNC, the particleboard resistance to water did not meet the ANSI A208.1specifications for non-structural applications.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Waste chicken feathers and wood sawdust are consists of potentially high-value products. Finding alternative and innovative uses for these wastes could transform the face of the poultry and sawmilling industry, both economically and environmentally. These biomass sources exist as large quantities of waste materials currently disposed of by ecologically unsustainable methods such as landfilling, incineration, or stockpiling on production sites. The diversion of all organic waste from landfills has now become mandatory in South Africa. These wastes can be beneficiated via bio-refinery technologies into high-value materials, including binders. Such usage could enable disposable of the biomass in an environmentally sustainable manner. In direct response to the necessity mentioned above, this study's key focus was to explore options for the beneficiation of chicken feathers and sawdust waste streams produced primarily from the poultry slaughterhouse and sawmilling industry. The main objective was to investigate the extraction and production of keratin protein from waste chicken feathers and cellulose nanocrystals from wood sawdust for binder application. Currently, there are no facilities in South Africa manufacturing keratin hydrolysate powder and CNC commercially to put this in context. In response to the rising demand for keratin and CNC-based products as green alternatives to fossil resources in skin and hair care products, bio-polymers and chemicals, significant efforts are underway to commercialize the production of keratin and cellulose nanocrystals (CNC) in South Africa.

This study considered the potential beneficiation of renewable and eco-friendly biomass as an alternative source of bio-binder in particleboard production. The purpose was to mitigate the harmful emissions from wood composite products fabricated with the formaldehyde-based adhesives on humans and the environment. The work optimized keratin protein extraction from waste chicken feather biomass using a novel hybrid extraction method. Keratin is a high-value product for hair care, cosmetics and bio-binder applications. These value-added products from waste chicken feathers and sawdust can provide additional income to the poultry and sawmilling industries across South Africa. This research is the first to extract keratin protein hydrolysate from waste chicken feathers using hybrid techniques and convert it to wood composites bio-binders. Besides, it utilized cellulose nanocrystals from wood sawdust as a binder for particleboard production. This chapter gives a summary of the primary findings of the study.

Firstly, Response Surface Methodology (RSM) using Box-Behnken design was used to investigate and optimized the keratin extraction operational conditions via a hybrid alkaline hydrolysis method. The effect of time, temperature, and sodium hydroxide and sodium bisulphite concentration as an extraction solvent on the keratin hydrolysate and protein yield were modeled and optimized. The coefficients of determination (R^2) of 0.77 and 0.74 were obtained for the dependent variables: keratin hydrolysate and protein yield models, respectively, which demonstrated the fitness of the models to navigate the optimization space. The optimum extraction conditions gotten from the study were (1.78%) sodium hydroxide and (0.5%) sodium bisulphite concentrations, (111 min) reaction time, and (87 ^{o}C) temperature. The results show the determined optimum physicochemical conditions of sodium hydroxide, sodium bisulphite, dissolution time and temperature significantly improved the protein yield. The FTIR, CHNS and SDS-page confirmed the amide and carboxylic structural groups of the extracted protein units, typical proteinous functional groups. It was evident that the implemented hybrid process conditions had not denatured the extracted protein structure. The results further underscore the importance of the extraction process parameters at the optimum conditions. An additional interest lies in the availability, renewability and the global challenge in the disposal of the waste chicken feathers, which fundamentally would influence the techno economics and the industrial process scalability.

The extracted keratin hydrolysate from waste chicken feather biomass was employed in synthesizing wood adhesives for particleboard production. The extraction was done to expose the polar functional groups buried within the folded protein structure for easy solubilization. These desirable active functional groups will eventually interact with the wood functional groups during the bonding process. The FTIR micrograph confirmed the targeted proteinous functional group's presence in the formulated keratin-based binder and the covalent bonding of the citric acid-based polyamide-epichlorohydrin azetidinium active group with the hydroxyl groups of the keratin protein in an effective co-crosslinking product. The bio-adhesive and the particleboard mechanical strength performance were desirable and comparable to synthetic adhesive upon evaluation. The formulated adhesives with 20, 25, and 30% solid content met the ANSI A208.1 requirements. The adhesive formulation (20%) solid content reinforced with cellulose nanocrystals (CNC) shows excellent performance in the fabricated particleboard panel. However, due to some functional groups of hydrolyzed keratin proteins' hydrophilic characteristics, the resistance to water of the adhesives produced did not satisfy the ANSI A208.1 specifications for structural applications. The particleboard manufacture in this research work is suggested to be utilized as a solid door core, indoor materials and dry

environment applications. The valorization of the waste chicken feather to value-added products such as wood composites bio-adhesives could be expanded to other slaughterhouses proteinaceous waste and commercialization.

Additionally, in this study, CNC from waste wood sawdust was successfully used as a bio-binder to produce particleboard panels. Similarly, the effect of different cross-linking agents on the CNC's performance as a bio-binder for particleboard panel production was evaluated. The undistorted functional composition of cellulose, its crystallinity, thermal stability and dimensions were obtained using FTIR, XRD, TGA and TEM, respectively. Impressively, the different cross-linkers in the CNC-adhesive formulation improved the particleboard panels' mechanical performance significantly. The panels fabricated with CNC associated cross-linkers satisfied the required manufacturing specifications of grade 1-L-1 panels. Hence, cellulose nanocrystals extracted from waste wood sawdust could be considered eco-friendly wood composites bio-adhesives and particleboard panels of high industrial quality.

This work opens an innovative area of insight into research based on the production of bio-based wood adhesives from waste chicken feathers and wood sawdust. This research provides an opportunity to recover valuable materials from waste chicken feathers and sawdust as a new feedstock for wood composite adhesive manufacturers. Thus, returning them into the local manufacturing economy as high-value products resulting in strengthening and encouraging the local Green Economy in South Africa.

6.2 RECOMMENDATIONS FOR FUTURE RESEARCH

As a way forward, the study's focus will be on the transfer of the novel hybrid keratin hydrolysate powder extraction technology to industry. However, new directions for future research were indicated by the findings of the present report. Nonetheless, some suggestions will be included for future research areas.

- Upscaling of extraction process for keratin from waste chicken feathers using the optimized methodology and upscaling of the keratin-based bio-binder process.
- Studying the production of plywood and other wood composites using keratin-based and cellulose nanocrystals as bio-binder.
- Investigating the effect of the addition of hydrophobic agents such as aqueous paraffin-based wax and hardener on the performance of the keratin-based bio-binder.
- Studies on the effect of incorporating other natural materials such as lignin, tannin and starch in the keratin-based bio-binder formulation.

- The techno-economic analysis of keratin extraction and keratin-based bio-binder production to assess the technology transition to Small, Medium and Micro Enterprises (SMMEs) will be necessary.

APPENDICES

APPENDIX A

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Optimization of keratin protein extraction from waste chicken feathers using hybrid pre-treatment techniques

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ABSTRACT

This study modeled and optimized the operational conditions for the extraction of keratin from waste chicken feathers via alkaline hydrolysis method. Response Surface methodology using Box-Behnken design was used to investigate the effect of time, temperature and the concentration of both sodium hydroxide and sodium bisulphite as an extraction solvent on the keratin hydrolysate and protein yield. The coefficients of determination (R^2) of 0.77 and 0.74 were obtained for keratin hydrolysate and protein yield models, respectively demonstrating the fitness of the models to navigate the optimization space. The optimum parameters were temperature (87 °C), time (111 min), sodium hydroxide (1.78%) and sodium bisulphite (0.5%). The yields of keratin hydrolysate and protein after validation were 68.3% and 65.2% respectively. Protein analysis via the CHNS analyser showed the elemental composition of 13.85% N, 47.25% C, 6.90% H and 2.8% S. The molecular weight of the extracted keratin ranged between 10-15 kDa and 3–10 kDa. Ultimate analysis by FTIR and NMR confirmed the presence of amide, carboxylic groups and alkyl side chains of amino acids. The results of this study proved that chicken feathers, typically disposed of by the poultry slaughterhouses, would be able to serve as a great sustainable source of keratin protein for the manufacture of value-added products like bio-adhesives.

1. Introduction

Chicken meat is one of the significant sources of animal protein mostly consumed worldwide. At the same time, its intake has resulted to the creation of a substantial number of feather waste byproducts from poultry slaughterhouses as near seven percent of chicken bodyweight is feathers. It was reported that the estimated number of chicken feathers generated each year globally is about 15 billion tons with South Africa, contributing about 258 million tons (Tesfaye et al., 2017). Currently, small amounts of feathers are beneficiated as animal feedstuff. Still, the majority of them produced from either small or large poultry operations in many countries ends up in landfills, creating environmental problems, except some that are incorporated in low-quality animal feeds (Chinta et al., 2013). However, these are renewable resources that are very rich in keratin protein: a rigid, fibrous type of proteins also found in hair, skin, hooves and nails (Sharma and Gupta, 2016). Since the disposal of waste feathers is not environmentally sustainable, research into their beneficiation is a necessity to produce cheap, biodegradable, sustainable, renewable, and abundantly available green materials (Tesfaye et al., 2018). Keratin protein obtained from other biomass such as nails, hair, horns and wool have been commercially incorporated in hair care, and cosmetic applications but globally chicken feather waste are the most abundant and sustainable keratinous material in nature that are yet to be adequately utilized, especially in bio-adhesive (Aluigi et al., 2007; Villa et al., 2013 ; Sharma and Gupta, 2016) Therefore, the drive of waste chicken feathers for the current study. Chicken feathers possess some excellent qualities such as its high keratinous protein and its hydrophobic structure, which give a better water resistance nature on the final product. Besides, the inherent anti-mildew property and fibrin units of feather protein promote its hydrolysation. These distinctive properties, as well as its abundant availability, sustainability and low cost, make chicken feathers a choice for many applications that require

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good tensile strength and elasticity (Nuutinen, 2017).

Thus keratin protein can be extracted from feathers and used as natural raw material for the production of films, fibres, hydrogels, binders, micro and nanoparticles for cosmetic, medical, textile, composite and other industrial uses (Khosa and Ullah, 2013). The keratin in feathers is a unique protein that has high cysteine content of about (7–13%) in the amino acid sequence (Shavandi et al., 2017), and cysteine that contains –SH groups which are responsible for the sulphur–sulphur (disulphide) bonding in the keratin. The high cysteine content stabilises the keratin by forming a network structure joined by adjacent polypeptides and disulphide cross-links (Saravanan and Dhurai, 2012). The chemical composition of keratin exhibits β-helix or β-pleated twisted sheets of small uniform size proteins with a low molecular weight of about 10 kDa (Khosa and Ullah, 2013; Sharma and Gupta, 2016).The cross-linked cystine bond between peptide chains components in keratin imparts high stability and xenobiotic nature. According to literature, keratin protein is not soluble in water, but this can be enhanced at mild and acidic pH, with the use of heat and in the presence of reducing agent (Donato and Mija, 2020). After the reduction, the crosslinks between the disulphide will be broken into free thiol (-SH) apart from the protonation of some $–NH_2$ and other groups, however, this will make their surface positive, and then, solubilisation will take place Khosa and Ullah, 2013(). After protonation reaction, different functional groups that have positive surface charges with high reactivity will be unfolded and exposed, and therefore, after chemical modification of keratin protein, they become pseudo cationic biopolymer (Khosa and Ullah, 2013). Thus, several methods are used to dissolve and extract keratin from chicken feathers. These methods include reduction hydrolysis, alkaline hydrolysis, sulphitolysis, and extraction with ionic liquids also, oxidation, supercritical water extraction and steam explosion; besides, microwave-assisted extraction, microbial and enzymatic methods have also been used for keratin extraction Shavandi et al., 2017). A lot of studies have revealed that using these methods that keratin protein from chicken feathers can be obtained by breaking the disulfide bonds in the cystine unit (Kamarudin et al., 2017). For instance, sodium sulphide, 2- mercaptoethanol, and sodium dodecyl sulfate were used to obtain a good yield of keratin protein from various animal sources (Kamarudin et al., 2017). Another previous study reported the addition of urea and sodium dodecyl sulfate in solutions containing 10 g/L Na_2S, 9 M urea, and 10 g/L sodium dodecyl sulfate. The results indicated that the presence of urea enhanced both the process rate and the product yield (Poole et al., 2011).

Sinkiewicz et al. (2017) reported the result obtained from the keratin extraction process using various reducing agents. It was shown that 84 and 82% yield of soluble keratin was obtained respectively after 2 h with the use of mercaptoethanol together with sodium bisulphite as the reducing agents, it also reported that the use of sodium bisulphite reduced the time of extraction to about 1 h with the same result (Sinkiewicz et al., 2017).

Besides, the author reveals that the chemical treatment of the feathers with 2.5 percentage concentration sodium hydroxide enhanced the efficiency of the extraction and was able to increase the yield obtained to 94% (Sinkiewicz et al., 2017). Feather keratins are not soluble in conventional polar and non-polar chemical solvents due to its tough structure: an extensive disulfide crosslinking and tightly packed α-helices and β-sheets in a polypeptide chain. Thus, an effective, low-cost process and scalable process to extract the keratin protein is desirable. Solvent for chicken feather solubilization should be a solution which enables the re-crosslinking process after dissolution and does not cause the deformation of the primary protein chains (Poole et al., 2011). There are important functional groups in the keratinous protein which should be considered during processing, regeneration and modification of feather keratin for further applications. These functional groups include sulfhydryl (SH), amino (NH_2) and carboxylic group (COOH). The choice of pre-treatment technique and chemical solvent chosen is based on the application to which keratin protein is to be utilized and to retain many of the protein attributes present in the native structure (Nuutinen, 2017, Barone and Schmidt, 2006). In some cases, mechanical treatment can be enough (Nuutinen, 2017). For instance, Poole *et al.* (2011) reported high exposure to the extremely alkaline conditions appears to be unfavourable to final product strength. Moreover, shorter exposure times could produce stronger regenerated products, since serious alkalinity chain damage can be significantly reduced (Poole et al., 2011). Scientifically, it is crucial to know and understand how to get valuable products from waste biomass efficiently and effectively. Sinkiewicz et al. (2017) stated that using acid hydrolysis for keratin extraction is very efficient, but this can harm some amino acids residues, such as tryptophan whereas alkaline hydrolysis is less effective, but the loss of amino acids residues will be minimal (Sinkiewicz et al., 2017). The yield of keratin from chicken feathers using hydrolytic processes depends on some crucial parameters which include, the solid to liquid ratio, the pH of the extraction medium, the level of temperature use and the time of reaction during the extraction process (Sinkiewicz et al., 2017). Besides, the state of the protein hydrolysate, whether it will be stable or soluble, is determined by the level of protein degradation during the extraction process (Sinkiewicz et al., 2017). However, thermochemical hydrolysis is used for feather keratin extraction to achieve a maximum hydrolysate yield. Nevertheless, it was reported that very high temperature could cause much damage to amino acids residues (Sinkiewicz et al., 2017), by denaturing both primary and secondary structure of the protein. The most common method for extraction of keratin is to use reducing agents in alkaline solution. There is insufficient knowledge in the literature on the use of optimized hybridization processes for the extraction of keratin protein from waste chicken feathers. Knowledge of the dynamics of optimized hybridization processes will ease the determination of optimum extractive conditions. The quality of extracted protein can be controlled via the solvent of extraction, dissolution time, and other physical parameters employed. These will reflect on the variability of the extraction parameters (Poole et al., 2011). Regardless of several studies devoted to valorisation of keratin-based materials such as chicken feather, there are still challenges due to the recalcitrant nature of keratin to various solvents, enzymes and physical conditions. As well as the likely negative effect such as protein deformation and aggregation of chosen methods. Hence, approach preventing detrimental changes of amino acids, leading to keratin of desirable dissolution and ultimately improved protein yield are needed. Optimised extraction conditions of such method, especially by the combination of two reducing agents: sodium hydroxide and sodium bisulphite in a solution with moderate concentrations have not been reported. The combined and interactive effect of sodium hydroxide and sodium bisulphite extraction solvents, dissolution time and temperature have thus far to be studied in keratin dissolution from waste chicken feathers.

Therefore, this research work aimed at optimizing keratin extraction process from waste chicken feather biomass using a combination of two reducing agents at concentration friendly to keratin protein structure. The extractive effectiveness of sodium hydroxide and sodium bisulphite combined were investigated and optimized using Response Surface Methodology. The extracted and purified keratin hydrolysate were then characterised for their structural and biochemical properties.

2. Materials and methods

Fresh chicken feathers were collected from a slaughterhouse in the province of KwaZulu-Natal, South Africa. Sodium hydroxide pellet, sodium bisulphite with 40% concentration in solution, sodium dodecyl sulphate (SDS), β-mercaptoethanol, Tris(hydroxymethyl) and hydrochloric acid were purchased from Sigma-Aldrich (South Africa).

Molecular weights of the keratin extracts were estimated by SDS-PAGE Electrophoresis whereby protein ladder Kaleidoscope (BioRad) with molecular weight ranges between 10 and 180 kDa and protein ladder Spectra™ Multicolor Low Range Protein Ladder (molecular weight range 1.7–40 kDa from ThermoFisher Scientific, South Africa)

Table 1
Variables used for designing the experimental.

Factors (independent variables)	Factors level		
	−1	0	1
Time (Minutes)	90	105	120
Temperature (°C)	80	85	90
NaOH (%)	1.5	1.75	2
$NaHSO_3$ (%)	0	0.5	1

were used separately as molecular weight markers. Coomassie Brilliant Blue R-250 (Bio-Rad was used as the staining solution for the protein separation gels.

The principal instruments used in characterization of the keratin samples were FTIR spectroscopy (Frontier Universal ATR-FTIR, by PerkinElmer), Nuclear Magnetic Resonance (NMR), PerkinElmer Simultaneous PerkinElmer CHNS/O analyzer series II, and Willey mill by Thomas Scientific; besides, small steel containers with tight seal were used in the extraction process.

2.1. Feathers pre-treatment

The fresh waste feathers collected from the poultry slaughterhouse were cleaned by separating the heads, intestines and blood from the feathers. After that, the feathers were washed with warm water. They were then decontaminated with 50:50 mixture of hydrogen peroxide and sodium hypochlorite and then oven-dried at $60^{\circ}C$ for 24 h. The dried feathers were defatted by soaking in ethanol for 24 h, washed with water and liquid soap; it was oven-dried for another 24 h and afterwards ground with Willey mill into a smaller particulate matter of about 1.5 mm. The ground feather biomass was stored in a cold room at 6 °C until further use.

2.2. The experimental design and optimization of the keratin protein extraction process

The design of the experiment was done with the use of design expert software, while the Box-Behnken design model was used for the optimization of the alkaline hydrolysis keratin extraction process. The four independent variables were between the ranges of 90–120 min for time, $80^{\circ}C$–$90^{\circ}C$ for temperature, 1.5–2% for sodium hydroxide, and 0–1% concentration for sodium bisulphite as shown in Table 1, with the keratin hydrolysate and the yield of protein as the dependent variables. The extraction parameters and the ranges used were selected according to the method used in the previous work that was reported in the literature (Brother and Binkley, 1946; Jiang et al., 2008; Kamarudin et al., 2017).

Twenty-nine experimental runs were generated for the preliminary experiment and performed (Table 2); the experimental data were fitted into two polynomial quadratic model equations, this is done to be able to link the keratin extraction process variables with the response variables which include the percentage yield of keratin hydrolysate and protein, according to Equation (1).

$$Y = \alpha 0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{33} X_3^2 + \alpha_{12} X_1 X_2 + \alpha_{13} X_1 X_3 + \alpha_{23} X_2 X_3 \quad (1)$$

Where: Y represents the response output, $\alpha 0$ is the intercept, $\alpha_1 X_1$ to $\alpha_3 X_3$ are the linear coefficients, $\alpha_{11} X_1^2$ to $\alpha_{33} X_3^2$ are the quadratic coefficients and $\alpha_{12} X_1 X_2$ to $\alpha_{23} X_2 X_3$ represents the interaction of the coefficients. The use of the analysis of variance was employed to authenticate if the model is significant.

2.3. Keratin extraction and purification process

20 g of the treated and milled feathers was weighed and added to a 100 ml of alkaline solution, which represent (1:5) in ratio containing sodium hydroxide (NaOH) and sodium bisulfite ($NaHSO_3$) in varying concentration according to the experimental design in (Table 2). The pH of the solution ranged between 12 and 13. Chicken feather samples were placed in small steel containers with tight seals that were sealed with a cap and immersed in a hot oil bath set at the appropriate temperature. The temperature and reaction times were inputted and varied according to the parameters generated by the experimental design (Table 2). The turbid solution was then filtered after cooling and then neutralised by

Table 2
The different process parameters affecting keratin hydrolysate and the protein yield.

Run	A: Time (Mins)	B: Temperature (°C)	C: NaOH (%)	D: $NaHSO_3$ (%)	Response 1 Hydrolysate (%)	Response 2 Protein yield (%)
1	90	80	1.75	0.5	71.51	49.91
2	105	90	1.5	0.5	63.00	62.61
3	90	85	1.5	0.5	60.50	51.48
4	105	85	1.5	0	60.50	51.09
5	105	85	1.75	0.5	69.50	64.04
6	105	80	1.75	1	62.00	53.52
7	105	80	1.5	0.5	53.00	46.81
8	120	85	1.75	0	65.00	59.59
9	105	85	1.75	0.5	69.50	64.04
10	105	85	2	1	71.00	57.39
11	90	85	2	0.5	61.00	54.65
12	120	85	1.5	0.5	64.60	55.61
13	120	85	1.75	1	58.40	50.09
14	120	80	1.75	0.5	62.00	55.66
15	105	85	1.75	0.5	69.50	64.04
16	90	85	1.75	0	60.00	52.43
17	105	80	2	0.5	54.00	49.28
18	105	85	1.5	1	34.00	29.39
19	120	90	1.75	0.5	59.00	60.47
20	105	85	1.75	0.5	69.50	64.04
21	105	85	2	0	41.00	35.07
22	105	90	1.75	1	53.00	62.79
23	120	85	2	0.5	61.00	60.21
24	105	80	1.75	0	54.50	50.77
25	105	85	1.75	0.5	69.50	64.04
26	105	90	2	0.5	60.80	60.22
27	90	85	1.75	1	50.00	47.62
28	105	90	1.75	0	53.00	53.46
29	90	90	1.75	0.5	38.70	39.61

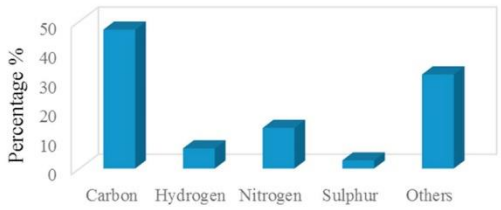

Fig. 1. Elemental analysis of keratin hydrolysate.

adding about 5 ml of HCl (2 M) dropwise. The filtrate was then dialysed using regenerated cellulose tubes (MWCO 3500-500 Da) in distilled water for 72 h, with changing of the outer water three times a day. The filtrate was removed after dialysis and freeze-dried to collect the keratin powder for storage in the cold-room.

2.4. Bradford assay

The Bradford assay was used for the detection and the quantification of protein concentration in the keratin hydrolysate; this was done using the Bradford (1976) procedure. The test works based on the capability of the protein solution to absorb the light on the wavelength from 595 nm (Bradford, 1976). Bovine Serum Albumin (Sigma-Aldrich, South Africa) was used to generate a linear calibration curve which was used as a standard. The sample used was prepared by weighing 35 mg of keratin hydrolysate powder and dissolved in 1 ml of deionised water, after that about 5μL of the prepared sample was mixed in a small tube with 200μL of the Bradford reagent (Sigma-Aldrich, South Africa). The keratin hydrolysate samples and the blank that contain deionised water and the Bradford assay reagent were allowed to stay for about 10 min before the absorbency was measured with a spectrophotometer (Promega GloMax Microplate Multimode Reader). After that, the amount of protein in the solution was determined by comparing the absorbance value obtained of the keratin samples to the calibration standard curve of BSA.

2.5. CHNS analysis

The protein content of the feather keratin powder was determined with the use of CHNS analyser (PerkinElmer, series II CHNS elemental analyser). The results from the equipment show the percentage of each element in the keratin hydrolysate, which are the content of carbon, hydrogen, nitrogen and sulphur in the keratin powder. After that, the content of nitrogen was used to quantify the percentage protein content in the keratin hydrolysate by using a factor that is commonly used to multiply the percentage of nitrogen that was given by the equipment, and this factor is 6.25 since most protein contains 16% nitrogen.

2.6. SDA-page electrophoresis

Electrophoretic separations of keratin protein molecular weight were done with the use of SDS-page gel electrophoresis technique. The analysis was carried out on 12% weight per volume polyacrylamide separating gel and 5% weight per volume polyacrylamide stacking gel for medium molecular weight (10–180 kDa). Low molecular weight (1.7–40 kDa) estimations were performed on 16% (w/v) tricine-sodium dodecyl sulphate polyacrylamide separating gel, and 5% (w/v) polyacrylamide stacking gel was also used (Haider et al., 2012). The sample preparation was done according to Wang et al. (2016) and Haider et al. (2012). 35 mg of the keratin extracts were dissolved with 1 ml of deionised water, and about 15 μL of protein sample mixed with 5 μL of 5μL of loading buffer containing about 0.25μL β-mercaptoethanol and 5 μL glycerinum. The denaturalisation of the protein was done by boiling the blend solution for about 6 min with loading buffer in a hot bath, after that about 7 μL of protein marker and 10 μL of denatured solution were loaded into gel well, respectively. The separation was performed at 80 V for 30 min, followed by 120 V for 60 min. After that, the gels were rinsed twice with distilled water before staining with Coomassie Brilliant Blue solution. The staining process was for about 30 min, after that the gel was destained overnight using 40% glacial acetic acid solution with shaking. Finally, the gel image was taken with an imaging instrument (Wang et al., 2016).

2.7. The chemical functional analysis using Fourier transform infrared spectroscopy (FTIR)

The chemical functional groups of keratin powder were analysed with the use of Fourier transform infrared spectroscopy (FTIR) spectroscopy. Universal attenuated total reflectance module was used for the spectra of the analysed samples in a wavenumber range between 3500 cm^{-1} and 550 cm^{-1}.

3. Results and discussions

3.1. Quantitative analysis of keratin hydrolysate

The quantitative analysis of keratin hydrolysate was determined by Bradford's assay method of protein estimation (Bradford, 1976). The process entails the dissolution of keratin powder in 1 ml of deionised water then 6 μL of the sample dilution was mixed with 200 μL of Bradford reagent. The assay was conducted in four replicates, and the protein concentration was determined by comparing the absorbance value of the keratin samples to the calibration standard curve of bovine serum albumin. The nitrogen content of the optimised sample was determined using a CHNS analyser (PerkinElmer, series II CHNS elemental analyser). The average protein concentration obtained with Bradford assay was 0.1 μg/ml whereas the average elemental composition of samples of keratin hydrolysate as shown by CHNS analysis was 13.85% of nitrogen, 47.25% of carbon, 6.90% hydrogen and 2.8% of sulphur, respectively (Fig. 1). The CHNS results are comparable to those reported in the literature (Tesfaye et al., 2017).

3.2. Molecular weight analysis using SDS-page

The molecular weight of the keratin hydrolysate was estimated by SDS-page using 12% polyacrylamide and 16% tricine-sodium dodecyl sulphate-polyacrylamide gel, respectively, which showed a big band of protein fractions between 10 and 15 kDa for the medium molecular weight and 3–10 kDa for the low molecular weight as shown in Fig. 2 a & b, respectively. The result of this study showed keratin with low molecular weight, which is comparable to the result obtained by Kamarudin et al. (2017) for the low molecular weight, as reducing agents was used in this study. This result also corroborates with the statement made by Floris and Slangen (2005) which stated that keratin protein mostly has a molecular weight of between 1 and 11 kDa (Floris and Slangen, 2007; Kamarudin et al., 2017).

3.3. Functional group analysis

FTIR spectra of the keratin protein hydrolysate are compared with feather meal, as shown in Fig. 3. The graph obtained from the keratin hydrolysate sample matched the standard graph of feather meal protein. The wavelength derived from the infrared spectroscopy also confirmed that C–N bond, N–H bond, C═O bond and disulphide bond are in the sample thus the peaks showed the presence of carboxyl group and amino groups the two common groups present in amino acids residues like cystine, threonine and glutamine are observed (Gupta et al., 2011). Therefore, the peaks in the spectra shows the characteristics of amide A, I, II and III conformation, besides it, was confirmed that the protein is present in the sample and comparable to what was obtained by other

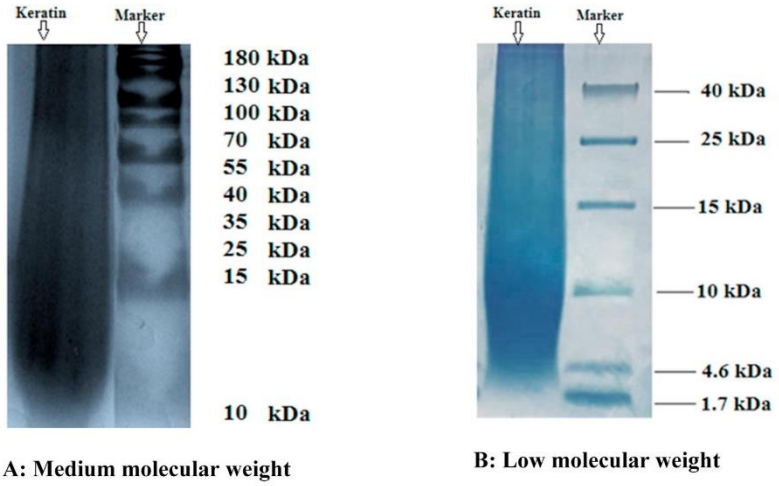

Fig. 2. SDS-PAGE of keratin protein hydrolysate.

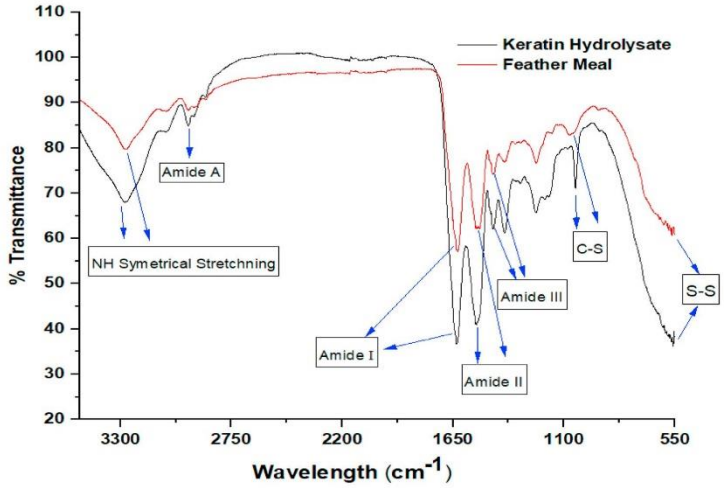

Fig. 3. FTIR spectra of keratin hydrolysate comparing with feather meal.

authors (Gupta et al., 2011; Wang et al., 2016; Tesfaye et al., 2017). The solid-state ^{13}C NMR spectrum revealed some chemical shift at 173–190 ppm, which is due to the carbonyl carbon of the amino acids. In addition, the aromatic carbons are seen at around 120–150 ppm while the α-carbon is seen at 60 ppm and the β-carbon at 42 ppm (Fig. 4). The alkyl side chains of the amino acids are shown between 10 and 30 ppm. The spectrum and the chemical shift observed were similar to what was seen by (Ghosh et al., 2019) from regenerated feather keratin, which used dimethyl sulfoxide (DMSO) to extract the keratin.

3.4. *The development of the response surface models*

The input data for the experiment and the output responses, which include, the percentage yield of keratin hydrolysate and the protein yield, are shown in Table 2. The data were used to form two quadratic polynomial model equations, which were developed to relate the percentage keratin hydrolysate and protein yield with the input variables, as shown in both Equations (2) and (3) below:

$$\text{Hydrolysate (\%)} = +69.50 + 2.36A - 2.46B + 1.10C - 0.47D + 7.45AB - 1.02AC + 0.85AD - 0.80BCE - 1.88BD + 14.13CD - 2.93A2 - 6.33B2 - 6.35C2 - 9.10D2 \quad (2)$$

$$\text{Protein yield (\%)} = +64.04 + 3.87A + 2.73B + 1.65C - 0.13D + 3.89AB + 0.36AC - 1.17AD - 1.22BCE + 1.64BD + 11.01CD - 4.46A2 - 3.49B2 - 7.35C2 - 8.67D2 \quad (3)$$

Where Y represents keratin hydrolysate yield and the percentage of

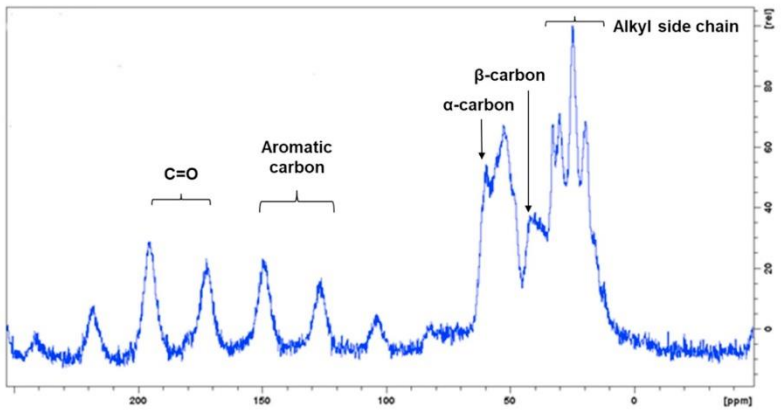

Fig. 4. The solid-state NMR spectrum showing the solubility of feather keratin hydrolysate.

Table 3
Analysis of variance (ANOVA) for hydrolysate and protein yield models.

Source	Sum of squares	Df	Mean square	F-value	P-value	R^2
Hydrolysate model	784.32	4.00	196.08	4.74	0.0125	0.77
Protein yield model	735.43	4.00	183.86	4.46	0.0156	0.74

Df: degree of freedom, F-value: Fisher-snedecor distribution value, P-value: probability value, R^2: coefficient of determination.

Table 4
Models coefficient of estimates with standard errors.

Factor	Hydrolysate CE	Hydrolysate SE	Protein yield CE	Protein yield SE
Intercept	69.50	2.88	64.04	2.87
A	2.36	1.86	3.87	1.85
B	−2.46	1.86	2.73	1.85
C	1.10	1.86	1.65	1.85
D	−0.47	1.86	−0.13	1.85
AB	7.45	3.21	3.89	3.21
AC	−1.02	3.21	0.36	3.21
AD	0.85	3.21	−0.17	3.21
BC	−0.80	3.21	−1.22	3.21
BD	−1.88	3.21	1.64	3.21
CD	14.13	3.21	11.01	3.21
A^2	−2.93	2.52	−4.46	2.52
B^2	−6.33	2.52	−3.49	2.52
C^2	−6.35	2.52	−7.35	2.52
D^2	−9.10	2.52	−8.67	2.52

CE-coefficient estimate, SE - standard error.

protein in the keratin hydrolysate for two equations, the letters A, B, C and D stand for the reaction time and temperature, percentage concentration of sodium hydroxide and sodium bisulphite, respectively, for Equations (2) and (3) respectively..

The authenticity of the fitted models was assessed with the use of the analysis of variance (ANOVA) as seen in Tables 3 and 4. The keratin hydrolysate and the percentage yield of protein models showed high F-values, that is, 4.74 and 4.46 with relatively low p-values of 0.0125 and 0.0156, respectively. While, the coefficient of determination (R^2) values for the two models were 0.77 (percentage yield of keratin hydrolysate) and 0.74 (percentage yield of protein), indicating that the models could account for about 77% and 74% of the variations in the experimental data. The p-values (<0.05) that are a bit low and the slightly high F calculated values shows that the polynomial models used were significance as shown in Table 3.

3.4.1. The linear effect of extraction variables on the hydrolysate and keratin protein yields

The yield of keratin hydrolysate and protein ranges between 34% and 71.51% and from 29.39% to 64.04%, respectively signifying how sensitive the recovery of the keratin hydrolysate to the input parameters.

3.4.2. Time and temperature

Extraction process carried out at the lowest reaction time (90 min), and temperature (80°*C*) gave a high keratin hydrolysate yield (71.51%) and slightly low protein yield (49.91%), respectively (Table 2). The process with the highest time (120 min) and temperature (90°*C*) gave keratin hydrolysate yield of (59%) and protein yield (60.47%), respectively. The process with time (120 min), temperature (85°*C*) and sodium hydroxide (1.75%) without sodium bisulphite (0%) produced a slightly high keratin hydrolysate yield (65%) and protein yield (59.59%). Compare to the previous studies, Kamarudin et al. (2017) obtained 43.8% and 41.5% keratin hydrolysate yield by treating feathers with sodium hydroxide and sodium sulphide respectively, with the temperature of 60°*C* for 2 h (Kamarudin et al., 2017); however, these results were lower than the yields obtained in this current study. Similarly, Poole et al. reported about 55% keratin yield using sodium sulphide at the temperature of 30 °*C* for about 24 h incubating under nitrogen gas (Poole et al., 2011), this yield is lower compared to the result in this present study. The variation in these studies with the current work could be due to differences in operation parameters and reducing agent employed. Because the structural nature of chicken feather biomass makes it difficult for smooth degradation, thus a suitable pre-treatment for extraction is required to release the locked up protein. Physical pre-treatment and extraction techniques, such as thermal treatment, inert atmosphere and chemical methods requiring acids or bases to break through the feather protective structures for the release of keratinous protein have been reported (Kamarudin et al., 2017). There are indications that the various extraction parameters and medium impact differently on the primary and secondary feather structure which result to variations in the keratin hydrolysate released and consequently protein yield (Poole et al., 2011). For instance, in the current study, extraction process carried out at 90 minutes reaction time, and 80°C process temperature gave a high keratin hydrolysate yield (71.51 %) and lower protein yield (49.91 %), compared to 120 minutes and 90°C

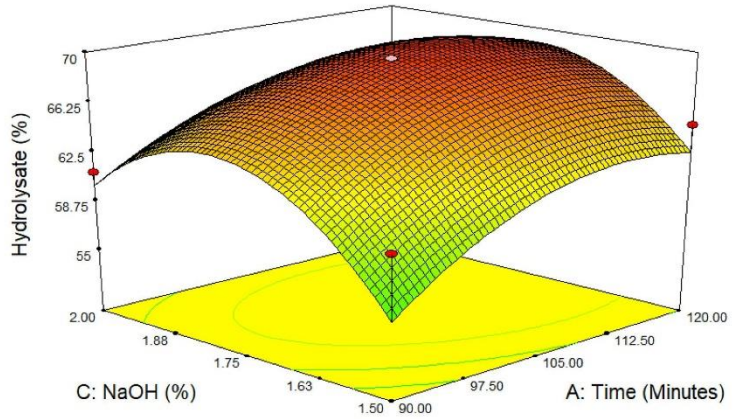

Fig. 5a. 3-D response surface plot showing the interaction of percentage NaOH and heating time on the keratin hydrolysate.

temperature process conditions, lower keratin hydrolysate yield of 59 % and higher protein yield of 60.47% were obtained. When the physical parameters interacted with the chemical parameters, a different set of results were observed. Process time of 120 minutes, temperature 85°C , sodium hydroxide of 1.75 % and sodium bisulphite (0 %) produced higher keratin hydrolysate yield (65 %) and protein yield (59.59 %). In comparison to previous studies, Kamarudin et al. (2017), which obtained 43.8 % and 41.5 % keratin hydrolysate yield by treating feathers with a mixture of sodium hydroxide and sodium sulphide, at 60°C process temperature for 2 h (Kamarudin et al., 2017). Similarly, the degree of degradation or solubilization of chicken feather is temperature dependant, when this is employed as a physical parameter. Hence, the need for optimum extraction parameters. The chicken feather structural becomes more accessible and solubilizes into desirable monomeric and soluble units when suitable chemical solvent and physical parameters such as heat are combined.

3.4.3. The concentration of NaOH and $NaHSO_3$

As shown in Table 2, the extraction carried out at 1.5% and 1% concentration of sodium hydroxide and sodium bisulphite, respectively showed a low yield of keratin hydrolysate (34%) and protein (29.39%), this might be because of high solid to liquid ratio and feather aggregation during the extraction process. However (Kamarudin et al., 2017), have reported a similar effect of these reducing agents concentration on keratin hydrolysate and protein yield from chicken feathers biomass. The highest keratin hydrolysate (71.51%) was observed in the mixture with sodium hydroxide (1.75%) and sodium bisulphite (0.5%) with a low temperature and time while the mixtures that contained the same concentration but with a relatively high temperature, (85°C) and time (105 min) gave the highest protein yields (64.04). This result is higher than the result obtained by Poole et al. (2011) which is 55% using sodium sulphide (Poole et al., 2011). Similarly, high keratin hydrolysate and protein yield were observed in experimental runs with sodium hydroxide (1.5% and 1.75%) without the addition of sodium bisulphite (0%) (Table 2).

3.4.4. The interaction of the experimental factors on the keratin hydrolysate and protein yield

The effects of the interaction of the extraction process variables were assessed with the use of the 3D response surface graphs. The assessment shows that the concentration of sodium hydroxide and the reaction time had a direct interaction on the yield of keratin hydrolysate (Fig. 5a)

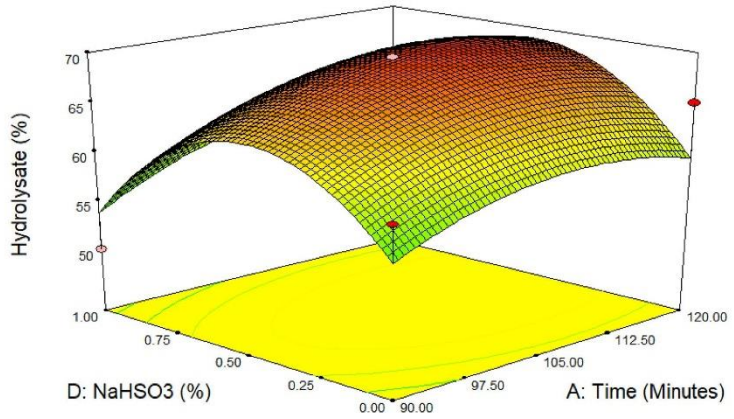

Fig. 5b. 3-D response surface plot showing the interaction of NaHSO3 and heating time on the keratin hydrolysate.

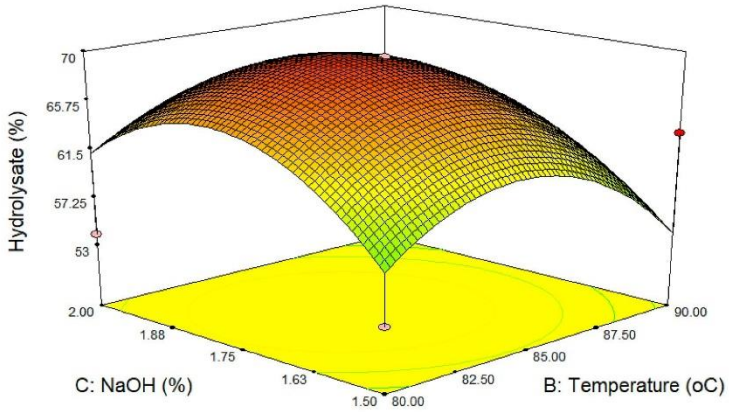

Fig. 5c. 3-D response surface plot showing the interaction of percentage NaOH and temperature on protein hydrolysate.

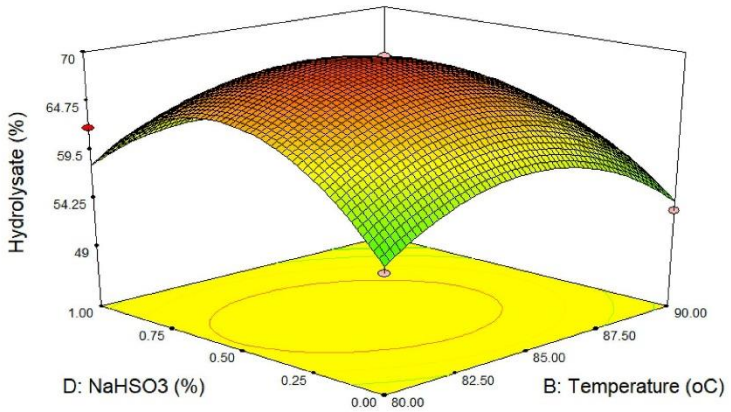

Fig. 5d. 3-D response surface plot showing the interaction of NaHSO3 and temperature on keratin hydrolysate.

when these parameters were varied from their lower to higher points. The same trend was observed with the protein yield. As shown in Fig. 5b, sodium bisulphite concentration and the extraction time similarly have a relationship that is linear on the keratin hydrolysate yield.

It was displayed in Fig. 5c, the graph shows that assuming the temperature of the reaction was maintained at around 86 °*C* and the concentration of sodium hydroxide was approximately 1.88%, the keratin hydrolysate yield also increased to about 70%. This pattern is similar to what was seen for sodium bisulphite and the temperature of the reaction (Fig. 5d).

The interactive effect of reaction time and the percentage yield of protein in the hydrolysate indicate that as the reaction temperature was maintained at the maximum level, as seen in Fig. 5e. The graph shows that the higher the reaction time and the temperature, the higher the protein yield obtained.

It can be seen in (Fig 6a-d) that the optimum condition is at the centre of the graphs; however, the maximum protein yield is observed at the sodium hydroxide and sodium bisulphite concentration of about 1.75% and 0, 5%, respectively and the reaction time of approximately 112 min. Likewise, the protein yield increases as the reaction temperature increases at sodium hydroxide and sodium bisulphite concentration of 1.88% and 0.5%, respectively. The influence of the extraction chemicals on the yield of protein is presented in Fig. 6e the graph shows that the highest protein yield is found at the sodium hydroxide concentration of (1.75% and 1.88%) and sodium bisulphite of about (0% and 0.5%), respectively. Hence, the strength of the keratin extraction solvent is one of the essential parameters because the higher the intensity, the more, the disulfide bonds will decrease resulting in shorter keratin particles (Kamarudin et al., 2017; Poole et al., 2011). The percentage keratin hydrolysate and the protein yield were higher than what was observed by (Kamarudin et al., 2017) that treated chicken feathers with sodium hydroxide and sodium sulphide respectively, with the temperature of 60 °C for 2 h. The outcome of these studies indicate that, the keratin yield of the hydrolysis depends on process pH, operating temperature and exposure time, and likewise on nature of acid or base and concentration of solvent used (Sinkiewicz et al., 2017).

3.4.5. *Validation of keratin extraction process*

The extraction parameters that include reaction time, temperature and the concentration of the reducing agents were optimised using design-expert software (Stat-Ease Inc., USA). The optimal setpoints based on the developed model were sodium hydroxide (1.78%), sodium

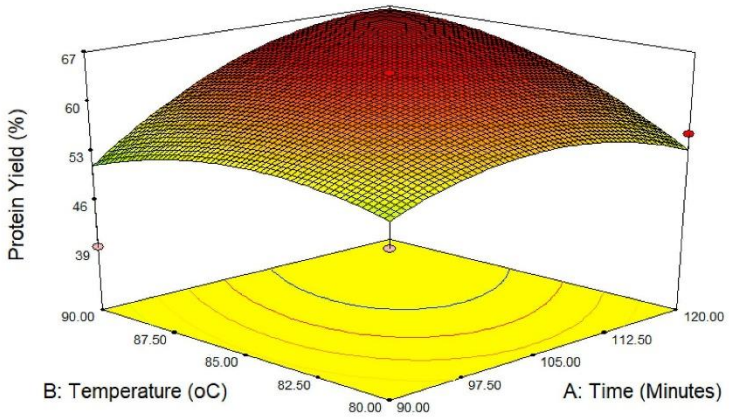

Fig 5e. 3-D response surface plot showing the interaction of temperature and heating time on the protein yield.

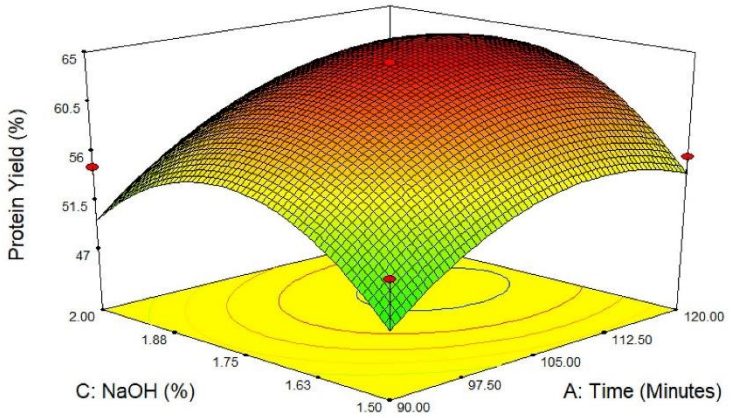

Fig. 6a. 3-D response surface plot showing the interaction of percentage NaOH and heating time on the protein yield.

bisulphite (0.5%) concentration, reaction time (111 min) and temperature (87 °C) respectively (Table 5). The optimised extraction process conditions based on the model predicted keratin hydrolysate yield and protein yield of 70.27% and 65%, respectively based on the optimal extraction parameters. The validation values were obtained from the software, and the experiment was carried out in triplicates and gave average keratin hydrolysate and protein yields of 68.29% and 65.21%, respectively (Table 5). This result shows a slightly lower keratin hydrolysate and protein yield than what was predicted by the model, which are 70.27% and 65.50% respectively. The difference might be because of higher decreased in disulfide bonds during the extraction process while most of the smaller keratin particles could have been lost during the dialysis process. This observation agrees with disulfide bonds spectrum of the FTIR analysis, where the disulfide bonds of the extracted hydrolysate were much lower compared to the feather meal used as control (Fig. 3). Furthermore, the reduction in amides A, I, II, and III as depicted by the spectra of this protein on the FTIR figure (Fig. 3), could also have accounted for the obtained lower protein yield compared to the predicted values. These results further underscore the importance of the choice of suitable extraction technique that reduces the negative impact of extraction conditions. Although, the yields observed were higher than, what was reported by (Kamarudin et al., 2017), which extracted keratin using sodium hydroxide and bisulphite mixture. Besides, compared to what was said by (Poole et al., 2011), using sodium sulphide at the temperature of 30 °C for about 24 h incubating under nitrogen gas, the yields from this current study were higher than the 55% yield that was observed by Poole et al.

Table 5
Optimum levels of variables during the keratin extraction process.

Independent variables		Predicted optimum levels
Time		111 Mins
Temperature		87 °C
NaOH		1.78%
$NaHSO_3$		0.5%
Response	**Predicted value**	**Observed value**
Hydrolysate	70.27%	68.29%
Protein yield	65.50%	65,21%

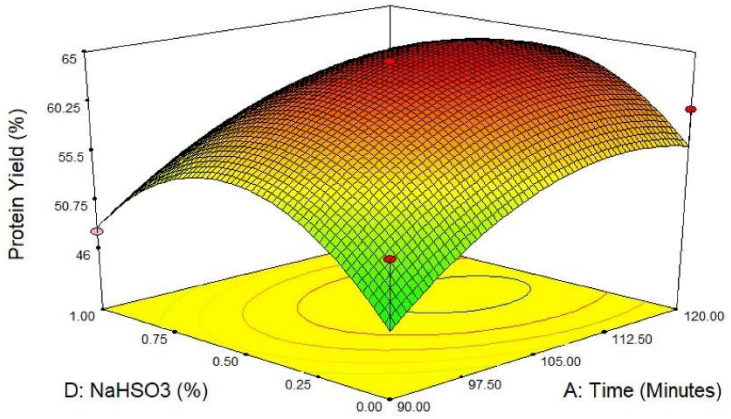

Fig. 6b. 3-D response surface plot showing the interaction of NaHSO3 and heating time on the protein yield.

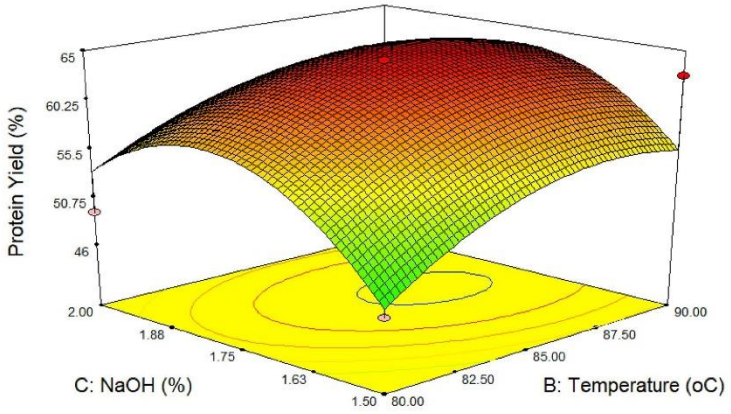

Fig. 6c. 3-D response surface plot showing the interaction of heating time and NaOH concentration on protein yield.

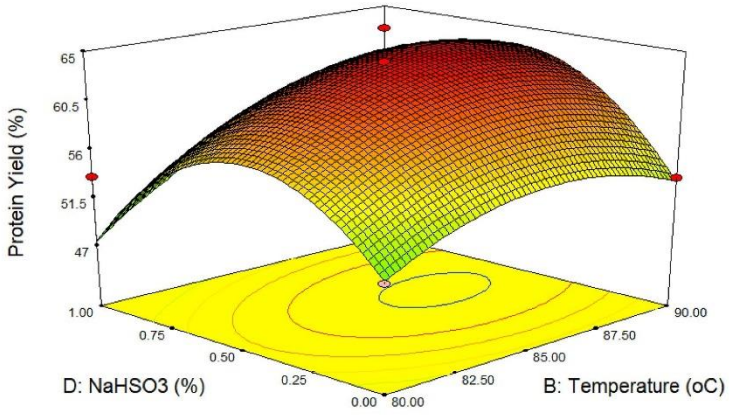

Fig 6d. 3-D response surface plot showing the interaction of NaHSO3 and temperature on protein yield.

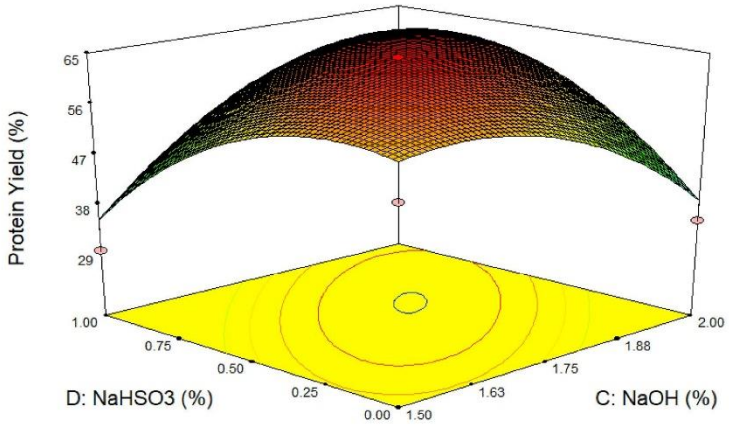

Fig 6e. 3-D response surface plot showing the interaction of percentage NaHSO3 and NaOH on protein yield.

4. Conclusions

The thermochemical process models connecting the extraction design of keratin hydrolysate and protein yield from chicken feathers subjected to hybrid pre-treatment of sodium hydroxide, sodium bisulphite and the heat was developed. The optimum extraction conditions were (1.78%) sodium hydroxide and (0.5%) sodium bisulphite concentrations, (111 min) reaction time, and (87 °C) temperature. The keratin hydrolysate and protein yield obtained from the optimum conditions were 68.29% and 65.21%, respectively. The determined optimum physico-chemical set points of sodium hydroxide, sodium bisulphite, dissolution time and temperature positively impacted protein yield from the waste chicken feathers. Under these conditions, the extracted protein structure was not denatured. The FTIR, CHNS and SDS-page results confirmed the extraction of the desired protein units, and this further underscores the suitability of operation parameters chosen. An additional interest lies in the availability, renewability and the global challenge in the disposal of the waste chicken feathers which fundamentally would influence the techno economics and the industrial process scalability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Olajumoke D. Fagbemi: Investigation, Methodology, Data curation, Writing - original draft, Formal analysis, Validation, Visualization. **Bruce Sithole:** Resources, Supervision, Funding acquisition, Writing - review & editing. **Tamrat Tesfaye:** Conceptualization, Supervision.

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APPENDIX B

CONFERENCE ABSTRACTS

EVENT: THE SOUTH AFRICAN INSTITUTION OF CHEMICAL ENGINEERS (SAICHE), DURBAN, SOUTH AFRICA, AUGUST 30/2017

VALORISATION OF CHICKEN FEATHERS: UTILISATION OF CHICKEN FEATHERS AS A BINDER IN THE FOREST PRODUCTS INDUSTRY

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Worldwide poultry consumption has led to the generation of large amounts of chicken feathers from poultry slaughterhouses; this is because about seven percent of chicken body weight is feathers. At present, the feathers from both small and large-scale poultry industries in most countries are disposed off in landfill, burned or processed to make a low-grade animal feedstock. Meanwhile, feathers are bio-resources with high protein content, which is a good source of natural adhesive. Feathers consists of about 91% keratin; a hard protein which can be chemically synthesized to produce a protein–based adhesive for use in wood composites industries. In this project, studies will be carried out to utilize chicken feathers for adhesive production. Feather keratin will be extracted using different extraction methods. The keratin will be modified using urea and phenol, also using different formulations, the binder will be characterised and the production process will be optimized. The binder properties will be characterised and the performances of the binder will be tested. The adhesive will be used as binder for medium density fiberboard(MDF), particleboard and plywood productions the physical and mechanical properties of the wood composites bonded with feather protein– based adhesives will be determined and compared to those using conventional resins like phenol and urea formaldehyde for their production.

Keywords: chicken feather, keratin, adhesive, wood composite

EVENT: THE SOUTH AFRICAN INSTITUTION OF CHEMICAL ENGINEERS (SAICHE), DURBAN, SOUTH AFRICA, AUGUST 15/2018

SYNTHESIS OF CELLULOSE NANOCRYSTAL BASED BIO-ADHESIVE FOR FOREST PRODCUSTS INDUSTRIES

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Chemical and natural bonding of wood components has played an essential role in the development and growth of the wood products industry and has been a key player in the efficient utilisation of the timber resources. Wood adhesives from natural sources were predominant adhesives used in the wood products industry, but displaced by synthetic adhesives derived from petrochemical sources. Synthetic adhesives used in wood-based industries are formaldehyde emitters, which causes environmental pollution and is detrimental to human health. This has led to renew interest in the natural sources of wood adhesives or starting materials for the production of wood adhesives. Cellulose nanocrystals/CNC are unique nanomaterials derived from the most abundant and almost inexhaustible natural polymer. These nanomaterials have several notable mechanical, optical, chemical, electrical and rheological properties. Their size, shape and charge lead to unique behaviour in solutions. The high chemical reactivity of the surface makes CNC customisable for various applications and their heat stability allows to be used in high temperature applications. Hence, they serve as a sustainable and environmentally friendly material for most applications. In this project, studies will be undertaken to utilise CNC obtained through acid hydrolysis for bio-based wood adhesive production. Due to hydrophilic nature of CNC biopolymer, polymer-grafting method will be used to modify it before adhesive production. The binder synthesis from CNC will be optimised by using different concentration of glyoxal aldehyde, CNC and other additives. Then the synthesised binder will be characterised for physical, rheological and mechanical properties. The performances of the binders will be tested on wood composites like particle-board and compared against commercial phenol and urea formaldehyde resins.

Keywords: CNC, keratin protein, adhesives, wood composites

EVENT: COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE RESEARCH DAY (CAES), UNIVERSITY OF KWAZULU-NATAL, DURBAN, SOUTH AFRICA, OCT 17/2019

BENEFICIATION OF WASTE CHICKEN FEATHERS AS SUSTAINABLE SOURCE OF BIO-ADHESIVE FOR WOOD COMPOSITE INDUSTRY

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Keywords: chicken feathers, keratin, bio-adhesive, wood composites

Poultry consumption has led to the generation of large amounts of waste chicken feathers from poultry slaughterhouses as about seven percent of chicken body weight is feathers. The amount of chicken feathers generated globally each year is estimated to be 15 billion tons with South Africa contributing about 258 million tons. At present, the majority of these feathers are disposed of in landfills or burned, creating environmental problems. These chicken feathers can be valorised by extracting valuable chemicals to be utilised as bio-binders. This is because, feathers contain about 91% keratin protein, a protein that can be chemically modified and be used as protein–based adhesives in the wood composites industries. The wood product manufacturers are the highest adhesive users, they are responsible for more than 65% by volume of the adhesives used and demand in the world. The adhesives from either synthetic or natural sources are one of the major bonding agents for wood composites production. While the synthetic adhesives commonly used in wood composites industries are formaldehyde-based, which emit formaldehyde, therefore causes environmental pollution and is detrimental to human health. Meanwhile, production of wood composites has continued to increase steadily because this has been playing an essential role in the development and growth of the wood products' industries. It has also been the key factor in the efficient utilisation of the timber resources. This review critically highlights the challenges posed by synthetic adhesives, and possible replacements by adhesives from natural sources including waste chicken feathers.

Beneficiation of waste chicken feathers as sustainable source of bio-adhesive for wood composite industry

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Key words: chicken feathers; keratin; bio-adhesive; wood composites

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VALORISATION OF CHICKEN FEATHERS: BENEFICIATION OF WASTE CHICKEN FEATHERS AS A BINDER

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Worldwide poultry consumption has led to the generation of large amounts of chicken feathers from poultry slaughterhouses; this is because about seven percent of chicken body weight is feathers. According to the literature an estimated 15 billion tons of chicken feathers are generated each year globally as a by-product of poultry processing. Chicken feathers are considered as a waste and currently their uses are economically marginal. At present, the feathers from both small and large-scale poultry industries in most countries are disposed of in landfills or burned creating environmental problem (small amounts are processed to make low-grade animal feedstock). However, feathers are bio-resources with high protein content, which is a good source of a natural adhesive. Feathers consists of about 91% keratin; a hard protein which can be chemically modified to produce a protein–based adhesive for use in wood composites industries. In this project, studies will be undertaken to utilize chicken feathers for adhesive production. Feather keratin will be extracted using alkaline hydrolysis extraction method. The keratin will be characterised and modified using formaldehyde and glyoxal as the crosslinking agents. In addition, different formulations will be used to form protein-based adhesives. Physical, rheological and mechanical properties of the binders will be characterized and the data used to optimise their production process. The performances of the binders will be tested on wood composites and particle board and compared against conventional phenol and urea formaldehyde resins. There is very high probability of producing effective binders from feathers and this will result in production of true bio- composite materials.

THEME: Material and Applications/ Processing and characterisation/ Analysis and Optimisation

KEYWORDS: chicken feathers, keratin, adhesives, wood composites

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SE-P-018

SYNTHESIS OF CELLULOSE NANOCRYSTAL BASED BIO-ADHESIVE FOR FOREST PRODUCTS INDUSTRIES

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Chemical and natural bonding of wood components has played an essential role in the development and growth of the wood products industry and has been a key player in the efficient utilisation of the timber resources. Wood adhesives from natural sources were predominant adhesives used in the wood products industry, but displaced by synthetic adhesives derived from petrochemical sources. Synthetic adhesives used in wood-based industries are formaldehyde emitters, which causes environmental pollution and is detrimental to human health. This has led to renew interest in the natural sources of wood adhesives or starting materials for the production of wood adhesives. Cellulose nanocrystals/CNC are unique nanomaterials derived from the most abundant and almost inexhaustible natural polymer. These nanomaterials have several notable mechanical, optical, chemical, electrical and rheological properties. Their size, shape and charge lead to unique behaviour in solutions. The high chemical reactivity of the surface makes CNC customisable for various applications and their heat stability allows to be used in high temperature applications. Hence, they serve as a sustainable and environmentally friendly material for most applications. In this project, studies will be undertaken to utilise CNC obtained through acid hydrolysis for bio-based wood adhesive production. Due to hydrophilic nature of CNC biopolymer, polymer-grafting method will be used to modify it before adhesive production. The binder synthesis from CNC will be optimised by using different concentration of glyoxal aldehyde, CNC and other additives. Then the synthesised binder will be characterised for physical, rheological and mechanical properties. The performances of the binders will be tested on wood composites like particle-board and compared against commercial phenol and urea formaldehyde resins.

Keywords: CNC, polymer grafting, adhesives, wood composites

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