



Extraction and assessing the reinforcing
properties of collagen derived from fish on the
mechanical properties of polymeric composite: A
case of epoxy resin and polyurethane

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ABSTRACT

Epoxy resin and polyurethane are among the commonly and widely used polymeric materials. However, the high brittleness of cured epoxy polymers promotes crack propagation and limits their impact resistance. Polyurethane (PU), on the other hand, while prized for its flexibility and biocompatibility, is mainly made from petrochemicals, which are now facing environmental concerns. In addition, natural biobased PU foams reportedly have a relatively low mechanical strength. To overcome this, reinforcing epoxy and PU with toughening agents such as collagen is proposed in the literature to enhance its mechanical properties. The fish scale has tremendous unexploited potential for adding these values to waste. Particularly, the fish scale consists of a high amount of collagen, a valuable biomaterial that has found many applications in many fields, including biomedical and pharmaceutical industries. Hence, fish scale-derived collagen has emerged as a promising natural filler, offering eco-friendly, cost-effective, high-performance reinforcement for polymeric composites. The study aims to extract fish scale-derived collagen (FSC) and assess its reinforcing properties as a filler on polymeric composite with special emphasis on epoxy and polyurethane.

A quantitative approach following an experimental research design was adopted in this study. The research design comprises three phases. In the first phase, collagen and milled fish scale powder were extracted from fish scale bio-waste using acid hydrolysis and ball-milling techniques, respectively. The extracted collagen was optimised by varying the extraction time, solvent concentration, solvent volume, and tris-glycine buffer. In the second phase, the extracted collagen (FSC) and milled fish scales powder (FS) were characterised with techniques such as X-ray diffraction, Fourier Transform Infrared spectroscopy, Scanning Electron Microscopy, and High-Transmission Electron Microscopy. Additionally, amino acid analysis was conducted to quantify and identify the type and amount of collagen extracted. In the third phase, epoxy (5, 10, 15, 20, and 25 wt.%) and polyurethane (0.5 and 1 wt.%), as well as laminates containing 2.5, 5, and 10 wt.%, were produced following ASTM standards. Mechanical properties, including tensile strength, elongation at break, tear resistance, and flexural strength, were evaluated according to ASTM standards for testing polymeric materials. The thermal stability of the composites was analysed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). One-way analysis of variance (ANOVA) was performed to assess differences in reinforcing properties ($\alpha = .05$). The validity of the study was verified using ASTM standards, while reliability was ensured through the processes of repeatability and reproducibility.

Paper 1 investigates the incorporation of milled fish scales (MFS) into ether-based polyurethane foam (PUF), revealing enhancements in mechanical properties such as tensile strength, elongation at break, and tear resistance, especially under heat-aging conditions. Paper 1 established that 0.5 wt.% FS improved tensile strength by 18.8% and tear resistance by 22% at elevated temperatures. Additionally, the composites demonstrated reduced density and enhanced flame-retardant properties, underscoring the material's suitability for lightweight and thermally resilient applications.

Paper 2 evaluates fish scale collagen (FSC) as a filler, highlighting its amino acid composition and superior mechanical performance. Paper 2 established that the addition of 2.5 wt.% FSC increased tensile strength by 12.66% during heat aging, while 5 wt.% FSC enhanced elongation by 6.65% at standard temperatures. Comparative analysis with traditional fillers, such as calcium carbonate, revealed that FSC could serve as a sustainable substitute without compromising mechanical properties. However, excessive FSC content negatively impacted material flexibility and strength, indicating an optimal concentration of 2.5 wt.%.

Paper 3 investigates the reinforcement of epoxy resin composites with fish scale-derived collagen (FSC) to enhance their mechanical and thermal properties. Results indicated significant improvements in tensile strength, Young's modulus, and thermal stability at lower FSC concentrations (5 and 10 wt.%), attributed to effective stress transfer and good interfacial bonding.

Overall, this research highlights the potential of natural materials to enhance mechanical properties while addressing sustainability concerns. The findings contribute to the development of eco-friendly and cost-effective materials for various applications, from construction to consumer products.



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DECLARATION

I, Deneshree Naidoo, hereby declare that this dissertation is wholly my work and that all the references to the best of my knowledge, are accurately reported. This work has not been submitted for a degree at any other university, and its only prior publication was in the form of conference papers, journal publications, and book chapters as listed below.

JOURNAL PAPER ARISING FROM THIS STUDY

Naidoo, D., Onwubu, S.C., Mokhothu, T.H., Mdluli, P.S., Mishra, A.K. 2023. Effect of milled fish scale powder reinforcement on physical properties of ether-based polyurethane foam composite. *Journal of Applied Polymer Science*. 2023, e54735. <https://doi.org/10.1002/app.54735>

S. Onwubu, **D. Naidoo**, Z. Obiechefu, T. Mokhothu, P. Mdluli, and A. Mishra, "Enhancing Mechanical and Thermal Properties of Epoxy Composites with Fish Scale-Derived Collagen Reinforcement," *Advances in Polymer Technology*, vol. 2024, no. 1, p. 8890654, 2024

D. Naidoo, S. Onwubu, T. Mokhothu, P. Mdluli, M. Makgobole, and A. Mishra, "Effectiveness of Fish Scale-Derived Collagen as an Alternative Filler Material in the Fabrication of Polyurethane Foam Composites," *Advances in Polymer Technology*, vol. 2024, no. 1, p. 1723927, 2024

Obiechefu, Z., Onwubu, S.C., **Naidoo, D.**, Mokhothu, T.H. and Mdluli, P.S., 2024. Comparison of Biowaste Fillers Extracted from Fish Scales and Collagen on the Mechanical Properties of High-Density Polyurethane Foams. *Polymers*, 16(19), p.2825

Makgobole, M.U., Onwubu, S.C., Baruwa, A., Mpofana, N., Obiechefu, Z., **Naidoo, D.**, Khathi, A. and Mkhwanazi, B., 2024. Optimization of Collagen Extraction from Fish Scales Using Tris-Glycine Buffer: A Taguchi Methodological Approach. *Marine Drugs*, 22(12), p.562.

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DEDICATION

This dissertation is dedicated to the cherished memory of my late grandfathers, J.A. Naidoo and D.G. Reddy, my late grandmother, A. Naidoo, and my late cousin, D. Govender. Their unwavering presence and wise guidance have profoundly influenced my academic journey. Additionally, I extend this dedication to the young ladies in my life, my sister Hailey Naidoo, and my nieces Ariyana Kieara Naidoo and Hailey Nikita Naidoo. May this thesis symbolize a pathway to their future achievements as woman and always strive for the best, as they continue to pursue their aspirations with resilience and determination.

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I extend my sincere gratitude to Dr. S C Onwubu for his unwavering commitment to my research. His constant assistance and dedication to excellence have been invaluable. He consistently goes above and beyond to ensure that every aspect of the work is thorough and of the highest quality. His attention to detail and willingness to help at every stage have been instrumental in the success of this project.

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ACRONYMS

ANOVA	Analysis of Variance
BF	Basalt microfiber
BP	Basalt powder
CFRPs	Carbon fiber-reinforced polymers
CWF	Cool white fluorescent
DOE	Design of Experiments
DSC	Differential Scanning Calorimetry
DUT	Durban University of Technology
EFC	Epoxy Fish Scale-Derived Collagen Composites
FBP	Fishbone-based
FESEM	Field Emission Scanning Electron Microscopy
FSC	Fish scale-derived collagen
FSC	Fish Scale Collagen
FTIR	Fourier Transform Infrared Spectroscopy
HS	Hard segment
MFSP	Milled Fish Scale Powder
PBS	Phosphate-buffered saline
PU	Polyurethane
PUF	Polyurethane Foam
RH	Rice husk
SA	Sunflower husk ash
SN	Signal-to-noise ratios
SS	Soft segment
TDI	Toluene Di-isocyanate
TFBPs	Thermally treated FBPs
TGA	Thermogravimetric Analysis

TPU	Thermoplastic polyurethane
XRD	X-ray Diffraction

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1.1 BACKGROUND AND CONTEXT OF THE STUDY

Polymeric composites are materials composed of a polymer matrix reinforced with fillers or fibers to improve their mechanical, thermal, and chemical properties. These composites are extensively used in industries such as aerospace, automotive, and construction due to their customizable properties, lightweight nature, and high strength-to-weight ratios (Giménez *et al.* 2022; Dallaev *et al.* 2023). Among commonly used polymer matrices, epoxy resins and polyurethane foams (PUF) stand out due to their versatility and superior performance. Epoxy resins are renowned for their excellent chemical resistance, mechanical strength, and thermal stability, but their intrinsic brittleness limits their impact resistance (Saba and Jawaid 2017; Zhao *et al.* 2022). Similarly, PUF materials are prized for their flexibility, biocompatibility, and tunable mechanical properties, making them suitable for applications ranging from insulation to automotive interiors (Alyamaç *et al.* 2022).

Despite their widespread applications, traditional polymer production heavily depends on petrochemical derivatives, raising significant environmental concerns (Gama, Ferreira and Barros-Timmons 2018; Członka *et al.* 2020a). Moreover, the mechanical properties of bio-based alternatives, such as soy-based PU, often fall short of those of their petroleum-based equivalents (Alyamaç *et al.* 2022). To address these issues, researchers have increasingly investigated the integration of natural fillers and reinforcements to improve the mechanical and thermal qualities of polymeric composites while promoting sustainable materials development (Lionetto and Esposito Corcione 2021). Natural fillers sourced from agricultural and marine waste are gaining popularity for their renewability, cost-effectiveness, and environmental advantages (Sienkiewicz, Dominic and Parameswaranpillai 2022). Previous research has demonstrated that natural fibres such as wood fibre (Choe, Sung and Kim 2018), sawdust (Tiuc *et al.* 2019), eggshells (Maamoun *et al.* 2023), and coconut husks (Kuranchie, Yaya and Bensah 2021) can substantially enhance the mechanical properties of polymer composites. Nevertheless, the potential of fish scales - a marine waste product rich in collagen — remains underexplored despite their promising mechanical and biocompatible characteristics.

Fish scales, a byproduct of the seafood industry, are a rich source of collagen, a structural protein characterised by high tensile strength, stiffness, and biodegradability (Lionetto and Esposito Corcione

2021; Naidoo *et al.* 2023). Unlike plant-based fibers, collagen exhibits a unique hierarchical structure that can enhance composite performance through improved adhesion and mechanical interlocking within polymer matrices (Rawat *et al.* 2021; Naidoo *et al.* 2023). Utilising fish scale-derived collagen (FSC) not only addresses waste management challenges but also aligns with the global sustainable development goals (Resolution 2015). Studies have demonstrated the effectiveness of natural fillers in enhancing polymer properties. For instance, Zhang and Hu (2019) found that hydroxyapatite fillers improved the surface wettability and biocompatibility of PUF composites, while Choe, Lee and Kim (2020) reported enhanced mechanical properties with calcium carbonate fillers. Matykiewicz *et al.* (2021) reported an increase in impact strength for epoxy resins reinforced with natural fillers such as basalt powder (BP), basalt microfiber (BF), and sunflower husk ash (SA). Given collagen's exceptional mechanical properties and abundance, fish scale-derived collagen (FSC) presents an opportunity to develop eco-friendly composites with superior performance.

Existing literature has predominantly focused on plant-based reinforcements or other marine-derived materials such as chitosan and hydroxyapatite (Mustafov, Sen and Seydibeyoglu 2020; Maamoun *et al.* 2023). While these studies highlight the potential of natural fillers, limited research has investigated FSC as a reinforcement material in polymeric composites, particularly in PUF and epoxy resin matrices. Furthermore, the influence of varying collagen concentrations and processing conditions, such as temperature, on the mechanical and thermal properties of these composites remains largely unexplored. According to Márquez Costa, Legrand and Fréour (2019), temperature can significantly influence the characteristics of composite materials, particularly their mechanical and thermal properties. Moreover, the successful incorporation of FSC into polymer matrices hinges on factors such as filler concentration, dispersion, and interaction with the polymer matrix (Šupová 2009; Šupová, Martynková and Barabaszová 2011). Ultimately, understanding these intricate molecular-level interactions is crucial for optimising composite formulation and processing, thereby ensuring enhanced mechanical properties and overall performance (David 2020; Amirrah *et al.* 2022). This study aims to address these gaps by systematically extracting collagen from fish scales and evaluating its reinforcing properties in epoxy resin and PUF matrices. By incorporating FSC in varying weight percentages, the study seeks to assess its impact on mechanical properties, such as tensile strength, elongation, and tear resistance, as well as thermal stability and surface morphology. Advanced characterisation techniques, including Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infra-red (FTIR), X-ray diffraction (XRD), and Thermogravimetric Analysis (TGA), were employed to provide comprehensive insights into the material's behaviour.

1.2 PROBLEM STATEMENT

The problem this study aims to address is twofold: the environmental concerns stemming from the excessive use of polymers derived from the petrochemical industry and the challenges associated with utilising natural fillers in polymer composites. The overuse of synthetic polymers has raised significant environmental concerns due to their limited biodegradability. These polymers, characterised by high molecular weight and hydrophobic properties, persist in the environment, contributing to pollution and ecological degradation (Angelini *et al.* 2015; Boaventura *et al.* 2020). According to Boaventura *et al.* (2020), the slow rate of environmental degradation for synthetic polymers necessitates innovative approaches to promote sustainability. One promising solution is the development of biodegradable materials by combining synthetic polymers with natural polymers or agricultural waste. This approach maximizes the use of renewable raw materials while minimising waste accumulation (Avolio *et al.* 2015; Cholake *et al.* 2017; Al-Oqla and El-Shekeil 2019).

Collagen, a highly abundant natural biopolymer, has gained attention for both biomedical and non-biomedical applications (Kulkarni and Maniyar 2020). Traditionally sourced from mammals such as pigs and cows, collagen extraction has faced challenges due to the risk of zoonotic diseases, such as bovine spongiform encephalopathy. Consequently, there is growing interest in alternative non-mammalian sources of collagen, such as fish scales, which are not only abundant but also cost-effective (Kulkarni and Maniyar 2020). Fish scales contain valuable organic components, including collagen, making them an attractive resource for developing environmentally friendly composites (Boaventura *et al.* 2020; Kulkarni and Maniyar 2020). Utilising fish scale-derived collagen for polymer matrix reinforcement aligns with sustainable development goals by adding value to marine waste while promoting a greener environment. While bio-based fillers, such as fish scale-derived collagen, offer environmental benefits and enhanced mechanical properties, their application poses significant challenges. These include inconsistencies in quality, high production costs, limited thermal stability, reduced durability, and poor compatibility with synthetic polymers (Bari, Morrell and Sistani 2019; Murawski *et al.* 2019; Chang, Mohanty and Misra 2020; Andrew and Dhakal 2022). Moreover, the performance of collagen-reinforced composites is often affected by the variability in collagen properties depending on its source and extraction method (Pati, Adhikari and Dhara 2010; Kane 2012). Collagen's susceptibility to degradation under environmental conditions, such as high humidity and temperature, further complicates its use in long-term applications (Pati, Adhikari and Dhara 2010; Sallach *et al.* 2010; Curto *et al.* 2021).

Additional challenges lie in the scaling up of fish-scale collagen production. These include optimising extraction methods, ensuring uniform dispersion within the polymer matrix, and enhancing mechanical and thermal properties to achieve consistent material performance (Bajpai 2019; Yin and Woo 2024b). As Sharabi *et al.* (2016) emphasise, the development of collagen-reinforced composites must prioritise addressing these challenges while optimising material properties for specific industrial uses. To overcome these challenges, further research is required to develop standardised extraction and purification methods for fish scale collagen and improve its compatibility with polymer matrices (Pati, Adhikari and Dhara 2010; Donkor *et al.* 2023; Var and Palamutcu 2024). This research aligns with global efforts to enhance the reliability and performance of collagen-based composites while supporting environmental sustainability (Gaikwad and Kim 2024; Var and Palamutcu 2024). By systematically addressing the outlined challenges, this study seeks to contribute to the advancement of sustainable composite materials with improved mechanical, thermal, and durability properties, enabling their application across various industries.

1.3 AIM

The study aims to extract fish scale-derived collagen (FSC) and assess its reinforcing properties as a filler on polymeric composite with special emphasis on epoxy and polyurethane.

1.4 OBJECTIVES

The research objective was achieved in three phases, namely:

Objective One: Preparation and extraction of fish scales collagen (FSC) from fish scale waste

The sub-objectives are to:

- Prepare milled fish scales (FS) using alkaline hydrolysis and mechanochemical method
- Extract collagen (FSC) from fish scale biowaste using acid hydrolysis and tris glycine buffer.
- Optimize the extraction conditions (Concentration of the acid, volume of the acid, soaking time, and buffer) using Minitab design software.

Objective Two: Physicochemical characterization of the extracted MFSP and FSC

The sub-objectives are to:

- Characterized the surface morphology, particle size, shape, and crystallinity, and functional group using different analytical techniques such as X-ray diffraction, Fourier Transform Infrared, Scanning Electron Microscope, and High Transmission Electron Microscope

- Quantify and determine the amount and type of collagen extracted using amino acid analysis

Objective Three: Assess the Mechanical, Physical, and Thermal Properties of the reinforced Composites

The sub-objectives are to:

- Determine the mechanical Properties of Epoxy Resin Composite, such as the tensile strength, flexural properties, and hardness reinforced with varying concentrations of FSC (5, 10, 15, 20, and 25 wt.%).
- Determine the tensile strength, elongation, tear resistance, and density of PUF foams reinforced with varying concentrations of fish scales (0.5 and 1 wt.%) and FSC (2.5, 5, and 10 wt.%) at different temperature conditions.
- Determine the cell count and visual colour differences of PUF foams under temperature conditions.
- Microscopically assess the microstructure of Epoxy Fish Scale-Derived Collagen Composites (EFC) as well as evaluate the cell structure of PUF foams.
- Assess the thermal properties and stability of Epoxy Fish Scale-Derived Collagen Composites (EFC) using thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC).
- Analysis of the microstructural interaction of the polymers and filler content of both epoxy and PUF foams using FTIR

1.5 RATIONALE AND SIGNIFICANCE OF THE STUDY

Although collagen has numerous applications in the biomedical field, its high cost remains a significant barrier to broader use (Kulkarni and Maniyar 2020). With the growing demand for collagen in the pharmaceutical industry, researchers over the past decade have focused on identifying cost-effective sources and optimising extraction methods. From an economic perspective, fish scales offer a promising alternative as an inexpensive biomaterial for industrial applications, a factor crucial for enhancing the sustainability of the South African economy. Often dismissed as waste, fish scales are rich in valuable organic and inorganic materials, containing 40–55% collagen, ~38%–46% inorganic components (calcium-deficient hydroxyapatite, calcium phosphate, etc.) and trace elements (such as magnesium, iron, zinc, calcium), making them a viable resource for collagen extraction (Boaventura *et al.* 2020; Kulkarni and Maniyar 2020). As such, fish scale waste has tremendous but rather unexploited biomaterial that could potentially add value to waste.

Fish scales, a readily available waste product, provide a valuable resource for collagen extraction. Repurposing this waste contributes to the circular economy, reduces environmental pollution, and promotes sustainable marine resource management (Kulkarni and Maniyar 2020; Yin and Woo 2024a). Furthermore, utilizing fish scale-derived collagen for developing biodegradable composites not only minimizes reliance on non-biodegradable synthetic polymers but also presents cost-effective alternatives to traditional materials. This, in turn, creates economic opportunities in waste management and composite manufacturing sectors (Castellan *et al.* 2011; Väisänen, Das and Tomppo 2017).

From an environmental perspective, the over-reliance on synthetic polymers derived from petrochemical sources has raised critical sustainability concerns. These polymers, characterized by their limited biodegradability, contribute to environmental pollution and disrupt ecological balance (Angelini *et al.*, 2015; Boaventura *et al.*, 2020). Addressing these issues necessitates the development of alternative, eco-friendly materials to replace or complement synthetic polymers. The incorporation of natural biopolymers like collagen into polymeric composites offers a promising solution to mitigate these challenges. Furthermore, the use of fish scale-derived collagen aligns with global sustainability efforts by promoting eco-friendly polymeric composites that address pressing environmental concerns (Al-Oqla and El-Shekeil 2019; Boaventura *et al.* 2020). These initiatives support South Africa's National Development Plan (NDP 2030) (National Planning Commission 2013) and contribute to achieving the United Nations Sustainable Development Goal (SDG 13), which emphasises climate action and fostering a greener environment (United Nations Development Programme 2023).

1.6 DELIMITATION/SCOPE OF THE STUDY

While collagen can potentially be extracted from a variety of bio-waste sources, including shells, porcine teeth and bones, and bovine bones, this study exclusively focuses on extracting FSC from fish scales. This decision is based on the abundance of waste materials available in South Africa. While there are numerous polymeric materials, the study solely focused on epoxy and polyurethane foams. This is motivated by their popularity and wide applications in various fields, including construction, biomedical, automobile, and aerospace.

1.7 STRUCTURE OF THE DISSERTATION

This study consists of five chapters:

Chapter 1: Introduction

This chapter provides a general background and provides a context for the study. This chapter also contains the problem statement, the aim, and the specific objectives of the research.

Chapter 2: Literature Review

An overview of the literature on polymer composites and their various applications. The review centres on epoxy and PUF as case studies. Collagen as a source of filler material was introduced. Particularly, the various sources of collagen extraction, including fish scales, will be presented. The chapter will also cover the mechanical and thermal properties of biobased composite materials and provide a detailed empirical review of their applications in epoxy and polyurethane.

Chapter 3: Research Methodology and Design

Detailing the specifics of the quantitative research paradigm, parameters, and experimental research design that is to be adopted in this study.

Chapter 4: Critical discussion

Provides a critical discussion of the study findings. The linkages of the published papers were presented with a view of addressing the research objectives.

Chapter 5: Conclusion and Recommendations

A conclusion based on the results obtained and the hypothesis will be given as well as recommendations on future work and appropriate actions based on the observations and results obtained.

1.8 CONCLUSIONS

Chapter one foregrounds the background and context of the need to reinforce polymeric composites such as epoxy and PUF foams. Although these composites are highly desirable, their mechanical properties have limited certain applications. The chapter, therefore, provides justification and rationale for reinforcing the composites to enhance their mechanical properties. The argument and rationale for the use of fish scale-derived collagen as natural bio-filler materials were provided in the chapter. The next chapter will provide a detailed overview of the properties of polymer composites as well as collagen derived from fish scales.

2.1 INTRODUCTION

The integration of natural materials into polymer composites to enhance mechanical properties while prioritizing environmental sustainability has gained significant attention in recent years. This chapter reviews literature related to the use of biowaste in reinforcing polymeric composites. The review delves into key research in this area, focusing on the use of natural materials, specifically fish scale-derived collagen as reinforcements in polymer composites to improve mechanical properties and support environmental sustainability.

2.2 INTRODUCTION TO POLYMER COMPOSITE

2.2.1 OVERVIEW OF POLYMERS

Polymer composites are materials composed of a polymer matrix reinforced with various fillers, fibers, or particles to enhance their mechanical, thermal, or chemical properties (Gupta, Kumar and Sachdeva 2024). The polymer matrix, typically thermosetting or thermoplastic, provides the shape and cohesion of the composite (Asim *et al.* 2018), while the reinforcement improves the overall performance, making polymer composites more versatile than pure polymers (Mittal *et al.* 2018). Polymer composites are widely used across various industries due to their excellent balance of lightweight, high strength, and resistance to environmental factors such as corrosion (Suyambulingam *et al.* 2024; Tusher, Imam and Khatun 2024). Polymers are key products in all industries, from food and beverage packaging (Vallejos *et al.* 2022) to transportation and the medical industry (Oladele, Omotosho and Adediran 2020). In the automotive industry, polymer composites are used to reduce vehicle weight, thus improving fuel efficiency and reducing emissions (Muhammad *et al.* 2021). In aerospace, they provide structural components with high strength-to-weight ratios (Singh *et al.* 2024). In biomedical applications, polymer composites are utilised in prosthetics, implants, and drug delivery systems because of their biocompatibility and durability (Egbo 2021). Construction industries use polymer composites for materials like fiber-reinforced concrete and structural panels, benefiting from their enhanced durability and resistance to weathering (Shafei *et al.* 2021).

In the polymer industry, the growth in bio-based, biodegradable, and recycled materials has a huge positive impact on the environmental research and development field (Chang, Mohanty and Misra 2020). With a massive growth in the development of science, especially in polymer fields, it is of good concern to develop and synthesize new and improved methods and materials used in our daily lives for cost-effectiveness, reducing toxic pollutants such as emissions, and using up waste products of everyday human lifestyle to develop newer biomaterials (Chang, Mohanty and Misra 2020). As many

of the polymers currently used in different industrial applications are harmful to the environment (Yao, Seong and Jang 2022; Groh *et al.* 2023), it is necessary to incorporate eco-friendly and recycled ways to manufacture polymeric materials to improve the quality (strengthening) of the product and reduce the build-up of food product waste, causing numerous environmental issues. Besides, the versatility of polymer composites comes from the ability to tailor their properties by selecting appropriate matrix materials and reinforcements (Suyambulingam *et al.* 2024). This adaptability allows composites to meet specific requirements for different industries, making them integral to advanced material engineering.

The key to improving the performance of polymer composites lies in the reinforcement materials, which can be in the form of fibers (e.g., glass, carbon, aramid), powders, or particles (e.g., nanoparticles) (Cantor and Watts 2011; Rashid *et al.* 2024). The main function of these reinforcements is to enhance the mechanical properties of the composite, including strength, stiffness, and durability, without adding significant weight (Majhool *et al.* 2019; Jafari *et al.* 2020; Rawat *et al.* 2021; Vaggar, Kamate and Nadaf 2022). Reinforcements work by distributing loads more efficiently throughout the composite (Awang Ngah 2013; Zhou, Fan and Chen 2016). Nilagiri Balasubramanian and Ramesh (2018) note that better improvement in mechanical, thermal, and electrical properties could be attained along the filler-aligned direction. Fibers, for instance, align along the direction of applied stress, allowing the composite to bear heavier loads and resist deformation. The inclusion of powders or particulate fillers, such as silica or calcium carbonate, can also improve the hardness and wear resistance of the composite, contributing to its longevity in demanding applications (Muraliraja *et al.* 2021; Fajdek-Bieda and Wróblewska 2024). Carbon fiber-reinforced polymers (CFRPs) are known for their high tensile strength and low weight, making them essential in the aerospace and automotive industries (Ozkan, Gok, and Karaoglanli 2020). Similarly, the addition of glass fibers into polymer matrices provides improved thermal and electrical insulation, ideal for electronic components (Dhanasekar *et al.* 2022). These reinforcements also contribute to improved thermal stability, chemical resistance, and even fire retardancy in certain composites. Given the beneficial properties of reinforcement in enhancing the properties of polymeric materials, this study envisaged reinforcing epoxy and polyurethane polymers with a filler using recycled marine waste material, such as fish scales and collagen. The next section reviews the properties of epoxy and polyurethane polymers.

2.2.2 EPOXY RESIN AND POLYURETHANE AS MATRIX MATERIALS

Two of the most widely used matrix materials in polymer composites are epoxy resin and polyurethane, both of which provide a range of desirable properties for industrial applications.

2.2.2.1 Epoxy

Epoxy resin is a thermosetting polymer known for its excellent mechanical strength, chemical resistance, and adhesive properties (Sukanto *et al.* 2021). It forms a highly cross-linked structure when cured, making it highly resistant to environmental factors such as moisture, heat, and chemicals. Because of its strength and durability, epoxy resin is commonly used as a matrix material in high-performance composites, especially in the aerospace, automotive, and construction industries. Epoxy polymers stand out as popular polymer matrices for composite applications owing to their exceptional mechanical properties, chemical resistance, and thermal stability (Giménez *et al.* 2022; Capretti *et al.* 2023). Despite these strengths, epoxy polymers exhibit limitations such as low impact resistance, poor toughness, and susceptibility to crack propagation (Saba and Jawaid 2017). To improve the physical and mechanical properties of this polymer, several current reinforcing materials, such as natural fibers, glass, and graphite, have been added to epoxy to such an extent that epoxies can be used in many structural applications (Saba *et al.* 2016a).

Epoxy resins are widely used in composites reinforced with glass or carbon fibers, where they enhance the material's tensile and flexural strength. In aerospace and automotive applications, epoxy-based composites provide a lightweight yet strong alternative to traditional metals, contributing to fuel efficiency and improved performance. Additionally, the biocompatibility of epoxy resins makes them suitable for use in biomedical devices such as prosthetics, implants, and medical adhesives. According to Fink (2013), epoxy resins are favoured in composite manufacturing because of their low shrinkage upon curing, excellent adhesion to a wide variety of substrates, and superior mechanical performance under a broad range of conditions. From a chemistry perspective, epoxy resins represent a crucial and highly adaptable category of cross-linkable polymers, derived from monomers containing at least two oxirane groups (Hodgkin 2001). These strained-ring structures, composed of one oxygen and two carbon atoms, are attached to a diverse array of aliphatic or aromatic organic molecules (Hodgkin 2001). Most commercially significant epoxy resins are synthesized through the reaction of polyphenolic compounds or other active hydrogen-containing molecules (such as amines or aliphatic alcohols) with epichlorohydrin in basic conditions. Among these, bisphenol A (diphenylolpropane) is one of the most widely used phenolic compounds, forming the foundation for an entire family of epoxy resins with a characteristic general structure (Figure 2.1). Note that the symbol “n” in epoxy resin denotes the number of repeating units in the polymer chain, which directly influences the molecular weight, viscosity, and physical properties of the resin.

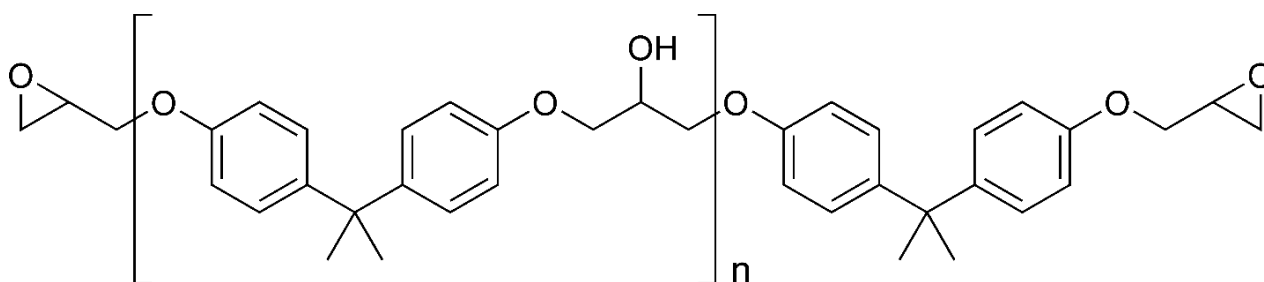


Figure 2.1: Structure of epoxy resins

2.2.2.2. Polyurethane

Polyurethane (PUF) is another versatile polymer matrix widely used in composites. Unlike epoxy resin, which is primarily a thermosetting polymer, polyurethane can be either thermosetting or thermoplastic, depending on its formulation. Polyurethane composites are known for their flexibility, abrasion resistance, and impact strength, making them useful in both structural and non-structural applications. Polyurethanes are used in the making of elastomeric fibers known as spandex and in the production of soft and rigid foam that can be found in mattresses, couches, liquid coatings, and paints, as insulation in walls and roofs, to thermoplastic polyurethane used in medical devices and footwear, to coatings, adhesives, sealants, and elastomers used on floors and automotive interiors, tough elastomers such as roller blade wheels, soft flexible foam toys, some elastic fibers, and many other applications as well.

Polyurethane foams (PUF), widely used in various industries, are versatile materials known for their lightweight nature, insulation properties, and affordability (Mohammadpour and Mir Mohamad Sadeghi 2020; Kaikade and Sabnis 2023). In engineering, for example, PUF is widely used due to its tunable mechanical and biocompatible properties, which can be tailored by the type and composition of its components (Alyamaç *et al.* 2022). In the construction industry, polyurethane-based composites are used for insulating panels and coatings due to their thermal insulation properties. In the automotive and transportation sectors, polyurethane composites provide shock-absorbing components such as bumpers and seat cushions. The biocompatibility of polyurethane also allows it to be used in medical devices such as catheters and artificial organs, where flexibility and durability are essential.

Polyurethane (PUF) polymers are synthesized through a reaction between diisocyanates and polyester diols (Gama, Ferreira and Barros-Timmons 2018; Kaikade and Sabnis 2023), a fundamental process in the polyurethane industry. The PUF macromolecule consists of alternating non-polar segments: the

hard segment (HS), derived from aliphatic or aromatic isocyanates, and the soft segment (SS), typically derived from macrodiols such as polyester, polyether, or polycarbonate-based compounds (Figure 2.2). Each segment contributes distinct properties to the polymer. The HS imparts stiffness, abrasion resistance, and film-forming capabilities, while the SS enhances elastomeric properties (Wang *et al.* 2015a). The chemical composition of these segments significantly influences the overall characteristics of PUF materials (Patti *et al.* 2021). For instance, aliphatic isocyanates in the HS improve environmental stability compared to their aromatic counterparts (Malik and Kaur 2018). Similarly, polyester-based polyols in the SS provide high tensile strength and excellent resistance to light and aging, while polyether-based polyols enhance chain flexibility and elasticity but are more susceptible to degradation by heat, light, and oxygen. Polycarbonate-based polyols, on the other hand, offer superior hydrolysis and aging resistance, along with good mechanical strength (Cakić *et al.* 2013). In diisocyanates, R is the variable organic group that separates the two isocyanate (–NCO) groups.

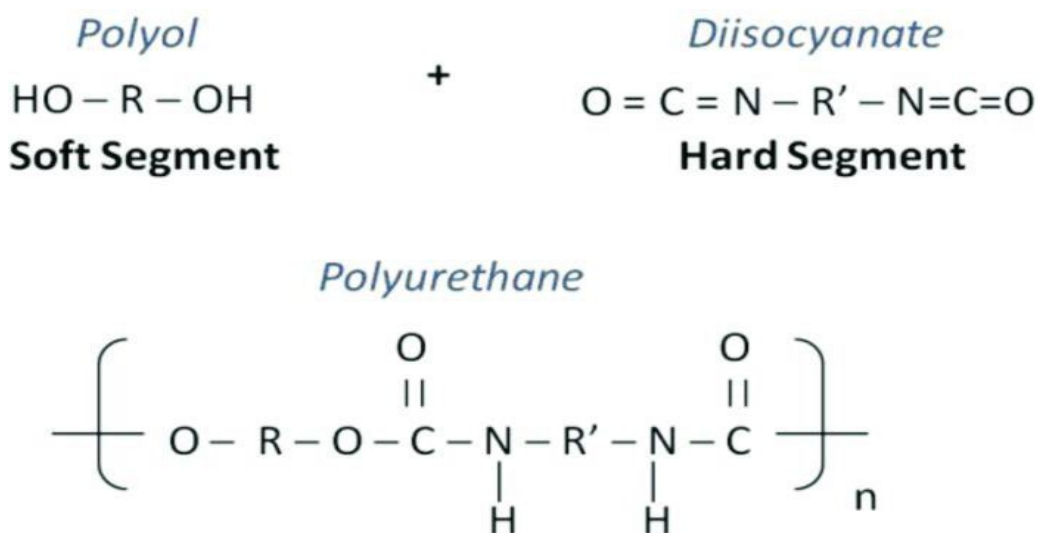


Figure 2.2: Polyurethane chemical structure (source: Patti *et al.* (2021))

Traditionally, the fabrication of PUF foams relies heavily on petrochemicals, which are now facing environmental concerns (Gama, Ferreira and Barros-Timmons 2018; Członka *et al.* 2020a). Consequently, these have led to the development and use of bio-based PUF fabricated from polyols derived from renewable resources such as soybeans (Alyamaç *et al.* 2022). While bio-based PUF foams with various functionalities and hydroxyl group contents can be produced (Özveren and Seydibeyoğlu 2017), they, however, have poor mechanical properties compared to those of petroleum-based PUF (Alyamaç *et al.* 2022). Durability is also a major disadvantage as it has a short life span of the product due to its hygroscopic tendencies, especially in furniture, adhesives, and sealants (Charles 2018). In this research, flexible polyurethane foam will be enhanced with biobased-type collagen to improve the mechanical properties of the foam. According to Alyamaç *et al.* (2022)

bio-based PUF formulations could be improved with reinforcing agents. Polyurethanes' ability to bond with various fillers and reinforcements, such as natural fibers, carbon nanofibers, or collagen, makes them adaptable for use in sustainable and high-performance composites. According to Sonnenschein (2014), polyurethanes' versatility in formulation allows them to be engineered for specific properties, including elasticity, hardness, and load-bearing capacity, making them a popular choice across many industries. The next section reviews various bio-based filler materials.

2.3 NATURAL FILLERS IN POLYMER CHEMISTRY

Natural fiber-reinforced polymer composites have a long history of use in civil engineering construction due to their advantageous properties and suitability for various applications (Keya *et al.* 2019). Ancient civilizations used natural materials like mud and straw in construction, and early composite products such as pottery and boats utilized straw for reinforcement. The modern development of composite materials began during the chemical revolution of the late 19th century, with the creation of synthetic resins and the introduction of fiberglass in the early 20th century, marking significant advancements (Keya *et al.* 2019). Synthetic materials like vinyl, polystyrene, phenolic, and polyester offered improved performance compared to their predecessors. Natural fiber composites have since gained prominence as eco-friendly alternatives to synthetic materials, offering benefits like low density, cost-effectiveness, and renewability, despite challenges such as high moisture absorption requiring chemical treatments (Taj, Munawar and Khan 2007; Keya *et al.* 2019).

Table 2.1 provides a comprehensive overview of the application of natural fillers in polymers. Natural fibers, such as plant fibers (e.g., jute, hemp) and animal fibers (e.g., wool, silk), have been extensively studied as reinforcements in polymer composites due to their renewability, low cost, and biodegradability (Taj, Munawar, and Khan 2007; Thakur, Thakur, and Kessler 2016). These natural fibers offer advantages such as high specific strength and stiffness, good thermal insulation properties, and reduced environmental impact compared to synthetic fibers (Taj, Munawar and Khan 2007; Thakur, Thakur and Kessler 2016). Natural fibers play a critical role in polymer chemistry, offering numerous advantages and applications (Tanasă *et al.* 2020). Sourced from renewable materials like plants and animals, these fibers are valued for their renewability, biodegradability, and enhanced mechanical properties (Thakur, Thakur and Kessler 2016). Incorporating natural fibers into polymer matrices improves composite materials' overall performance by enhancing strength, stiffness, and toughness (Kalia, Kaith and Kaur 2009).

Table 2. 1: Comprehensive Overview of Research Papers on Natural Fibers/Fillers in Polymer Composites.

Research paper title	Reference	Type of natural fibers/fillers	Application of fiber/filler	Historical dates found and used
From natural to synthetic fibers	(El Nemr 2012; Gibson 2012; Lucas and Harris 2012)	Cotton, wool, silk, jute, flax, wood fibers, or straw	Textiles, clothing, construction materials	Pre-20th century to present
Plastics materials: introduction and historical development	(Gilbert 2017; Ma and Elkin 2022)	Cellulose, wood fibers	Early plastics formulations or their contemporary applications in biocomposites	1930s-1940s
Plant-based natural fiber reinforced composites: a review on fabrication, properties, and applications	(Syduzzaman <i>et al.</i> 2020)	Fibers like jute, hemp, flax, and bamboo	Automotive, construction, packaging	1950s
Natural fiber-reinforced composites for bioengineering and environmental engineering applications" by Hoi-yan Cheung.	(Cheung <i>et al.</i> 2009)	Jute, coir, sisal, bamboo,	Bioengineering and environmental engineering, soil erosion control, biodegradable materials, wastewater treatment	1960s
Natural fiber-reinforced polymer composites"	(Taj, Munawar and Khan 2007)	Fibers like jute, kenaf, hemp, flax, sisal	Automotive parts, construction materials, packaging	1970s
Recent advances of natural fiber-based green rubber composites: properties, current status, and future perspectives	(Roy <i>et al.</i> 2021)	Cellulose, hemicellulose, and lignin. Ute, ramie, hemp, kenaf, silk, wool, angora, mohair	Structural components, consumer products, building, construction, packaging, and automotive industries	Late 20th century to present

A review: nanomaterials as a filler in natural fiber-reinforced composites	(Hosseini 2017)	Carbon nanotubes, graphene	Aerospace, electronics, automotive	21st century
Influence of filler material on properties of fiber- reinforced polymer composites: a review	(Ramesh <i>et al.</i> 2022)	Epoxy, polyurethane	Marine, automotive, civil engineering	Present
Recent progress on natural fiber hybrid composites for advanced applications: a review	(Mochane <i>et al.</i> 2019)	Hybrid composites	Tailored performance requirements	Present and future

Previous research has focused on optimizing processing techniques, surface modification of fibers, and compatibility with polymer matrices to maximize the mechanical performance of natural fiber-reinforced composites (Taj, Munawar, and Khan 2007; Thakur, Thakur, and Kessler 2016). The dispersion and alignment of these fibers within the polymer matrix significantly influence the mechanical properties, underscoring the importance of optimizing filler dispersion for maximum performance (Tanasă *et al.* 2020). Additionally, natural fibers contribute to lightweight and cost-effective composite materials, making them suitable for various industrial and consumer applications (Faruk *et al.* 2012). With their biocompatibility and thermal insulation properties, natural fibers are also utilized in medical devices and building materials (Chang, Mohanty and Misra 2020). In addition, the applications of natural fiber-reinforced polymer composites span various industries, including aerospace, automotive, construction, and packaging (Bismarck, Mishra and Lampke 2005). These composites find utility in manufacturing household furniture, fencing, windows, decking, flooring, sports equipment, and lightweight automobile components, among others (John and Thomas 2008; Kozłowski and Władyka - Przybylak 2008). Overall, the utilization of natural fibers in polymer composites aligns with sustainable practices and offers versatile solutions for addressing environmental and performance challenges (Satyanarayana, Arizaga and Wypych 2009). Figure 2.3 provides an overview of the different natural and synthetic fillers and their source of origin.

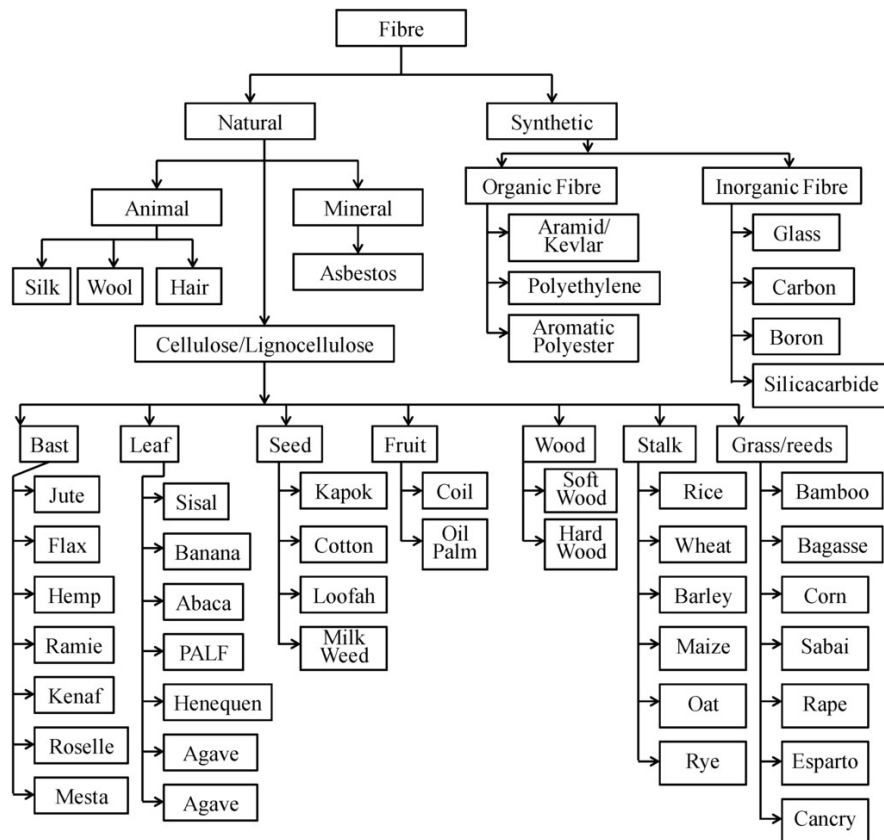


Figure 2.3: Sources of Natural and Synthetic Fibers Sourced : (Saba, Md Tahir and Jawaaid 2014)

Although natural fiber composites have gained prominence as eco-friendly alternatives to synthetic materials, offering advantages such as low density, cost-effectiveness, and renewability (John and Thomas 2008), they, however, also present challenges, including high moisture absorption, which necessitates chemical treatments to control (Kozłowski and Władyka-Przybylak 2008). Research endeavors have focused on enhancing the mechanical properties of natural fiber composites by optimizing factors such as fiber length and matrix adhesion (Bismarck, Mishra, and Lampke 2005).

Nevertheless, bio-based materials, derived from renewable resources, offer unique properties that make them suitable for various applications, particularly in the biomedical field (Gilbert 2016; Gupta 2016; Huang, Xiao and Shi 2019; Murano 2021). These materials are increasingly used to enhance polymer composites' performance and develop sustainable solutions across multiple industries. For instance, fish scale collagen and milled fish scale powder have advanced polymer composites by improving mechanical properties in polyurethane and epoxy resin matrices, resulting in stronger, more durable composites essential for high-performance applications (Ge *et al.* 2022; Lee *et al.* 2022; Qin *et al.* 2022). Fish-scale collagen is particularly significant in biomedical applications like wound healing, tissue engineering, and drug delivery, due to its biocompatibility and cell growth promotion capabilities (Jafari *et al.* 2020; Lee *et al.* 2022).

Beyond fish scale-derived materials, other bio-based fillers also enhance polymer composites. Natural fiber-reinforced polymer composites are increasingly utilised in civil engineering construction due to their advantageous properties and suitability for various applications. These composites incorporate fibers such as jute, kenaf, pineapple, sugarcane, hemp, and flax, offering benefits such as environmental friendliness, renewability, and satisfactory mechanical properties. Cellulose nanofibers from plant sources increase mechanical strength, thermal stability, and biodegradability, making them useful in the packaging, automotive, and construction industries (Dufresne, Thomas and Pothan 2013; Isogai 2013). Chitosan from crustacean exoskeletons is used in biomedical applications such as wound dressings and drug delivery systems due to its biocompatibility and antimicrobial properties (Kumar 2000; Dutta, Dutta and Tripathi 2004). Starch from plants like corn and potatoes is employed to create biodegradable plastics and composites for packaging and agricultural films (Averous and Boquillon 2004; Halley 2005). Lignin, a byproduct of the paper and bioethanol industries, improves UV resistance and mechanical properties of polymer matrices, reducing environmental impact (Laurichesse and Avérous 2014). Soy protein serves as a sustainable alternative in adhesives, coatings, and biodegradable plastics, applicable in agriculture and packaging (Thrane *et al.* 2017).

The adoption of bio-based materials aligns with sustainability and environmental conservation principles. Utilising waste products like fish scales and agricultural residues helps reduce environmental pollution and promotes a circular economy (Jafari *et al.* 2020). These materials generally have a lower carbon footprint than synthetic alternatives, helping to reduce greenhouse gas emissions (Huang, Xiao and Shi 2019). Incorporating biodegradable materials into products helps mitigate the long-term environmental impact of plastic waste, supporting eco-friendly practices (Averous and Boquillon 2004). The incorporation of bio-based materials as natural fillers in polymeric materials will lead to the departure for the next section.

2.4 COLLAGEN AND ITS SOURCES OF EXTRACTION

Collagen is a constitutive protein that plays an important part in the development of nearly all tissues and organs in the human body (Kulkarni and Maniyar 2020). Due to its strong direct cell adhesion capacity, low antigenicity, high stability and binding capacity, and remarkable biocompatibility, collagen has been regarded as an ideal biomaterial for the creation of tissue engineering constructions and wound dressing systems (Kulkarni and Maniyar 2020). As a result, collagen's characteristics make it a good choice for polymer reinforcement because of its strength. As highlighted in Table 2.2, collagen is sourced from various natural materials, including fish scales, skins, mammalian tissues,

poultry by-products, and marine organisms, offers a versatile biomaterial with vast industrial potential (Pati, Adhikari and Dhara 2010; Cherim *et al.* 2019; Davison-Kotler, Marshall and García-Gareta 2019; Noorzai *et al.* 2020; Mudhafar *et al.* 2022). Type I collagen, predominantly found in skin, tendons, and bones, is commonly sourced from bovine and marine collagen(Liu *et al.* 2019).

Bovine collagen is typically derived from cow hides and bones, while marine collagen is extracted from fish scales and skin (Lin and Liu 2006). Type II collagen, crucial for cartilage health, is primarily obtained from the chicken sternum and trachea. Type III collagen, essential for the structure of organs and blood vessels, is often sourced from bovine and porcine origins. Bovine and porcine skins are significant sources of Type VII collagen, which plays a vital role in anchoring cells to the extracellular matrix (Keene *et al.* 1987). Each source of collagen presents various benefits and applications across industries, including skincare, pharmaceuticals, and biomedical research (Muthukumar *et al.* 2018; Lupu *et al.* 2020; Rezvani Ghomi *et al.* 2021).

Table 2.2: Common Sources of Collagen

Origin	Source and supporting literature
Mammalian	Bovine (cattle) collagen: Derived from the skin, bones, and connective tissues of cattle (Silvipriya <i>et al.</i> 2015)
	Porcine (pig) collagen: Obtained from the skin, tendons, and bones of pigs (Silvipriya <i>et al.</i> 2015)
	Equine (horse) collagen: Extracted from the skin and tendons of horses (Gallo <i>et al.</i> 2020)
	Ovine (sheep) collagen: Derived from the skin and connective tissues of sheep(Gao <i>et al.</i> 2018)
Aquatic	Fish collagen: Derived from the skin, scales, and bones of fish, such as cod, salmon, or tilapia (Huang <i>et al.</i> 2016)
	Marine collagen: Obtained from various marine sources, including fish, shellfish, and other sea creatures like jellyfish or squid(Coppola <i>et al.</i> 2020)
	Shark collagen: Extracted from the cartilage and skin of sharks (Jeevithan <i>et al.</i> 2014)
	Cephalopod collagen: Derived from the skin and cartilage of cephalopods, such as squid, octoPUFs, or cuttlefish(Tapia-Vasquez <i>et al.</i> 2021)

	Seaweed collagen: Although not from animal sources, some seaweed species contain collagen-like proteins that are used in cosmetics and skin care products(Makgobole <i>et al.</i> 2024)
	Oyster shell collagen: Derived from the shells of oysters (Ulagesan <i>et al.</i> 2022)
	Mussel shell collagen: Obtained from the shells of mussels(Wu <i>et al.</i> 2019)
Shells	Crab shell collagen: Derived from the shells of crabs. (Bhattacharjee <i>et al.</i> 2019)
	Shrimp shell collagen: Obtained from the shells of shrimp (Dang <i>et al.</i> 2020)
	Plant collagen: Derived from plant sources such as soybeans, wheat, or corn (Zhang, Baez and Glatz 2009)
Plant and Algae	Algal collagen: Extracted from various types of algae, such as seaweed or microalgae (Berthon <i>et al.</i> 2017)
	Hydroxyapatite collagen: Collagen mineralized with hydroxyapatite; a form of calcium phosphate found in bone.
Mineral	(Du <i>et al.</i> 2000)
	Magnesium-enriched collagen: Collagen supplemented or enriched with magnesium ions. (Qu <i>et al.</i> 2014)

While collagen is generally abundant in mammalian skin, the risks of disease transfer have necessitated the need for an alternative source of collagen (Bhuimbar, Bhagwat, and Dandge 2019). Marine collagen, specifically derived from fish scales, has become a focus of research due to its lower risk of disease transmission compared to mammalian sources, along with its excellent mechanical and thermal properties. Fish scale collarticular offers a sustainable alternative with minimal environmental impact, making it a valuable resource in the production of biocomposites. Fish-scale collagen, for example, possesses excellent biocompatibility and low antigenicity, making it suitable for biomedical applications like wound dressings and tissue engineering scaffolds (Lim *et al.* 2019). Fish scales are essential skeletal elements that provide protection to a fish's body against external threats and predators (Arola *et al.* 2018; Rawat *et al.* 2021). In recent years, fish scales have garnered significant attention due to their unique laminated composite structure, low bending resistance, and exceptional specific strength and toughness (Murcia *et al.* 2017; Rawat *et al.* 2021).

Structurally, fish scales possess a unique laminated composite structure, which consists of alternating layers of collagen and hydroxyapatite minerals (Pon-On *et al.* 2016; Rawat *et al.* 2021). This natural structure provides properties, making fish scales an intriguing candidate for reinforcing polymers (Figure 2.4). These scales also contain other chemical constituents, including calcium phosphate, and can be categorized into four types based on the species of fish, namely placoid, ganoid, elasmoid, and cosmoid scales (Ehrlich and Ehrlich 2015). Of particular interest in this study are elasmoid scales investigated and extracted as a filler as milled fish scale powder and fish scale collagen (Zainol *et al.* 2012; Quan *et al.* 2020; Rawat *et al.* 2021; Mudhafar *et al.* 2022). Elasmoid scales are primarily composed of type I collagen fibers and apatite, both of which are naturally occurring within mineralized systems (Ikoma *et al.* 2003; Quan *et al.* 2020). Collagen, a fibrous protein abundant in animal tissues, particularly in the dermal layers of fish scales, plays a crucial role in providing structural support, flexibility, and resilience to fish scales (Arola *et al.* 2018). This study endeavors to harness fish scales for collagen and milled fish scale powder, leveraging their abundance and inherent safety as a renewable resource (Keya *et al.* 2019).

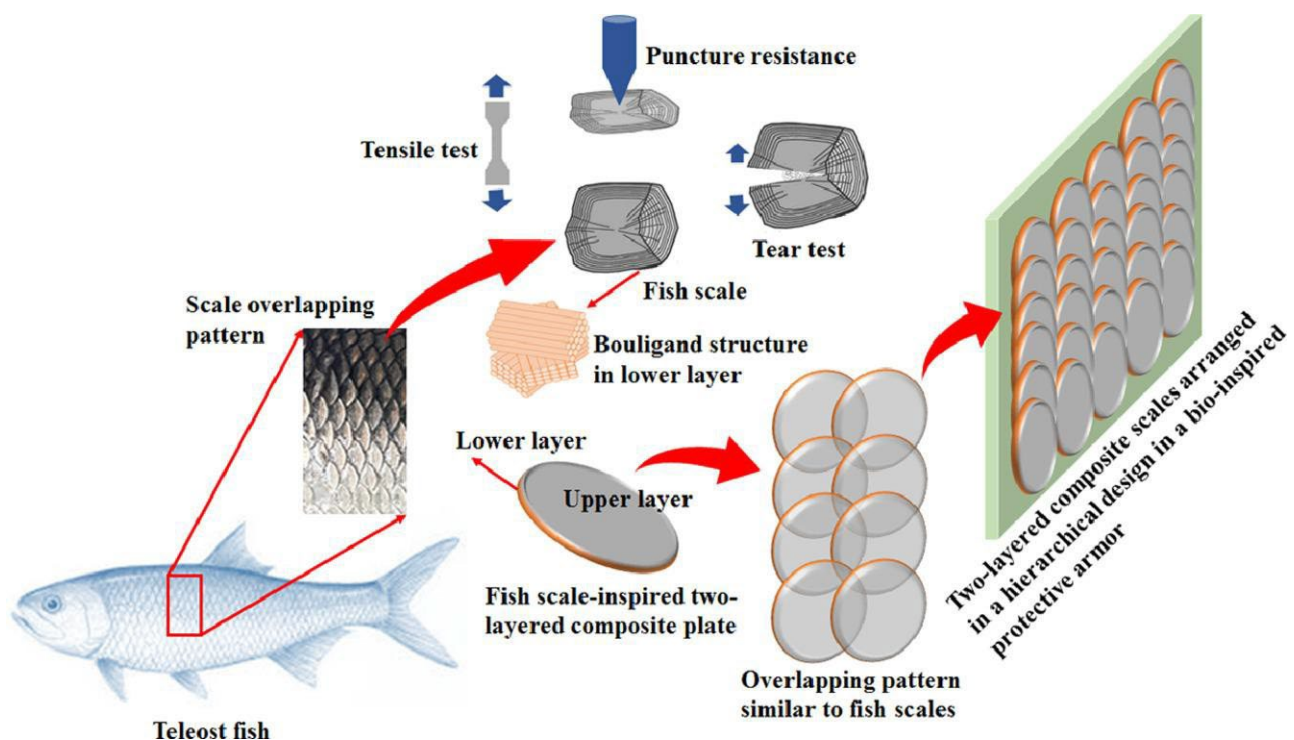


Figure 2.4: Structural Properties of Fish Scales Sourced : (Rawat *et al.* 2021)

Furthermore, fish scale-derived materials can be processed into bioplastics, offering an eco-friendly alternative to traditional plastics (Tennakoon *et al.* 2023). These materials also find use in cosmetics due to their moisturizing and anti-aging properties (Avila Rodríguez, Rodríguez Barroso and Sánchez

2018). In polymer science, fish scale-derived materials hold promise due to their unique properties and sustainability (Qin *et al.* 2022). Fish-scale collagen can be processed into biodegradable films and coatings, providing sustainable options for food packaging and reducing environmental impact (Lim *et al.* 2019). Moreover, fish-scale collagen incorporation into polymer matrices enhances mechanical properties and biocompatibility in tissue engineering scaffolds (Raftery *et al.* 2016). Additionally, fish scale-derived nanomaterials offer functionalities like antimicrobial activity and controlled drug release in polymer composites (Qin *et al.* 2022). This interdisciplinary approach between polymer science and biomaterials fosters the development of sustainable and biocompatible materials with diverse applications, reducing reliance on non-renewable resources and mitigating environmental pollution (Choppakatla 2023) It is reasonable to point out that collagen can also be derived from other parts of fish (See Table 2.3).

Table 2.3: Past Research Based on Collagen Application

Author	Source of Collagen	Key Points
(Nagai, Izumi and Ishii 2004)	Fish scales	The preparation and initial characterization of collagen using the Ish scale method.
(Pati, Adhikari and Dhara 2010)		Explores the isolation and characterization of fish scale collagen, emphasizing its notable thermal stability and potential applications.
(Bhagwat and Dandge 2016)		The isolation, characterization, and potential applications of fish scale collagen in food and agriculture industries.
(Shalaby <i>et al.</i> 2020)		The preparation, characterization, and application of fish scale collagen in wound healing.
(Arumugam <i>et al.</i> 2018)	Fish skin	The extraction, optimization, and characterization processes of collagen from sole fish skin.
(de Souza <i>et al.</i> 2022)		The experimental animal studies evaluate the efficacy of fish collagen in skin wound healing.
(Alves <i>et al.</i> 2017)		The cosmetic applications of marine fish skin collagen, exploring its potential benefits for enhancing skin health and appearance.
(Blanco <i>et al.</i> 2017)		The potential of producing hydrolysates of fish skin collagen as a means to valorise fish industry by-products, offering opportunities for utilization in various applications
(Nagai and SUZUKI 2000)	Fish bones	The preparation and characterization of various fish bone collagens, elucidating their structural properties and potential applications.
(Darmanto <i>et al.</i> 2014)		Fishbone collagens can enhance food quality, potentially impacting texture, moisture retention, and flavour in different food products.

2.5 EXTRACTION METHODS FOR COLLAGEN

2.5.1 METHOD OF EXTRACTION AND QUANTIFICATION OF COLLAGEN FROM FISH SCALES

Fish scale collagen exhibits a triple helical structure, enhancing mechanical strength and stability in collagen fibrils, making it suitable for diverse biomedical and biomaterial applications (Jafari *et al.* 2020). Fish scale collagen is particularly valued for its biocompatibility, biodegradability, and excellent mechanical strength due to its unique triple helical structure composed mainly of glycine, proline, and hydroxyproline amino acids (El-Rashidy *et al.* 2015; Rajabimashhadi *et al.* 2023). The extraction process for fish scale collagen typically involves methods like acetic acid treatment to preserve collagen's integrity, although it raises concerns about the suitability for human consumption and the potential denaturation of collagen peptides (Liu *et al.* 2012; Chinh *et al.* 2019; Rajabimashhadi *et al.* 2023). Figure 2.5 illustrates a typical chemical structure of collagen and its various constituents found in biological tissues.

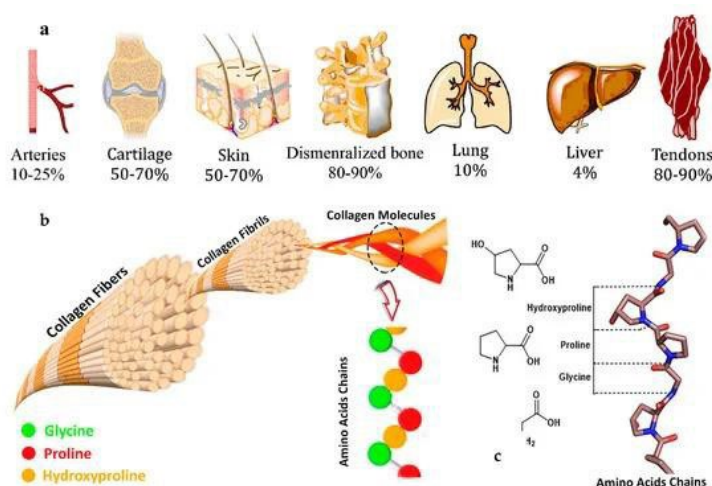


Figure 2.5: Collagens Chemical Structure: Different Tissues, Structure of Collagen Fibers and Amino Acid (Jafari *et al.* 2020)

Fish waste contains numerous contaminants such as lipids, non-collagenous proteins, pigments, calcium, and other inorganic materials, which can be removed in the pre-treatment methods. Various techniques for collagen preparation have been documented and categorised into distinct groups, including acid/alkali treatment, enzymatic digestion, demineralisation, purification, deproteinization, extraction with organic solvents, enzymatic digestion, fermentation, coprecipitation, sol-gel synthesis, precipitation, and hydrothermal synthesis. (Pati, Adhikari and Dhara 2010; Boudemagh *et al.* 2019; Cherim *et al.* 2019; Yorgancioglu, Başaran and Sancakli 2020; Matinong *et al.* 2022; Ingwattanapok, Sakunrak and Tangboriboon 2023). While acknowledging the plethora of methods available for extracting collagen from waste materials such as fish scales, fish bones, bovine, and

porcine animal sources (Table 2.4), this study emphasises the acid extraction, demineralisation, and purification approach for obtaining valuable biobased materials from waste.

The extraction process for fish skin collagen typically involves methods that preserve collagen's integrity, such as avoiding heat or enzyme treatments (Fernández-Arias *et al.* 2021). In contrast, extracting collagen from fish scales often requires the use of acetic acid, a process that raises concerns regarding the suitability of the resulting collagen for human consumption (Huang *et al.* 2016; Coppola *et al.* 2020). The heat involved in the extraction process for fish scale collagen can potentially denature collagen peptides, affecting their beneficial properties (Jafari *et al.* 2020). The extraction of collagen from fish waste consists of numerous steps. Fish waste is subjected to various pre-treatments followed by the extraction method. Before the pre-treatments, fish waste is segregated, such as bone, scales, skin, and swim bladder. The pre-treatment is given to the segregated fish waste to remove the impurities as well as to increase the quality of extracted collagen. The extraction process from fish scale powder begins with thorough washing and disinfection to remove contaminants and reduce microbial load (Chandra and Shamasundar 2011). Subsequently, the scales undergo demineralization and acid hydrolysis to break down mineral components and non-collagenous proteins, preparing them for milling (Dhara *et al.* 2013; Xu *et al.* 2017; Jafari *et al.* 2020; Furtado *et al.* 2022).

Mechanical processing methods, such as grinding, milling, or pulverization, physically separate collagen fibrils from fish scales, which include reducing fish scales to fine particles and are often used in conjunction with chemical or enzymatic treatments to enhance extraction efficiency and yield (Chandra and Shamasundar 2011; Huang *et al.* 2016; Nawaz *et al.* 2020; Arokiasamy *et al.* 2022). This method is suitable for large-scale production and can be adapted to different types of fish scales and collagen sources (Qin *et al.* 2022)). These processes are crucial for obtaining hydroxyapatite (HAp) and other valuable components from fish scales. Mechanochemical processing not only contributes to solid waste management but also offers dual benefits in terms of value-added product generation and environmental conservation (Hajiali *et al.* 2022). In this study, the acid-soluble collagen method of extraction was followed.

Acid-base extraction is used to separate organic compounds from each other based on their acid-base properties. This method rests on the assumption that most organic compounds are more soluble in organic solvents than they are in water (Chris Schaller 2019). Acid-base extraction is used because it removes non-collagenous proteins and pigment, which makes an easy extraction of collagen from Fish. The extraction of collagen is done by the treatment of an acid and a base by primary

decalcification, which decreases the number of minerals in fish scales, and hydrolysis to remove undesirable surface proteins and residual salts, and further purification using a tris glycine buffer, most efficient for protein extractions and using a dialysis membrane with a molecular cut-off weight around 12-14 kDa due to the molecular mass of fish scales for extraction to obtain acid-soluble collagen. All the extraction processes are performed at 4 degrees Celsius to minimise the collagen from deteriorating and to reduce temperature-induced defragmentation of collagen (Bhagwat and Dandge 2016).

Table 2.4: Various Extraction Methods of Collagen

Type of material	Source	Extraction Method	References	
Bovine, porcine, ovine, hircine, and galline	Animal Skin, bones, and tendons	Acid/alkali treatment, demineralisation, enzymatic digestion, Acid Hydrolysis, salt solubilization, and enzymatic hybrid/alkali hydrolysis, Ultrasound-Assisted, microwave, purification, Dialysis, Precipitation	(Zhou <i>et al.</i> 2016; Gorlov <i>et al.</i> 2018; Matinong <i>et al.</i> 2022; Rana <i>et al.</i> 2023)	
	Fish	Skin, Scales,	Acid/alkali treatment, enzymatic digestion, purification,	(Felician <i>et al.</i> 2018; Ahmed, Verma and Patel 2020; Hiransuchalert <i>et al.</i> 2021; Farooq <i>et al.</i> 2024)
	Jellyfish	Umbrella,	Demineralisation,	
	Squid	Tentacle, Cartilage	deproteinization	
	Shrimp	Shell		
Crab				
Plant and Algae	Plant	Extraction with organic solvents	(Liao and Shi 2005)	
	Algae	Enzymatic digestion, Fermentation	(Xu <i>et al.</i> 2023)	
Mineral	Hydroxyapatite	Coprecipitation, sol-gel synthesis	(Le, Natesan and Pranti-Haran 2015; Pandharipande and Sondawale 2016)	
Mineral	Calcium phosphate	Precipitation, hydrothermal Synthesis	(Hong <i>et al.</i> 2022)	

Beyond serving as a rich collagen source, fish scales themselves hold intrinsic advantages that make them valuable for composite and biomaterial applications. Structurally, fish scales are composed of

collagen fibrils reinforced by hydroxyapatite, forming a natural composite with excellent tensile strength, stiffness, and resistance to penetration (Yang *et al.* 2013; Zhu *et al.* 2012). This unique hierarchical structure has inspired the development of bioinspired materials with enhanced mechanical properties and durability. Additionally, fish scales are lightweight, abundant, biodegradable, and represent a sustainable avenue for valorising fishery by-products that would otherwise contribute to environmental waste streams (Pati, Adhikari and Dhara 2010). These characteristics align with principles of the circular economy and green material innovation.

However, certain disadvantages limit the direct use of fish scales. Their natural variability, depending on species and habitat, can result in inconsistent mechanical and biochemical properties (Ikoma *et al.* 2003; Rawat *et al.* 2022). Furthermore, fish scales tend to have lower thermal stability compared to mammalian-derived biomaterials due to differences in collagen composition, especially lower hydroxyproline content (Shoulders and Raines 2009). This thermal sensitivity restricts their application in environments requiring high heat resistance. Processing challenges also arise, as acid- or enzyme-based extraction methods may partially denature collagen or alter mineral phases, thereby affecting bioactivity and structural integrity (Singh *et al.* 2011).

An important limitation is the microbial contamination risk associated with fish scales. As an external tissue directly exposed to aquatic environments, scales serve as potential reservoirs for pathogenic and spoilage microorganisms. Studies have identified bacteria such as *Pseudomonas*, *Vibrio*, *Aeromonas*, and *Shewanella* species on fish scales and skin, all of which are linked to spoilage and opportunistic infections (Chandra and Shamasundar 2015; Cardoso *et al.* 2020; Manchanayake *et al.* 2023). These bacteria not only compromise the safety and quality of extracted biomaterials but also raise concerns for biomedical applications where sterility is paramount. Effective pre-treatment strategies, including washing, disinfection, and sterilisation, are therefore essential to minimise microbial risks and ensure safe downstream applications of fish scale-derived materials (Gómez-Guillén *et al.* 2011).

2.5.2 OPTIMISING THE EXTRACTION PARAMETERS

Research into optimal extraction methods for obtaining collagen and milled fish scale powder is crucial for maximising fish waste utilisation and developing sustainable biomaterials (Huang *et al.* 2016; Raftery *et al.* 2016; Jafari *et al.* 2020; Rajabimashhadi *et al.* 2023). The properties of collagen and its recovery are directly affected by the collagen extraction method. Several parameters are reported in the literature to affect the extraction process. These include the extraction temperature, the extraction time, and solvent concentrations (Jafari *et al.* 2020).

2.5.2.1 Effect of Extraction Temperature

The collagen extraction temperature depends on the type of substrate for collagen extraction. Extant literature reported that an increase in temperature will induce a drop in the viscosity of the extraction solution, thus resulting in the rise of the overall mass transfer rate, while increasing the temperature above the denaturation point of collagen would lead to thermal degradation of the isolated proteins (Jafari *et al.* 2020). According to Jafari *et al.* (2020), collagen extraction from fish sources is mainly carried out between 4 and 10 °C, which allows pepsin to cleave the crosslinks in the collagen triple helix without damaging the structure of the peptides. Cognisance of this will be taken in this study to avoid the degradation of collagen.

2.5.2.2 Effect of Extraction Time

According to Jafari *et al.* (2020), the mass transfer rate might be used to explain the influence of extraction time on collagen yield. Extraction is significantly influenced by the diffusion process, which is time-dependent; as a result, collagen recovery will increase as the extraction period is extended. Excessive extraction times, on the other hand, may cause the leached peptides to degrade (Jafari *et al.* 2020). The acid solution begins to break down the collagen chains in this scenario, causing their breakdown and lowering the ultimate extraction yield. Furthermore, long extraction times would make the extraction process unsuitable for industrial scale-up (Jafari *et al.* 2020). da Trindade Alfaro *et al.* (2014) studied the extraction times (3-15h) of collagen from fish scales. The authors concluded that an increase in extraction time has a significant influence on the yield of collagen extracted. This study intends to optimise the appropriate extraction that will give an increased yield of collagen.

2.5.2.3 Effect of solvent concentration

According to Arumugam *et al.* (2018), higher molarities would cause degradation of the peptides, thus decreasing both the yield and purity of the final product. While inorganic acids, for example, hydrochloric acid, could be used in the extraction of collagen, Jafari *et al.* (2020), however, noted that their efficiencies are lower when compared to organic acids. Consequently, organic acids are regarded as more effective in the solubilisation of non-crosslinked collagen as well as breaking the inter-strand crosslinks in collagen (Liu *et al.* 2015). Owing to this, this study will be using organic acids for the extraction of collagen.

2.5.2.4 Effect of buffer in collagen extraction

Buffer systems play a significant role in maintaining pH stability and providing an optimal environment for protein solubilization during collagen extraction. The commonly used buffers in collagen extraction include phosphate-buffered saline (PBS), acetic acid, and tris-based buffers (e.g., tris-HCl, tris-glycine), each having distinct effects on collagen yield and quality. Tris-glycine buffer has shown promise in the extraction process due to its buffering capacity at neutral pH, which is crucial for maintaining the structural integrity of collagen during extraction. Tris (tris(hydroxymethyl)aminomethane) is a widely used biological buffer due to its effective pH range of 7.0 to 9.0. It ensures the physiological conditions under which collagen molecules remain stable and soluble, minimising protein denaturation or degradation. Glycine, an amino acid with buffering properties, complements tris by enhancing solubilization and maintaining the appropriate ionic strength, which is important for the interaction between collagen fibers and extraction solutions.

The tris-glycine buffer system helps preserve the triple-helix structure of collagen, which is essential for its functional and mechanical properties. Furthermore, it minimises proteolytic degradation, often seen in enzymatic extraction methods, thus improving the yield of intact collagen. Studies have shown that maintaining the buffer system at neutral pH (around 7.4) leads to more effective collagen solubilization from tissues such as skin, tendons, and bones, particularly in mammalian sources. Maintaining the pH of the tris-glycine buffer is crucial in collagen extraction. Neutral pH conditions (pH 7.0–7.4) are ideal for minimising collagen denaturation. In acidic environments, collagen tends to denature, while in overly basic conditions, hydrolysis of collagen occurs, reducing yield. Using a tris glycine buffer helps maintain the pH and ionic strength of the collagen solution, preserving its stability and solubility (Chen *et al.* 2016; Rýglová, Braun and Suchý 2017; Rana *et al.* 2023). For instance, Zhang *et al.* (2014) reported that the yield of collagen extraction using tris-based buffers at pH 7.2 from bovine skin tissues was higher than that obtained with acidic solutions. This highlights the importance of using tris-glycine buffer in providing pH stability throughout the extraction process.

2.6 MECHANICAL AND PHYSICAL PROPERTIES OF COMPOSITES WITH BIO-BASED REINFORCEMENTS

As previously mentioned in this chapter, the mechanical properties of polymer composites are significantly influenced by the incorporation of bio-based reinforcements, such as natural fibers, and biopolymers. These materials offer sustainability, biodegradability, and favourable mechanical performance, making them attractive for various industrial applications. This section examines empirical studies focusing on tensile strength, flexural strength, impact strength, hardness, and other relevant mechanical attributes.

2.6.1. TENSILE STRENGTH

Tensile strength is a critical mechanical property that determines how a composite material can withstand pulling forces without failure (Awais *et al.* 2021). It is typically defined as the maximum force that can be applied in this manner before the material fractures (Ismail, Khulbe and Matsuura 2019). Saba, Jawaid and Sultan (2019) note that tensile strength evaluates the force needed to fracture a composite or plastic specimen and determines how much the specimen stretches or elongates before reaching the breaking point. According to Ismail, Khulbe and Matsuura (2019), a tensile test is conducted using standard testing equipment following the ASTM D638-10 method for measuring the tensile properties of plastics. Saba, Jawaid and Sultan (2019) clarified that tensile testing of composites typically involves basic tension or flat-sandwich tension testing, following standards such as ISO 527-4, ISO 527-5, ASTM D638, ASTM D3039, and ASTM C297. These tests generate stress-strain diagrams that are used to calculate the tensile modulus.

When a material is exposed to a tensile pulling force, it stretches until it fractures. The results are displayed as a curve illustrating the material's response to the applied forces (Ismail, Khulbe and Matsuura 2019). Tensile testing also measures properties such as tensile strength (both at yield and break), tensile modulus, tensile strain, elongation, and percent elongation at yield, as well as percent elongation and elongation at break (Saba, Jawaid and Sultan 2019). These parameters help evaluate a material's appropriateness for specific applications (Rahman and Putra 2019). Numerous empirical studies have shown that bio-based reinforcements, particularly natural fibers can enhance the tensile strength of polymer composites (Rahman and Putra 2019; Awais *et al.* 2021). For instance, Khan *et al.* (2020) investigated the tensile properties of epoxy composites reinforced with jute fibers. The results demonstrated a significant improvement in tensile strength and modulus compared to pure epoxy. The tensile strength increased by 25% with the inclusion of 20% jute fiber, attributed to the effective load transfer from the matrix to the fibers due to good interfacial adhesion. Jute fibers in epoxy matrices demonstrated a 10.06% increase in tensile strength, showcasing the reinforcement's effectiveness (Shah *et al.* 2023).

Mohammed *et al.* (2021) assessed the tensile behaviour of composites reinforced with sisal fibers. The tensile strength increased by 30% with 15% fiber addition, although the performance was highly dependent on fiber-matrix bonding quality. Sisal fiber-reinforced epoxy composites achieved a tensile strength of 69.1 MPa, significantly outperforming neat epoxy (Huzaifa *et al.* 2024). Sisal fiber reinforced with polyurethane composites enhanced tensile strength by up to sevenfold (17.0 MPa)

compared to neat polyurethane resin, with dry fibers improving fiber-matrix adhesion while humid fibers offered higher elongation (11.3%) (Milanese, Cioffi and Voorwald 2011).

Kenaf/Epoxy composites exhibited the highest tensile strength, 3.1 times greater than the neat resin, and achieved the highest Young's modulus at 50% fiber volume fraction (Hafizah *et al.* 2014). Hybrid composites of bi-directional glass fiber and rice husk (RH) fiber in epoxy matrices achieved the highest tensile strength (215.42 MPa) and modulus (1.8 GPa) with 25 wt.% glass fiber and 5 wt.% RH fiber. Increasing RH content to 15 wt.% weakened interfacial bonding, reducing tensile properties by 25% (Ismail *et al.* 2020). A more recent study by Onwubu *et al.* (2024) analyzed collagen-extracted fish scale-reinforced epoxy composites. The addition of 5-10% fish scale collagen improved tensile strength by 18% compared to the neat epoxy. The improvement was largely attributed to efficient load transfer between the particulates and the polymer matrix.

2.6.2. FLEXURAL STRENGTH

Flexural strength indicates a material's ability to resist deformation under bending forces (Mehndiratta *et al.* 2018), and bio-based reinforcements have shown considerable potential in enhancing this property in polymer composites. The data is frequently utilised to choose materials for components that must bear loads without bending (Saba, Jawaid and Sultan 2019). The most common methods for flexural testing of plastics, polymer composites, and large fiber-reinforced plates are three-point and four-point bend tests, conducted following ISO 14,125, ISO 178, ASTM D790, and ASTM D6272. These tests provide valuable insights into material properties and ensure their suitability for specific applications under various conditions. Flexural properties testing delivers both raw and adjustable data, including flexural stress and strain at yield, flexural stress and strain at break, flexural stress at 3.5% deflection (ISO) or 5.0% deflection (ASTM), flexural modulus, and stress-strain curves. The flexural modulus serves as a measure of a material's stiffness when subjected to bending (Saba, Jawaid and Sultan 2019).

An empirical study by El-Shekeil *et al.* (2012) on thermoplastic polyurethane (TPUF) reinforced with 20–50 wt.% kenaf fibers reported increased flexural strength from 6.55 MPa (PUFre TPUF), with higher fiber content improving both strength and modulus. Bamboo fiber-reinforced epoxy composites demonstrated superior flexural strength compared to virgin epoxy and jute fiber composites, with optimal performance at 5% NaOH-treated fibers (Parbin *et al.* 2019). Alkali-treated sugar palm fiber-reinforced epoxy composites achieved the highest flexural strength (96.71 MPa, a 24.41% improvement) with 10 wt% reinforcement using 0.25 M NaOH for 1 hour. Flexural modulus

reached 6948 MPa (a 148% increase) with 0.5 M NaOH for 4 hours (Sapuan, Hamdan and Bachtiar 2010). Bi-directional glass fiber and rice husk (RH) fiber composites showed the best flexural strength (275.51 MPa) and modulus (9.79 GPa) with 25 wt.% glass fiber and 5 wt.% RH fiber, decreasing by 27% and 32%, respectively, at 15 wt.% RH fiber (Ismail *et al.* 2020).

2.6.3. IMPACT STRENGTH

Impact strength is the material's ability to absorb energy during a sudden force or impact (Saba, Jawaaid and Sultan 2019). The impact test is specifically designed to assess the toughness, brittleness, notch sensitivity, and impact strength of engineering materials in their ability to withstand high-rate loading (Saba *et al.* 2016b; Saba *et al.* 2016c). Bio-based reinforcements, including natural fibers and collagen-based materials, have been found to enhance the impact resistance of polymer composites in various studies. In an empirical study by Ribeiro *et al.* (2021), hemp fiber-reinforced epoxy composites showed an increase of 7.5% in impact strength compared to the unreinforced epoxy. This improvement was due to the fibers' ability to dissipate energy during impact, thus enhancing toughness. Similarly, Venkatachalam *et al.* (2024) tested the impact of coir fiber-reinforced epoxy composites. The result reveals the maximum Impact strength (98.35 J/m^2) by combining coir fibre (5 wt.%), and coir fiber powder size ($225 \mu\text{m}$).

Furthermore, hybrid composites of bi-directional glass fiber and RH fiber achieved peak impact strength (447.19 J/m) with 25 wt.% glass fiber and 5 wt.% RH fiber, outperforming other configurations (Ismail *et al.* 2020). The impact strength of *Moringa oleifera* bio-filler-reinforced epoxy composites increased up to 12% v/v bio-filler content but declined beyond 16–24% v/v due to filler agglomeration (Sridevi and Giri 2024). Epoxy-based composites with 3 wt.% Rohu fish scale residue powder achieved an impact strength of 1908.2 J/m^2 , a 54.72% increase over neat epoxy (Kumar, Bansal and Singh 2019). Impact strength peaked at 22.22 kJ/m^2 with 30 wt.% fiber content in thermoplastic polyurethane composites but decreased beyond this level due to fiber breakage (Radzi *et al.* 2017).

2.6.4. HARDNESS

Bio-based reinforcements offer other mechanical properties such as hardness, toughness, and wear resistance. Hardness indicates a composite's resistance to surface deformation. The hardness of polymer composites reinforced with bio-based materials was investigated by Martins *et al.* (2021), where bamboo fiber-reinforced polyester composites showed a 12% increase in hardness compared

to pure polyester. The bamboo fibers' inherent stiffness and rigidity contributed to the improved surface hardness, making the composites more resistant to indentation. In addition, an empirical study by Agnihotri *et al.* (2022) examined the wear of bio-based hybrid composites reinforced with hemp and silk fibers in an epoxy matrix. The wear rate was reduced by 30% compared to the neat epoxy, highlighting the reinforcing effect of the bio-based fibers in reducing material loss under friction.

Sisal-glass fiber-reinforced epoxy composites exhibited the highest hardness value (24.73 RHN) (Huzaifa *et al.* 2024). Bamboo-coir epoxy resin composites achieved the highest Rockwell Hardness Number (38 RHN), outperforming bamboo epoxy (20 RHN) and bamboo-jute epoxy (37 RHN) composites (Bansal, Ramachandran and Raichurkar 2017). WPC with 50% finely ground wood filler achieved the highest indentation hardness (56 MPa, a 125% improvement) and indentation modulus (0.77 GPa, a 201% increase). Coarsely ground wood filler exhibited a hardness of 49 MPa (99% increase), while slate fillers achieved 40 MPa (62% increase). These enhancements highlight the role of filler type and content in improving WPC properties for applications such as tiling and window frames (Dočkal *et al.* 2020).

2.7 THERMAL PROPERTIES OF COMPOSITES REINFORCED WITH PARTICULATE FILLERS

According to Haque *et al.* (2021), the mechanical properties of polymers are significantly influenced by temperature. Specifically, as temperature increases, the elastic modulus and tensile strength decrease, while ductility improves (Balani *et al.* 2015). To address these challenges, extensive research has focused on enhancing the thermal properties of various polymers and developing heat-resistant polymers for high-temperature applications requiring reliable performance (Haque *et al.* 2021).

Thermo-gravimetric analysis (TGA) is a widely used method to evaluate the thermal stability of materials. TGA measures changes in a material's physical and chemical properties as a function of increasing temperature at a constant heating rate or over time at a constant temperature or mass loss (Azammi *et al.* 2020). This technique provides insights into chemical processes such as desolvation (e.g., dehydration), decomposition, and solid-gas reactions like oxidation or reduction. It also identifies physical phenomena, including phase transitions, vaporisation, sublimation, absorption, and desorption (Sahu, Mondloe and Upadhyay 2017).

Sahu, Mondloe and Upadhyay (2017) highlighted that TGA is instrumental in assessing the thermal

stability of polymers, ensuring their physical and chemical properties remain unchanged at elevated temperatures. Researchers use TGA to determine the temperature at which a composite begins to degrade, the weight loss with temperature increase, and the residue left behind (Azammi *et al.* 2020). Thermal stability within the desired temperature range is characterised by negligible mass loss and minimal slope on the TGA curve. Additionally, TGA determines the upper application temperature beyond which a material starts degrading (Sahu, Mondloe and Upadhyay 2017).

Numerous studies have employed TGA to analyse epoxy composites. For instance, Wang *et al.* (2015b) investigated the thermal stability of pure epoxy and epoxy composites filled with 4.5 wt.% KH550@EG. They observed initial decomposition temperatures of 318, 329, and 348 °C for untreated and treated samples, respectively, concluding that surface treatment enhances compatibility and thermal stability. Similarly, Gu *et al.* (2016) examined graphene nanoplatelets (GNP) and functionalized graphene nanoplatelets (f-GNP) composites. Their findings revealed improved thermal stability with increasing f-GNP content, with heat resistance indices rising from 192 °C for pristine epoxy to 203 °C for composites with 30 wt.% f-GNPs.

Xu and Chung (2000) analysed TGA data for composites filled with boron nitride and aluminium nitride particles, comparing surface-treated samples with those treated using acetone or acids. They reported that silane-treated samples exhibited higher weight loss compared to acid- or acetone-treated samples. Liang and Wong (2017) studied nanocomposites with 1 wt.% SiO₂ or Al₂O₃ fillers and hybrid composites containing 1 wt.% nano-fillers and 20 wt.% micro-fillers. Their results showed that both micro- and hybrid composites exhibited significantly lower weight loss compared to neat epoxy, with SiO₂-filled samples displaying similar behaviour to Al₂O₃-filled specimens.

Hani *et al.* (2021) explored the thermal properties of fishbone-based (FBP) epoxy composites using TGA and DSC. Their results demonstrated that incorporating FBPs enhanced thermal stability compared to neat epoxy. The onset decomposition temperature (T_{onset}) increased with FBP content, peaking at 345 °C for composites containing 10 wt.% and 15 wt.% FBPs. Furthermore, composites with thermally treated FBPs (TFBPs) exhibited an additional 21 °C increase in T_{onset} for 10 wt.% TFBPs. Differential scanning calorimetry (DSC) analysis revealed that the glass transition temperature (T_g) of epoxy composites improved with FBP incorporation, rising from 58 °C for neat epoxy to 72 °C for composites with 10 wt.% FBPs.

The above studies collectively highlight the importance of optimising filler content and surface treatment to

enhance the thermal stability and mechanical performance of polymer composites. Cognisance of the effect of filler content will be taken into consideration.

2.8 EMPIRICAL REVIEW OF POLYURETHANE AND EPOXY RESINS REINFORCED WITH NATURAL FILLERS

The integration of bio-based fillers into polymer matrices has garnered significant attention due to their potential to enhance mechanical properties while promoting sustainability. Table 2.6 summarises empirical studies investigating the effects of different bio-based fillers on epoxy and polyurethane properties. The narrative is presented as cases.

Case 1: Enhancing the mechanical properties of epoxy composites using natural fillers

Shah *et al.* (2023) explored the incorporation of jute fibers at 30% weight in epoxy resin and observed a 10.06% increase in tensile strength and a 4.70% improvement in flexural strength. This enhancement demonstrates better load-bearing capabilities attributed to the strong interfacial bonding between jute fibers and the polymer matrix. Similarly, Huzaifa *et al.* (2024) utilised sisal glass fibers (50%) in epoxy, achieving a tensile strength of 69.1 MPa and a high hardness value of 24.73, indicative of superior resistance to deformation. These results highlight the suitability of natural fibers for structural applications where high strength and durability are critical.

Boopalan, Niranjanaa and Umapathy (2013) studied a hybrid composite of jute and banana fibers (50%) in epoxy, reporting enhanced mechanical and thermal properties, along with reduced moisture absorption. The reduction in moisture uptake is significant as it improves the composites' durability and dimensional stability, making them more viable for applications in humid environments. These findings align with prior studies emphasising the benefits of hybrid natural fibers for synergistic improvements in polymer performance (Kumar *et al.* 2022).

Wood *et al.* (2011) investigated the addition of lignin (1–10%) in epoxy, finding the highest tensile and flexural strengths at 2.5 wt.% filler (86.16 MPa). The decline in strength at higher filler content suggests filler agglomeration, leading to stress concentration and matrix disruption. This underscores the importance of optimising filler content for achieving peak mechanical performance, a recurring observation in polymer-filler studies (Natrayan *et al.* 2023).

Majhi, Samantarai and Acharya (2012) introduced rice husk (5–20%) in epoxy and observed a 10% reduction in abrasive wear loss, with wear resistance maximised at optimal filler content. The use of surface-treated rice husk further amplified these benefits, emphasising the role of chemical modifications in enhancing filler-polymer interactions. Such treatments mitigate filler incompatibility with hydrophobic matrices, improving composite properties (Mohammed *et al.* 2022; Olonisakin *et al.* 2022).

Case 2: Enhancing the mechanical properties of Polyurethane Composites using natural fillers

Członka *et al.* (2020b) added grounded clove (1–5%) to polyurethane, achieving an 18% increase in compression strength, 11% improvement in flexural strength, and 8% enhancement in impact strength at 1–2 wt.% filler. However, beyond this range, diminishing returns were noted, aligning with findings from Fan *et al.* (2012), who investigated glass microsphere and nanoclay fillers (1–7%) in biobased rigid polyurethane. Although foam volume increased with filler content, compressive strength decreased at 7% filler due to lower density and weaker cell wall structures. The impact of fiber type and content on polymer properties is exemplified by Głowińska, Datta and Parcheta (2017), who incorporated sisal fibers (5–15%) into polyurethane. While hardness improved at lower filler contents, tensile strength and elongation properties declined at higher percentages, attributed to poor dispersion and matrix weakening. Husainie *et al.* (2020) also reported similar trends with cellulose, chitin, hazelnut, and eggshell fillers (1–5%), where tensile strength peaked at 1 wt.%, but properties such as split tear strength deteriorated significantly at higher concentrations.

Ozgur Seydibeyoglu *et al.* (2017) demonstrated the potential of the olive kernel and nutshell fibers (2.5–7.5%) in polyurethane, with the highest compressive strength recorded at 2.5 wt.% olive kernel filler, enhancing structural performance. This improvement highlights the strategic role of rigid fillers in boosting foam stability and load-bearing capacity, which is critical for lightweight structural applications.

Table 2.5: Summary of empirical work on enhancing the mechanical properties of epoxy and polyurethane with natural fillers

Authors & Year	Type of Bio-based Fillers	Weight %	Polymer Material	Outcome of Mechanical Properties
Shah <i>et al.</i> (2023)	Jute fibers	30%	Epoxy	Tensile strength increased by 10.06%, while flexural strength improved by 4.70%, highlighting better load-bearing capabilities.
Huzaifa <i>et al.</i> (2024)	Sisal glass fibers	50%	Epoxy	Achieved a tensile strength of 69.1 MPa, demonstrating superior strength, and recorded the highest hardness value of 24.73, indicating excellent resistance to deformation.
Boopalan, Niranjanaa and Umapathy (2013)	Jute/banana fiber	50%	Epoxy	Enhanced mechanical and thermal properties with a notable reduction in moisture absorption, improving durability and stability.
Wood <i>et al.</i> (2011)	lignin	1-10%	Epoxy	Tensile and flexural strengths significantly increased at 2.5% w/w filler, reaching 86.16 MPa. Strengths declined at higher filler content.
Majhi, Samantarai and Acharya (2012)	Rice husk	5-20%	Epoxy	Abrasive wear loss was reduced by 10% with improved wear resistance at optimal filler content. Surface-treated rice husk enhanced these effects.
Członka <i>et al.</i> (2020b)	Grounded clove	1-5%	Polyurethane	Compression strength increased by 18%, flexural strength improved by 11%, and impact strength enhanced by 8% at 1-2 wt.% filler content.
(Fan <i>et al.</i> 2012)	Glass microsphere and nanoclay fillers	1-7%	Biobased rigid Polyurethane	Foam volume increased with filler content. However, compressive strength decreased at 7% filler due to lower density and weaker cell walls.

Głowińska, Datta and Parcheta (2017)	Sisal fibers	5-15%	Polyurethane	Hardness slightly improved at 5% filler content, but tensile strength and elongation decreased with higher filler percentages.
Husainie <i>et al.</i> (2020)	cellulose, chitin, hazelnut, and eggshell	1-5%	Polyurethane	At 1 wt.%, tensile strength and elongation improved. However, properties, including split tear strength, decreased significantly at 5 wt.%.
Ozgur Seydibeyoglu <i>et al.</i> (2017)	olive kernel and nutshell fibers	2.5-7.5 %	Polyurethane	The compressive strength of polyurethane foam increased at 2.5 wt.% olive kernel, enhancing structural performance.

The above-reviewed studies collectively illustrate the significant role of bio-based fillers in enhancing the mechanical, thermal, and durability properties of polymer composites. Optimal filler content, fiber-matrix compatibility, and surface modifications are critical determinants of the resultant composite properties. The incorporation of natural fillers aligns with the global shift towards sustainable materials, offering an eco-friendly alternative to synthetic reinforcements (Lionetto and Esposito Corcione 2021). However, challenges such as filler dispersion, moisture absorption, and trade-offs at higher filler concentrations warrant further investigation to maximise the potential of bio-based composites.

Interfacial interaction between composites and filler particulates plays a critical role in enhancing the mechanical properties of polymeric materials. For instance, research by Hani *et al.* (2021) reported that strong interfacial interactions between epoxy and fishbone particles (FBPs), along with mechanical interlocking, significantly improve the thermal properties of epoxy/FBP composites. When FBPs are embedded in epoxy, they form mechanical interlocks with epoxy molecules, creating a robust interfacial layer. Additionally, epoxy molecules form a layer at the interface with FBPs, further strengthening the composite structure. Similarly, Husseinayah and Mostapha (2011) observed increased interfacial adhesion between filler particles and the resin matrix under scanning electron microscopy. Their findings underscore the importance of interfacial adhesion and mechanical interlocking in enhancing the compatibility and overall performance of filler particulates within polymer matrices.

2.9 CONCLUSIONS

This chapter provides a comprehensive review of the literature on polymer composites and their reinforcement using natural biowaste, particularly fish scales. The focus is on epoxy and polyurethane due to their extensive applications in industries such as biomedical, construction, automotive, and aerospace. The properties, characteristics, and applications of these two polymers are discussed in detail.

The review underscores the fact that while polymeric materials exhibit favourable mechanical properties, their performance can be significantly enhanced through reinforcement with filler materials. Natural biowaste is highlighted as an environmentally friendly and cost-effective option for reinforcement.

The chapter examines the properties and characteristics of biowaste as a filler material, supported by an analysis of existing literature and scholarly work. Particular emphasis is placed on collagen, a biobased filler, with an extensive discussion of its structure and sources. The chapter also elaborates on the structural features of fish scales that make them an ideal source for collagen extraction. It is noted that the efficiency of collagen extraction from natural sources depends on several factors, including temperature, extraction time, solvent concentration, and buffering conditions.

Additionally, the mechanical properties of polymeric materials—such as tensile strength, hardness, flexural strength, and impact resistance—are reviewed. The chapter concludes with an empirical analysis of the role of natural fillers in enhancing both the mechanical and thermal properties of epoxy and polyurethane composites.

The subsequent chapter presents the research methodology and outlines the experimental procedures for extracting collagen and using it to reinforce epoxy and polyurethane polymers.

3.1 INTRODUCTION

The methodology and design employed in this study are detailed in this chapter. The research design consists of several phases, with the initial phase centred on developing a systematic approach for extracting fish scale collagen and milled fish scale powder utilising acid extraction and ball milling techniques. The process of collagen extraction from fish scales involves a series of steps, including preparation, demineralisation, purification, and freeze-drying. This method is compared to existing collagen extraction techniques and optimisation parameters documented in the literature. While adhering to established collagen extraction protocols, adjustments to optimisation parameters can enhance the efficiency and yield of the process based on specific research objectives. Subsequently, the extracted materials are integrated as reinforcements into polymer composites, notably epoxy resin and polyurethane, followed by extensive testing of the mechanical and chemical properties of the resulting products. Additionally, detailed elaboration on the methodology employed in subsequent phases, involving various experimental procedures and analyses, is provided.

3.2 BACKGROUND TO THE RESEARCH METHODOLOGY

Research is generally stimulated by several methods, such as the scientific method of inquiry and critical thinking, deductive reasoning, and inductive reasoning, amongst others (Welman 2005; Berg and Latin 2008). Deductive reasoning provided the basis for the initiation of this study. The methodology employed in this research draws upon established techniques and principles to address the primary objectives of the study. It has been suggested in the literature that the combination of polymeric materials and natural fillers maximises the use of raw materials and minimises the accumulation of waste (Boaventura *et al.* 2020). In line with this, this study sought to extract fish scale-derived collagen (FSC) and assess its reinforcing properties as a filler on polymeric composite with special emphasis on epoxy and polyurethane. The methodology employed in this research adheres to a quantitative research approach and an experimental research design strategy, aligning with established principles in scientific inquiry. According to Johnson and Christensen (2024), a quantitative approach involves testing hypotheses with empirical data to ascertain their validity, providing a robust framework for data analysis and interpretation. To facilitate the proposed research design and methodology, a three-phase research methodology was considered most appropriate (Figure 3.1). This section provides a comprehensive overview of the materials utilised and the methodologies employed in the extraction of fish scale collagen from fish scales, as well as the

optimization process carried out. Subsequently, these extracted materials are integrated into polymer composites, specifically polyurethane and epoxy resin, to evaluate their mechanical properties, characterization, and overall performance enhancement.

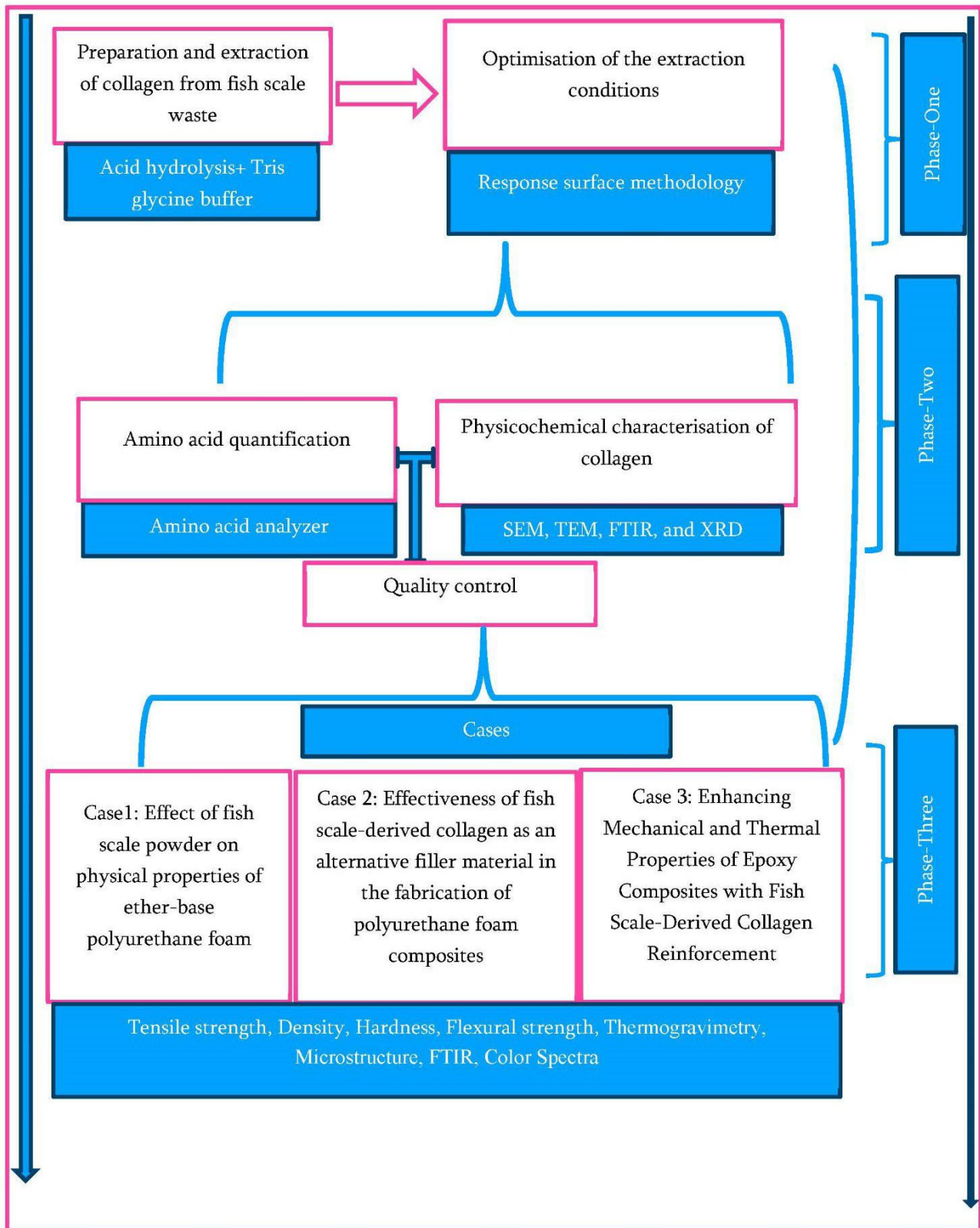


Figure 3.1: Experimental research design outline

3.3 MATERIALS AND METHOD

Waste fish scales were collected from local fish markets in Durban, South Africa. Sodium hydroxide (NaOH), acetic acid, sodium chloride (NaCl), and tris-glycine buffer were obtained from Merck Chemicals (South Africa). Household bleach was used for initial disinfection of fish scales. Polyol, toluene diisocyanate (TDI), methyl chloride, and auxiliary additives (catalysts and surfactants) for polyurethane foam synthesis were supplied by MinTek (Randburg, South Africa). Epoxy resin and hardener were procured from AMT Composites (Durban, South Africa). Dialysis membranes with a molecular weight cut-off of 12–14 kDa were purchased from Sigma-Aldrich (USA). The methods are structured in phases as outlined below:

3.3.1 PHASE ONE: PREPARATION AND EXTRACTION OF FISH SCALE COLLAGEN AND MILLED FISH SCALE POWDER FROM FISH SCALE WASTE

The extraction of fish scale collagen serves as a crucial initial step, guided by methodologies outlined in prior literature (Zhang *et al.* 2011; Huang *et al.* 2016). Acid extraction and ball milling methods are chosen for their efficacy in isolating collagen and producing fine powder from fish scales, as documented by previous studies (Chalamaiah *et al.* 2012; Dehghani *et al.* 2019). The methodology is designed to build upon existing knowledge while incorporating innovative approaches to address specific research objectives. Fish scale collagen and milled fish scale powder from fish scale waste were extracted and characterised as described in the sections below.

(a) Preparation of fish powders (FSP) from fish scales

In preparation for FSP from waste fish scales, fish scales were first collected from local fish shops within Durban, South Africa. All preparation and chemical extraction of the fish scales were done at the Biochemistry and X Laboratory (Durban University of Technology, Durban, and MinTek, Randberg, South Africa). Following the techniques demonstrated by Onwubu (2016), the fish scale powder was prepared with slight modifications based on the formulae below:

- **Preparation for Demineralization:**

Fish scales are defrosted to room temperature to facilitate the removal of bacteria, such as *Pseudomonas*, *Vibrio*, *Aeromonas*, and *Shewanella* species.

- **Washing and Disinfection:**

Fish scales (500g) are washed and disinfected using distilled water and 5 ml of household bleach. Scales are then air-dried for 2 days to eliminate excess water and thereafter oven-dried at 80 °C for 3 hours (Figure 3.2).



Figure 3.2: Dried fish scales (A) Air dried; (B) Oven dried

- **Ball Milling:** The oven-dried fish scales were subsequently crushed into powder (Figure 3.3). This was achieved by placing 30 g of the fish scales in a 250 ml stainless steel jar (inner diameter of 100 mm), together with 20 stainless steel balls of 10 mm diameter and dry milling in a planetary ball mill (Retsch[®] PM 100) at 500 rpm for 1 hour (Figure 3.4).



Figure 3.3: Milled fish scales

- **Mechanical sieving:** As shown in Figure 3.3, the milled fish scale powder was sieved using a mechanical shaker for 60 minutes at a speed of 100 rpm using a sieve with a mesh size of 25 μm .

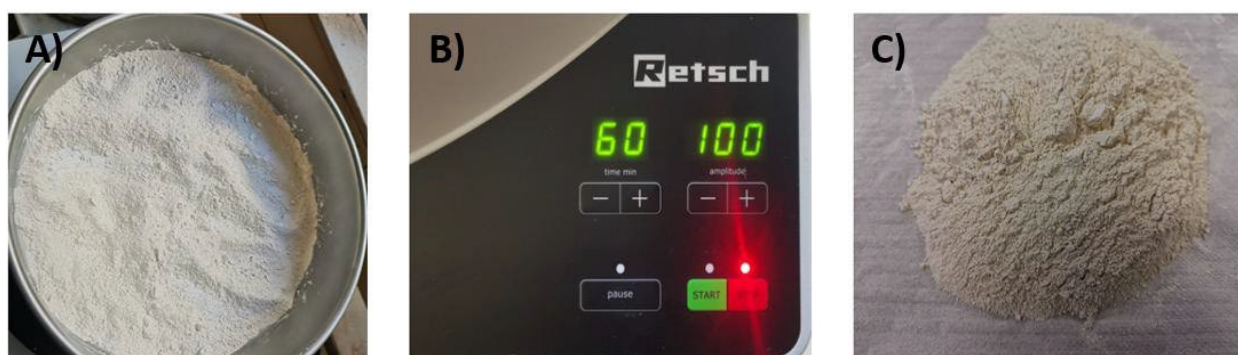


Figure 3.4: Mechanical sieving process: (A) fish scale powder in sieve mesh, (B) sieving conditions, (C) fine fish scale powder

(b) Preparation and extraction of fish scale collagen (FSC) from fish scales

Using the collected fish scales reported in section 3.3.1.2, fish scale collagen was extracted through acid hydrolysis (Figure 3.5). All preparation and chemical extraction of the fish scales were done at the Biochemistry and X Laboratory (Durban University of Technology, Durban, and MinTek, Randburg, South Africa). Following defrosting to room temperature to facilitate bacteria removal, the scales were air-dried for 2 days to eliminate excess water. FSC was extracted according to a well-established protocol involving demineralisation and acid hydrolysis (Wahyuningsih *et al.* 2018). The scales were treated with 0.1 N NaOH for 2 days to eliminate non-collagenous proteins and pigments. After washing and drying for 2-3 weeks, collagen extraction was carried out using varied parameters. Collagen extraction involved soaking 100 g of scales in 0.5 M acetic acid for 48 hours at 4 °C with intermittent stirring. The solution underwent centrifugation at 3000 rpm for 30 minutes at 4 °C. Supernatants were salted out by adding 9% NaCl, stirred automatically for 1 day, and suspended in a tris glycine buffer. Pellets were collected via centrifugation, washed with distilled water, and subjected to dialysis using a dialysis membrane (MWCO 12-14 kDa) to eliminate residual salts. The resulting material was freeze-dried at -80 °C under vacuum conditions. Characterisation of the resulting collagen was conducted using different analytical techniques.

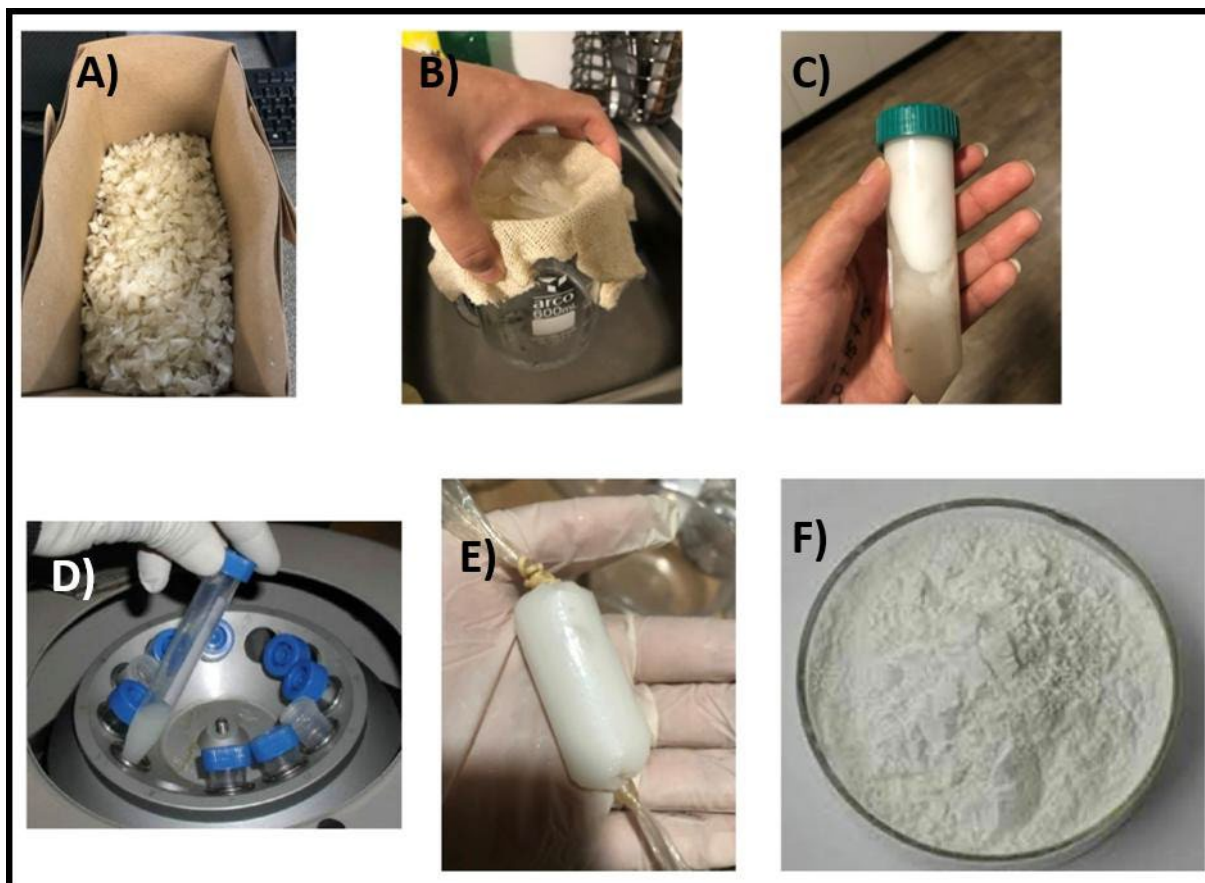


Figure 3.5: Schematic illustration of FSC extraction (A) Washed and Dried Fish Scales; (B) Soaked Fish Scales after 48 hours separating using a muslin cloth; (C) Acetic acid solution ready for centrifugation; (D) Samples in Centrifuge; (E) Dialysis tubing with sample; (F) Vacuum Dried Fish Scale Collagen

(c) Optimisation of the extraction parameters

As advocated in the literature, the Design of Experiments (DOE) was used to determine the number of screening experiments to optimise the parameters (Dixit and Kumar 2015). A four-factor-three-level at the center point and 36 experiments. The optimal experimental conditions are determined by comparing the averages. Fisher RA emphasized the importance of identifying the optimal combination of factors and levels in the DOE to achieve desired outcomes. Subsequently, the results are validated through Analysis of Variance (ANOVA) focusing on dominant factors, as indicated by prior studies (Agarwal *et al.* 2016; Meena, Kumar and Pandya 2017). Table 3.1 illustrates the primary factors and their corresponding levels, while Table 3.2 outlines the extraction conditions. Utilising MINITAB 17 software, this methodology enables the evaluation of test outcomes in the form of signal-to-noise ratios (SN).

Collagen yields were calculated as follows:

$$Yield (\%) = \left(Dry\ collagen \frac{weight}{Dry} scales\ weight \right) * 100$$

Signal-to-Noise Ratio: Nominal is best.

Formula: $10\log_{10}(Y\bar{Y}^2 / s^2)$

Table 3.1: Coded values and independent variables used for optimisation

Factors	Symbols	Coded Level		
		-1	0	+1
Concentration of acetic acid	A	0.5	1	5
Volume of acetic acid (mL)	B	100	300	500
Time of soaking fish	C	30	60	120
Buffer (mL)	D	2,5	5	10

Table 3.2: Plan of experiments (extraction conditions) as per 36 runs

Run	Concentration of acetic acid	Volume of acetic acid (mL)	Time of soaking fish	Buffer (mL)	Yield
1	0,5	100	30	2,5	0
2	0,5	100	60	2,5	0
3	0,5	100	120	2,5	0
4	0,5	300	30	2,5	0
5	0,5	300	60	2,5	0
6	0,5	300	120	2,5	0
7	0,5	500	30	2,5	0
8	0,5	500	60	2,5	0
9	0,5	500	120	2,5	0
10	5	100	30	2,5	0
11	5	100	60	2,5	21,73
12	5	100	120	2,5	3,48
13	5	300	30	2,5	0
14	1	300	60	2,5	0
15	1	300	120	2,5	0
16	1	500	30	2,5	0
17	5	500	60	2,5	0
18	5	500	120	2,5	0
19	0,5	100	30	5	0
20	0,5	100	30	5	0
21	0,5	100	60	5	4,53
22	5	100	60	5	0

23	5	100	60	5	11,94
24	5	100	60	5	11,04
25	0,5	100	30	10	11,64
26	0,5	100	60	10	11,85
27	0,5	100	60	10	10,89
28	5	100	30	10	11,56
29	5	100	30	10	14,81
30	5	100	60	10	11,5
31	0,5	100	30	10	9,19
32	0,5	100	30	10	20,9
33	0,5	100	60	10	22,28
34	5	100	30	10	20,13
35	5	100	30	10	22,03
36	5	100	60	10	20,28

3.3.2 PHASE TWO: PHYSICOCHEMICAL CHARACTERISATION OF THE EXTRACTED FSP AND FSC

Phase two details various characterisation techniques applied to determine the physicochemical properties of fish scale powder (FSP) and the extracted fish scale collagen (FSC). This was done in compliance with the international standards for quality management and quality assurance (ISO 9001: 2008). The particle size, shape, phase change, and crystallinity of FSP and FSC were determined by using various analytical techniques. These are detailed in the sections below.

3.3.2.1. Fourier transform infrared spectroscopy analysis

Perkin Elmer Universal ATR was used to identify the functional groups present in the prepared MFSP. Prior to scanning, a background investigation was carried out. Afterwards, a small amount of the prepared sample powders was placed in a sample holder and scanned between 400 and 4500 cm^{-1} with a resolution of 4 cm^{-1} .

3.3.2.2. X-ray diffraction analysis

An X-ray diffraction (XRD) analysis was carried out on the crystalline structure of the prepared MFSP. The D8 Advance BRUKER AXS instrument from Germany, using Cu-K α radiation ($\lambda_{\text{K}\alpha 1}=1.5406$), was used to capture the patterns within the 10 to 80 (2 theta) range. The parameters used for the analysis included 40 kV of voltage, 40 mA of current, and 0.5 s of pass time.

3.3.2.3. Field Scanning Electron Microscope Analysis

The surface morphology was studied using scanning electron microscopy (SEM) (field emission, Carl Zeiss) operating at 20 kV under controlled air conditions. A thin, electrically conductive gold coating was applied to the surface to prevent an electrostatic charge build-up before SEM observation.

3.3.2.4 Determination of Amino acid composition of FSC

The Kjeldahl method was utilised to ascertain the amino acid content of the extracted FSC, while the Stegmann method (Hurych 1962) was employed to evaluate the hydroxyproline concentration. An automatic amino acid analyser was used to get the results of the amino acid composition tests for FSC, which are shown in Paper 2 (Table 2). Amino acids are the building blocks of proteins, and their distribution is crucial in determining the overall characteristics of the protein.

3.3.3 PHASE THREE: ASSESSING THE MECHANICAL PROPERTIES OF THE REINFORCED COMPOSITES

This phase assesses the fitness of the purpose of the prepared FSP and FSC as filler material in polymeric materials (Polyurethane and Epoxy). The fillers were integrated into polymer composites, namely epoxy resin and polyurethane. Mechanical and chemical testing of the composites follows, utilising standardised procedures to assess properties such as tensile strength, flexural strength, and hardness (Haque *et al.* 2021).

3.3.3.1 Preparation of polyurethane-based composites

This section details the method of preparing the polyurethane foam (PUF) using fish scale powder (FSP) and fish scale collagen (FSC) as the fillers in these composites. The preparation and fabrication process are detailed in the subsections below.

Case 1: Preparation of Polyurethane-based composites using FSP as the filler

Table 3.3 highlights the general formula used for the preparation of the PUF-based composites (Paper 1). Flexible polyurethane foams were prepared by mixing MFSP with the PUF using a mechanical stirrer. Different weight percentages of MFSP (ranging from 0 to 1 wt.%) were added to the PUF to prepare composites with varying levels of reinforcement. The amounts of each chemical component were chosen to obtain a target density of 16-17 kg/m³. The experiment was conducted in a Foam Laboratory equipped with a manual mixing apparatus. The apparatus includes a stirrer with two

operating speeds (600 and 1300 rpm) and is powered by a 7.5 HP motor, allowing for efficient mixing of the components. The chemical materials were measured and deposited into the large 3L container by hand dosages. The materials used in this paper comprise a standard polyurethane foam recipe.

Table 3.3: The general formula for the PUF-based composites

Materials	Sample groups by weight %		
	Std	Std +0.5g Fish	Std +1.0g Fish
		Scale Powder	Scale Powder
POLYOL -1906	170.01	170.01	170.01
TDI-T80	123.79	123.79	123.79
WATER	9.81	9.81	9.81
CATALYST MIX-TIN/MESAMOL	2.75	2.75	2.75
AMINE MIX-1906/B18	0.69	0.69	0.69
SILICONE-L620	3.11	3.11	3.11
BLOWING AGENT -METHYL CHLORIDE	29.01	29.01	29.01
COLOUR STABILIZER -CS-15	3.01	3.01	3.01
FILLER -KULU POWDER	50.01	50.01	50.01
MILLED FISH SCALE POWDER	0	0.50	1.00

The procedure for preparing PUF-based composites involved two steps. The first step entails weighing the polyol and all other ingredients, such as methyl chloride, catalysts, other additives, and water, to specific amounts in the 3 L container. Thereafter, MFSP was added as a filler and followed by stirring for 20-30 seconds. Then the second step follows by weighing out the Toluene Diisocyanate (TDI) specific amounts separately in a 500 ml cup and adding to the stirred mixture of the polyol, water, and additives and stirring again for 10-15 seconds before it starts creaming in the container. This is immediately transferred into a 305 x 235 x 305 mould where cream and rise times are recorded (Figure 3.6A). After the polyurethane foam is at a full rise, it is transferred to a 70 °C oven to cure for 10 minutes. This step is repeated 5 times with different amounts of filler swapped and added to the standard formulation. This is then cut using a band knife and a clicking press for the correct dimensions for the physical property testing.



Figure 3.6: PUF (A) creaming and rising; (B) formed PUF foam

Case 2: Preparation of Polyurethane-based composites using collagen as the filler

The general formula utilised to prepare the PUF-based composites is highlighted in Table 3.4 (Paper 2). Following a standardised process similar to that described by Naidoo and Onwubu (Naidoo *et al.* 2023), the PUF foam samples were manufactured, and their qualities were methodically assessed. In this process, a mechanical stirrer was used to combine FSC and PUF to make PUFs. To create composites with various reinforcement levels, different weight percentages of FSC (2.5, 5, and 10 g) were added to the conventional PUF mix. In addition, 50 g of FSC (swap sample) was used in place of Kulu powder or calcium carbonate in the usual formula. This was done to assess the possibilities of replacing the commonly used filler material in the foam industry. To get the target density of 16–17 kg/m³, the composition was changed. In the experimental setting, a manual mixing device was included in the Foam Laboratory. Weighing the polyol, additives, and additional ingredients, such as Kulu powder, to precise proportions in a 3 L container was the process for making PUF-based composites. FSC in varying concentrations was then added as a substitute and an additional filler, followed by stirring. In a separate cup, Toluene Di-isocyanate (TDI) with an index value of 113.6 was weighed and added to the stirred mixture of polyol, water, and additives. The mixture was stirred again before being transferred into a mould for creaming and rise time recording. The foam was dried for ten minutes at 70 °C in an oven following a full rise. Three trials of this procedure were conducted, one with a standard sample and two with varying FSC doses. Before being subjected to physical property testing, the foam blocks were sliced using a band cutter and clicking press to ensure

dimensional correctness. Correctly sized cut samples were subjected to two different temperatures: 24 °C in a controlled laboratory setting and 140 °C in an oven for heat aging.

Table 3.4: Formulation details for producing foams made of PUF composites using fillers made of FSC and CaCO₃.

	Std	Swap Out Filler (Kulu powder) with Fish Scale Collagen	Std + 2.5g Fish Scale Collagen	Std + 5g Fish Scale Collagen	Std + 10g Fish Scale Collagen
POLYOL	170.0 ₁	170.01	170.01	170.01	170.01
TDI	123.7 ₉	123.79	123.79	123.79	123.79
WATER	9.81	9.81	9.81	9.81	9.81
CATALYST MIX	2.75	2.75	2.75	2.75	2.75
AMINE MIX	0.69	0.69	0.69	0.69	0.69
SILICONE	3.11	3.11	3.11	3.11	3.11
BLOWING AGENT	29.01	29.01	29.01	29.01	29.01
COLOUR STABILIZER	3.01	3.01	3.01	3.01	3.01
FILLER -Kulu powder (CaCo ₃)	50.01	0	50.01	50.01	50.01
FISH SCALE COLLAGEN	0	50	2.5	5	10
CREAM TIME	10	12	11	10	12
RISE TIME	115	129	139	144	148

Note: std=Standard sample, all preparations were in weight.

Case 3: Preparation of epoxy laminate using collagen as a filler

The epoxy laminate composite was prepared through a conventional resin casting technique. The epoxy resin is hand-mixed with the FC and then mixed with the hardener and poured into the glass sheets. A rectangular glass sheet (300 x 300 x 4 mm) served as a mould during the casting process (Figure 3.7), ensuring homogeneity and consistency in the laminate formation. A 4 mm rubber was used in between the glass sheets to determine a thickness and tightened with bolts and screws till cured for 24 hours. The epoxy laminate sample after 24 hours of curing at room temperature was removed from glass sheets and placed into an oven at 50 degrees Celsius for 16 hours for post-curing.



Figure 3.7: Conventional casting of epoxy resins

Five composite specimens ($n = 5$) were fabricated with different volume fraction percentages of FC, and epoxy polymer as shown (Table 3.5).

Table 3.5: Sample groups for epoxy composites

Sample codes	Epoxy resin (g)	Hardener (g)	Fish scale collagen (g)	Fish scale collagen (%)
Neat	84	316	0	0
EFC 5%	84	316	20	5
EFC 10%	84	316	40	10
EFC 15%	84	316	60	15
EFC 20%	84	316	80	20
EFC 25%	84	316	100	25

The reinforced composites were then machined to obtain mechanical test specimens (Figure 3.8). The reinforced and modified composites were tested to obtain the mechanical results of the epoxy resin.



Figure 3.8: Machining out epoxy specimens

3.3.4 MECHANICAL AND PHYSICAL PROPERTIES OF POLYURETHANE COMPOSITES

The mechanical and physical properties of the PUF-based composites were characterized using standard test methods. Tensile strength, elongation at break, and compression strength were measured using an Instron Universal Testing Machine. Physical testing properties are conducted under 3 temperatures: room temperature humidity aging and heat aging. Four composite specimens were reinforced with different volume fraction percentages of fish scale powder and polyurethane polymer as shown in Table 3.1. The reinforced composites are then machined to obtain the mechanical test specimens. The reinforced and modified composites are then tested to obtain the physical results of the ether-based polyurethane foam.

3.3.4.1 Tensile strength test

The tensile test was conducted as per SABS 640 – 1976 Section 6.8 standards using a 2kN Instron. The dimension of the specimen is 150 x 27 x 17 mm with a dog bone shape die. Using the die, 3 samples with a thickness of at least 12.5 mm were cut (Figure 3.9).



Figure 3.9: PUF tensile specimens

During the test, the test piece is clamped in the jaws of the machine, making sure that the sample is vertical so that the tension is distributed uniformly over the cross-section. The machine speed is set to 500 mm/min, and the gauge length is set to 45 mm. The machine is operated until the sample ruptures and records the tensile strength results in kPa (Figure 3.10). The tensile strength was calculated using the formula:

$$\text{Tensile strength (kPa)} = \frac{\text{Max force applied (N)}}{\text{Thickness of sample (mm)}} \times \frac{1000}{13}$$



Figure 3.10: Testing tensile strength of PUF

3.3.4.2 Elongation at breakpoint

After conditioning, and using the die, 3 samples with thicknesses of at least 10 to 15 mm are cut without skin. The gauge lengths on the sample are marked. The test piece is clamped in the jaws of the machine, making sure that the sample is vertical so that the tension is distributed uniformly over the cross-section. The machine speed is set to 500 mm/min and the gauge length is set to 45mm. The machine is operated until the sample ruptures and records the elongation in %. Tensile strength is conducted simultaneously with the elongation at the break test. This test is conducted as per SANS 883:2009 refers to ISO 1798. The dimension of the specimen is the same as reported in section 2.41. The elongation at break was calculated using the formula:

$$\text{Elongation at Break (\%)} = \frac{\text{Final gauge length} - \text{Original gauge length}}{\text{Original gauge length}} \times 100$$

3.3.4.3 Tear resistance test

The force needed to tear a piece of foam of standard thickness is measured. This test indicates the tear resistance of the foam in end-use. From the appropriate slab, 3 samples each of size 150 x 25 x 25 mm were cut longitudinally down the center of each specimen to form two tongues of length 40 mm. Thereafter, the tongue was clamped in the jaws of the machine (Figure 3.11). The machine speed was set to 100 mm/min. The machine was operated until the tear propagated through the center of the sample. The maximum force (N) was recorded during the test. This test is conducted as per SABS 640 – 1976 SECTION 6.9. The tear resistance was calculated using the formula:

$$\text{Tear resistance (N/mm)} = \frac{\text{Max. Force (N)}}{\text{Width of sample (mm)}}$$

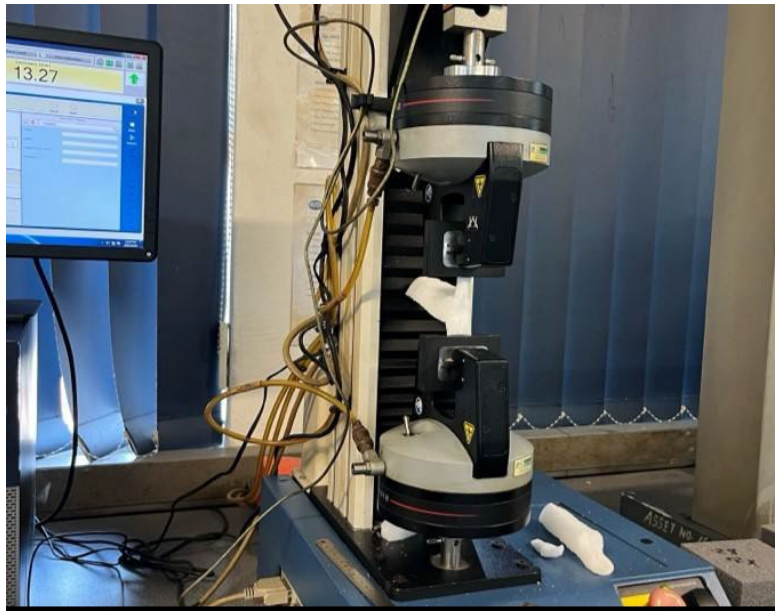


Figure 3.11: Measuring the tear resistance of PUF

3.3.4.4 Density

Density was conducted using a sample size of 100 x 100 x 50 mm. The sample was measured with a steel ruler graduated in millimetres and accurate to 1mm for length, breadth, and height. The sample was thereafter weighed for the mass of the sample using a balance having a sensitivity of 0.1 g. Density was kept standard as all samples were cut from the bottom of the rise profile. The mass per unit volume of a sample was measured and calculated as per the formula below.

$$D = \frac{M}{V} \times 1000$$

Where D = Density, Kg/m³, M = Mass, kg and V = Volume, m³

3.3.5 MECHANICAL AND PHYSICAL PROPERTIES OF EPOXY LAMINATE COMPOSITES

The physical properties of the epoxy resin composites were assessed using standardised test methods.

3.3.5.1 Tensile strength of epoxy composites

Tensile strength was evaluated according to ISO 527-2 standards, employing a 2kN Instron apparatus known for its standardised methodology. This test aimed to characterise key mechanical properties, including tensile strength, elastic modulus, and elongation at break. Dog bone-shaped specimens with dimensions conforming to ASTM D638 (20 mm width, 4 mm thickness, and 165 mm length) were utilised to ensure consistency with industry norms. To ensure statistical reliability, five specimens were tested to obtain mean values and deviations. Careful attention was given to the sample clamping to ensure uniform tension distribution. Operational parameters such as a speed of 1 mm/min and a load cell size of 100 kN were meticulously controlled to ensure reproducibility (Figure 3.12).

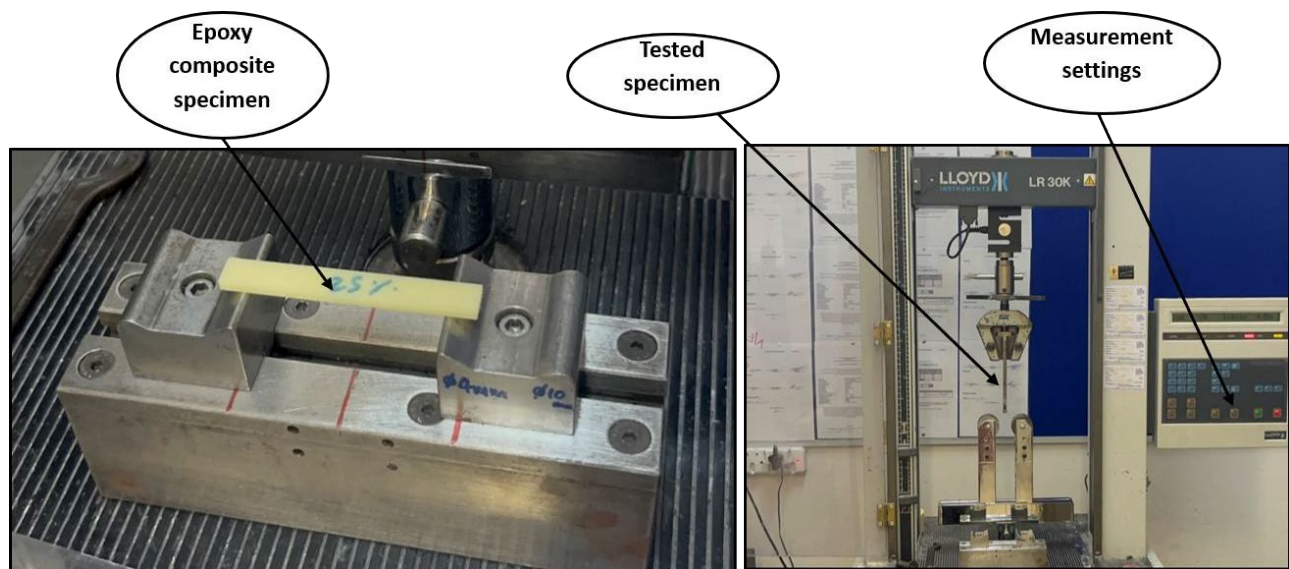


Figure 3.12: Testing of epoxy laminates

3.3.5.2 Hardness of epoxy composites

The Barcol hardness of cured epoxy resin laminates was evaluated by ASTM D2583 standards (Figure 3.13). This method involved indenting the material's surface with a sharp steel point and measuring the resulting hardness, providing insights into the laminate's strength and suitability for various applications.



Figure 3.13: Barcol hardness tester.

3.3.5.3 Flexural properties of epoxy composites

Flexural properties were assessed following ISO 178 standards. Specimens with dimensions of 80 x 10 x 4 mm were tested using a Lloyd's tensile tester. The test aimed to determine flexural strength and modulus, crucial parameters indicative of material stiffness and deformation resistance. The experimental setup included a specified crosshead speed of 2.2 mm/min, a span of 68 mm, and a load cell size of 30 kN. This testing configuration ensured adherence to standardised procedures and facilitated comparisons with existing literature.

3.3.6 MICROSCOPIC ASSESSMENT

This section provides details of the microscopic assessment carried out on both PUF and epoxy composites.

3.3.6.1 Microscopic assessment of PUF-based composites cell structure reinforced with FSP

Using a handheld instrument Dino Lite Digital microscope. Dino Lite is a 1.3 Megapixel resolution with a 10-70X & 200X Magnification containing 8 LED white lights with an infrared cut filter of more than 650 nm. The DinoXcope (Mac OS) software is downloaded on the laptop, connecting to the USB 2.0 device and the laptop. Multiple pictures are taken as per the zoom magnification point chosen (50 and 225 are used in this study). This is repeated with all samples and labelled and saved on the computer after each analysis to compare cell structure, cell membrane, and cell size change with the addition of MFSP (Paper 1).

3.3.6.2 Microscopic evaluation of PUF-based composites cell structure reinforced with FSC

Using a Carl Zeiss scanning electron microscope (SEM) with field emission, the surface morphology of PUF composites was examined. The SEM was run in controlled air conditions at 20 kV. Before SEM inspection, a small layer of electrically conductive gold was applied to the surface to prevent an accumulation of electrostatic charge.

3.3.6.3 Microscopic assessment of epoxy-fish scale-derived collagen composites (EFC)

The surface characteristics of EFC composites were analysed via scanning electron microscopy (SEM) using a field emission microscope from Carl Zeiss. The SEM was operated at 20 kV under controlled air conditions. Prior to observation, a fine layer of electrically conductive gold coating was deposited on the surface to mitigate the risk of electrostatic charge accumulation.

3.3.7 *FOURIER TRANSFORM INFRARED EVALUATION OF THE POLYMERS*

To examine the structural alterations in the PUF composite, the Perkin Elmer Universal ATR was employed. An investigation into the background was done before scanning (Paper 2). Subsequently, every sample that had been cut was put in a sample holder and scanned at a resolution of 4 cm^{-1} between 400 and 4500 cm^{-1} . Perkin Elmer Universal ATR was also used to identify the changes in the Epoxy composite's structure (Paper 3). Prior to scanning, a background investigation was carried out. Afterwards, each of the cut samples was placed in a sample holder and scanned between 400 and 4500 cm^{-1} with a resolution of 4 cm^{-1} . A similar procedure was followed to assess the interactions between the filler content and the epoxy matrix (Paper 3).

3.3.8 *TESTING OF THE THERMAL PROPERTIES*

This section provides details of the various thermal assessments conducted on both the PUF and epoxy composites.

3.3.8.1 Colour Spectra Light II Lightbox

The Spectra Light II is a visual colour evaluation system to identify colour variation in material. The results of this visual test indicate the colour variation of samples compared to standards using the Macbeth Spectra Light II Lightbox (Paper 1). Test specimen and standard sample undergo 4 light sources to determine if there are any visual differences under different light conditions:

- daylight (D65) – (main visual test needed)

- horizon (early sunrise/afternoon sunset simulation)
- Illuminate A (incandescent home lighting)
- Cool white fluorescent (CWF)

3.3.8.2 Thermogravimetric assessment of epoxy composite

Thermal properties of the samples were measured using Thermogravimetric analysis (TGA). TGA testing employed small quantities of samples (20 mg) in platinum crucibles. The temperature range was fixed between 30 to 600 °C, with a nitrogen flow rate of 30 ml /min, and the heating rate was maintained at 20°C /min (Paper 3).

3.3.8.3 Calorimetry assessment of epoxy composite

Calorimetry analysis was performed by a differential scanning calorimeter (DSC), Mettler Toledo (model: TAGC 10, model: DSC 822e). DSC testing took a few amounts of samples (20 mg). A platinum crucible was used to carry the samples. The temperature ranges were kept fixed between 30 and 600 °C. Nitrogen flow rate and heating rate were maintained at 30ml min⁻¹ and 20°C/min, respectively, during testing (Paper 3).

3.4 STATISTICAL ANALYSIS

Using the statistical package for social science (SPSS, IBM, version 29), One-way analysis of variance (ANOVA) was applied to test the mean differences in tensile strength, elongation at breaking point, and tear strength at $\alpha.05$.

3.5 VALIDITY AND RELIABILITY

The two most important aspects of experimental research design are centered on the validity and reliability of the data, as this ensures the study's credibility. In this study, the internal validity was ensured by following the ISO 527-2, ASTM D2583, ASTM D638, SABS 640, and SANS 883:2009 requirements for the preparation and testing of sampled composites (polyurethane and epoxy resin laminates).

In terms of the study reliability, it is advised that the reliability of an experimental research design be determined by the repeatability and reproducibility of the tests (Onwubu 2016). Repeatability is the variability of the measurement obtained by one person measuring the same item repeatedly, whilst

reproducibility, by contrast, is the variability of the average values obtained by several observers measuring the same item (Slezak and Waczulikova 2011; Vitek and Kalibera 2011). In this study, the repeatability and reproducibility were assured by the different number of sample laminates used in assessing the mechanical properties of both polyurethane and epoxy resins using FSP and FSC as a filler.

3.6 CONCLUSION

In summary, this chapter has exhaustively explained the research approach and the experimental design strategy carried out in the study. The different phase of the research approach was provided, as well as the study's validity and reliability process. The next chapter provides the results and discussion, which were presented based on papers published and or submitted to DHET-accredited journals.

4.1 INTRODUCTION

Polymeric composites are widely used in various industries due to their versatile properties, including high strength-to-weight ratios, durability, and resistance to environmental factors (Ganeshkumar *et al.* 2024). However, the incorporation of reinforcing agents can further enhance these properties, leading to improved performance and extended application ranges. Traditional reinforcing agents, such as glass and carbon fibers, although effective, pose environmental concerns due to their non-biodegradable nature (Sun 2018; Vinod *et al.* 2020). In this context, the exploration of natural fillers, such as fish scale collagen, is both timely and essential.

Natural fibers offer a cost-efficient means of producing polymeric composites while retaining their intrinsic properties (Kuranchie, Yaya and Bensah 2021). Fish scales as a natural fiber have gained tremendous attention for their cost-effectiveness, environmental friendliness, and efficiency in reinforcing polymeric composites (Babu *et al.* 2020). Fish scales are composed of collagen fibers, which are known for their high strength, stiffness, and toughness (Rawat *et al.* 2021). Despite the recognised potential of collagen as a reinforcing agent, there is limited research on its application in polymeric composites, particularly those involving fish scale-derived collagen. The mechanical properties of epoxy resin and polyurethane composites, when reinforced with fish scale collagen and milled scale powder, remain underexplored. This gap in knowledge hinders the development of sustainable composite materials that can leverage the benefits of natural biopolymers. Hence, this study aimed to extract fish scale-derived collagen (FSC) and assess its reinforcing properties as a filler on polymeric composite with special emphasis on epoxy and polyurethane. This chapter provides a critical review of the published paper that addresses each phase of the research objectives highlighted in Chapter One, Section 1.4. The critical review is presented in line with the published papers 1 to 3.

4.2 REINFORCEMENT OF POLYURETHANE COMPOSITES WITH MILLED FISH SCALE POWDER

Polyurethane foam (PUF) is a versatile material that is widely used in various industries due to its excellent mechanical properties, low cost, and lightweight (Kaikade and Sabnis 2023). Polyurethane materials are highly versatile due to their chemical composition, making them suitable for a wide range of applications where other materials may not be appropriate. While polyurethane materials have many practical applications, their production relies heavily on petrochemicals, which are now facing environmental concerns (Gama, Ferreira and Barros-Timmons 2018; Czlonka *et al.* 2020a).

The demand for a more sustainable environment has spurred the development of innovative green materials that rely on natural resources (Alyamaç *et al.* 2022). In this, biobased materials such as vegetable oil, which is plentiful and widely used, are the primary renewable source for producing raw materials, particularly polyols for PUF products (Agrawal, Kaur and Walia 2017; Członka *et al.* 2020a). However, these natural biobased materials, PUF foams, reportedly have a relatively low mechanical strength (Paberza, Cabulis and Arshanitsa 2014). To address these drawbacks, there is a growing interest in developing bio-based PUF foams that incorporate natural fillers derived from renewable sources. Considering this, it became pertinent to investigate the reinforcing properties of biowaste from fish scales. Paper 1 investigated the impact of incorporating milled fish scale powder (MFS) into ether-based polyurethane foam composites. The research focuses on understanding how the addition of this natural reinforcement affects the physical properties of the composite material, particularly at different temperature conditions. According to Márquez Costa, Legrand and Fréour (2019), temperature can have a significant impact on the properties of composite materials, including their mechanical and thermal properties. It thus meant that there was a need to investigate the effect of temperature on the physical properties of PUF-based composites reinforced with MFS.

The MFS was prepared using a mechanochemical approach. This technique is reported to be gaining increasing attention among researchers due to its simplicity, low cost, reproducibility, and environmental friendliness (Onwubu *et al.* 2019). The milling condition of 20 g of dried fish scales was dry milled in a 250 mL bowl at 500 rpm for 1 hour. The physicochemical characteristics of the MFS were established using different traditional characterisation techniques such as Field Scanning Electron Microscope (FESEM), X-ray Diffraction (XRD), and Fourier Transform Infrared (FTIR). The physical properties of the composites, namely tensile strength, elongation at break, tear resistance, density, colour, and cell structure, were characterised under different temperature conditions.

Paper 1 established that MFS contains collagen type I (amide I, amide II, and amide III), carbonate ions, and calcium-deficient apatite. The surface morphology of the MFS shows that the particles appear as angular, rod-like, and irregularly shaped fragments with various sizes and surface features. The tensile and elongation at breaking point results demonstrate that the inclusion of fish scale powder (0.5 and 1 wt.%) enhances the mechanical properties of the polyurethane foam, leading to improved tensile and compression strength. However, it was found that temperature had a significant effect on the mechanical properties. For instance, the results showed that at standard temperature (23 °C), the addition of 0.5 wt.% MFS resulted in an 18.8% increase in tensile strength and a 15.7% increase in elongation at break, while tear resistance exhibited significant improvements under heat aging, with a 22% increase (0.5 wt.% MFS) and a remarkable 37% increase (1 wt.% MFS) when compared to the

unreinforced sample. The finding from Paper 1 suggests that the addition of MFS can improve the mechanical properties of PUF foams, particularly at heat aging. The observed improvement under higher temperatures was attributed to an increase in stiffness and strength of the PUF foams due to good dispersion of MFS particles in the matrix.

It was also reported in Paper 1 that the addition of fish scale powder caused a reduction in the mass of the material without significantly affecting its volume. This is a desirable outcome as it suggests that the addition of fish scale powder can be used to enhance the mechanical properties of the composite material without significantly affecting its weight. The slight decrease in density observed with the addition of MFS can have certain implications. A lower-density material can offer advantages such as improved lightweight properties (Jassim 2017), making it easier to handle and transport. This is important in applications where weight is a critical factor, such as in the automotive and aerospace industries, as reported by *Sun et al.* (2022).

More so, in Paper 1, it was observed that the addition of MFS enhances the flame-retardant properties of PUF foams. The observed resistance to discolouration from the scorching test was attributable to the high protein content in the fish scales, which could have helped dissipate heat and reduce scorching. The finding aligns with the study by Agrawal, Kaur and Walia (2017) that the addition of natural filler materials to polyurethane foam enhances the mechanical, thermal, and flame-retardant behaviours. This is further supported by the microscopic image reported in Paper 1. An increase in the number of cells packed closely together was observed with the addition of MFS. This consequently resulted in the creation of semi-rigid foam, which is suitable for use in insulation or packaging materials, attributable to its increased strength and durability. Overall, Paper 1 highlights the potential of using fish scale powder as an eco-friendly and sustainable reinforcing agent in polymer composites, offering a viable alternative to traditional synthetic fillers.

Despite the enhanced mechanical properties of the polyurethane foam, leading to improved compression strength and thermal stability reported in Paper 1, some limitations and gaps were observed. Firstly, the maximum weight percentage of the milled fish scale (MFS) used in the study was 1 wt.%, as the polymer became very rigid beyond this percentage. This suggests that the optimum filler content of MFS in the polyurethane (PUF) matrix is 1 wt.%. Another gap observed in the study is that the interaction between the PUF matrix and the MFS at the molecular level was not reported. This was attributed to the difficulty of observing any changes due to the low weight percentage of MFS. These observed limitations and gaps provide a basis for further research to explore higher weight percentages of MFS and to investigate the molecular interactions within the composite matrix.

4.3 COLLAGEN-REINFORCED POLYURETHANE COMPOSITES (PAPER 2)

Building on the promising but compositionally complex results of MFS in polyurethane foams, Paper 2 explores the effectiveness of fish scale-derived collagen (FSC) as a filler material in the fabrication of polyurethane (PUF) foam composites. The rationale for the extraction of collagen from fish scale powder is premise on the assumption that the enhanced mechanical properties reported in Paper 1 were attributable to the collagen constituents in the MSF. Besides, Paper 2 attempts to address some of the shortcomings highlighted in Paper 1. In the Paper, collagen was extracted through demineralisation and acid hydrolysis. The amino acid composition of the collagen was analysed using the Kjeldahl Method. The composition of the amino acids in 100 g revealed 17 different types of amino acids. The high percentages of glycine (20.98%), proline (15.43%), and hydroxyproline (11.51%) suggest a significant presence of structural proteins like collagen (Gauza-Włodarczyk, Kubisz and Włodarczyk 2017).

The composites were prepared with varying FSC concentrations (2.5, 5, and 10 wt.%) with the standard PUF matrix, while calcium carbonates in the standard sample (STD) were completely substituted with 50 wt.% of collagens. Tensile, shear, elongation, and FSEM tests were used to describe the mechanical and physical characteristics of the PUF-based composites at both standard temperature (23 °C) and heat aging. The FTIR was used to provide a detailed understanding of the molecular changes occurring in the composite materials. In Paper 2, it was reported that the peaks around $1,650\text{ cm}^{-1}$ and 550 cm^{-1} were related to amide groups associated with the incorporation of FSC into the polyurethane matrix. The concentration-dependent effects of collagen on mechanical properties were examined, revealing nuanced relationships with tensile strength, elongation, tear resistance, and ductility. The results reveal that the addition of 2.5 wt.% FSC increased tensile strength by 12.66% during heat aging, while the addition of 5 wt.% at standard temperature increased elongation by 6.65%.

Comparison between collagen and traditionally used filler material in the foam industry (Kulu powder-calcium carbonate) revealed no significant difference in tensile strength among sample groups ($P = 0.805$), suggesting that variations in composition, including collagen addition and calcium carbonate substitution (swap), do not substantially affect tensile strength under normal operating conditions. This implies that FSC could potentially substitute Kulu powder in the foam industry, offering environmental benefits by utilising fish scale waste (Araújo et al. 2018). By contrast, tensile strength significantly differed among sample groups under heat aging ($P = 0.023$). It was observed that the substitution of calcium carbonate with 50 g of collagen in the “swap” sample led to a substantial decrease in tensile strength (65.0 ± 5.6), while the addition of 2.5 g of collagen resulted in

a remarkable increase in tensile strength (86.9 ± 8.1), approximately 12.66% higher than the STD. The conditions with 5 and 10 g collagen exhibited decreases in tensile strength (71.1 ± 7.4 vs. 71.49 ± 9.5), albeit to a lesser extent than observed at standard temperature (77.1 ± 7.4). The improvement in tensile strength measured at 2.5 g was attributed to the positive influence of collagen concentration. The finding was noted to contrast with studies suggesting a loss of strength and modulus with increased temperature (Song *et al.* 2009; Horak *et al.* 2020).

The elongation at the breaking point of the samples also revealed subtle differences at standard temperature and under heat ageing, suggesting the influence of temperature on PUF. At standard temperature, for example, no significant difference was observed among the sample groups, while under heat ageing, the samples differ significantly (Moo-Tun, Iñiguez-Covarrubias, and Valadez-Gonzalez 2020). Under heat ageing, for instance, the “swap” condition with calcium carbonate substitution resulted in a substantial, significant decrease (65.0 ± 5.6) of approximately -15.63% . Conversely, the addition of 2.5 g collagen led to a significant increase (86.9 ± 8.1), approximately 12.66%. This suggests that completely substituting collagen may negatively impact PUF flexibility, while reinforcing the PUF with 2.5 g of collagen significantly improves the material's flexibility and ductility despite elevated temperatures. These findings underscore the intricate interplay between PUF filler contents and environmental conditions in determining elongation at the breaking point. The finding is consistent with Moo-Tun, Iñiguez-Covarrubias and Valadez-Gonzalez (2020), who reported that the use of FSC as a filler material may be advantageous in the real world for creating flexible and long-lasting polyurethane-based composites, especially in applications exposed to moderate temperatures.

The tear resistance and density of the reinforced PUF were also evaluated under standard conditions and through heat aging. Tear resistance is a vital mechanical property, reflecting the material's ability to withstand tearing forces, which is crucial for PUF's longevity and durability. In Paper 2, temperature played a significant role in the resistance observed in the sample groups. For example, a notable difference was found in tear resistance at standard temperature with the “swap” involving 50 g of collagen, resulting in a substantial and statistically significant increase (0.3867 ± 0.06 N/mm), marking an 84.85% improvement compared to other groups. Additionally, tear resistance consistently increased with higher collagen content, suggesting that more filler results in better tear resistance. No difference was observed under heat aging, indicating that elevated temperature does not markedly affect PUF. However, conditions with 5 and 10 g of collagen showed non-significant decreases of approximately -10.85% and -6.63% , respectively, hinting at a potential reduction in tear resistance when collagen is added under heat aging conditions. This decline is likely due to subtle changes in

the material's properties at higher temperatures and aligns with (Wang *et al.* 2022).

The density, on the other hand, remains consistent at both standard temperature and elevated temperature under heat aging. This implies that temperature does not play a role in the density of the PUF materials. Nonetheless, the filler content did influence the density of the material. It was reported that the higher the filler content, the higher the density of the PUF. This is supported by the SEM images reported, which show that increases in filler content led to an increase in the quantity of densely packed cells and a decrease in cell size. Further morphological assessments indicate a consistent closed-cell structure across all samples, with collagen potentially contributing to reinforcement. The findings suggest that all samples maintain a closed-cell structure, regardless of standard temperature or heat aging, implying that temperature conditions do not impact the morphology of the PUF foams. The greater closure of pores in FSC–PUF composites compared to unfilled PUF was attributed to enhanced filler-polymer interaction, increased crosslinking density, optimised filler dispersion, and the physical barrier effect provided by FSC particles. The increase in closed cell structure as the filler content increases was attributed to the bulk viscosity being raised to withstand cell expansion as the concentration of inorganic fillers rose (Moo-Tun, Iñiguez-Covarrubias and Valadez-Gonzalez 2020). The finding from Paper 2 supports the sustainable use of fish scale-derived collagen as a filler, addressing waste management challenges and aligning with principles of environmentally conscious material development.

In summary, Paper 2 showed that FSC can effectively reinforce polyurethane foams, delivering competitive performance with conventional fillers under standard conditions and superior adaptability under heat aging. However, its lack of flame-retardant benefits compared with MFS suggests that neither phase alone can provide a complete solution. Instead, the complementary nature of MFS (organic + inorganic) and FSC (organic only) highlights the need for hybrid reinforcement strategies that integrate both collagen and mineral fractions. Such an approach could balance mechanical performance, flame resistance, and thermal stability, thereby maximising the potential of fish scale waste as a sustainable reinforcement in polyurethane composites.

4.4 REINFORCEMENT ON PHYSICAL PROPERTIES OF EPOXY RESIN COMPOSITES (PAPER 3)

Epoxy resins, like polyurethane, represent a special class of organic macromolecules that are comprehensively used for different industrial applications. The wide adoption as well as utilisation of epoxy polymers is due to their exceptional mechanical properties, chemical resistance, and thermal stability (Giménez *et al.* 2022; Capretti *et al.* 2023). Nevertheless, epoxy polymers exhibit limitations such as low impact resistance, poor toughness, and susceptibility to crack propagation (Saba and

Jawaid 2017). More so, the brittleness of cured epoxy resins - attributable to extensive cross-linking- diminishes their impact strengths. To overcome these drawbacks and improve on their industrial applications, epoxy is often reinforced with other materials. The utilisation of FSC as a reinforcing material for epoxy resins is motivated by the need to develop sustainable and eco-friendly materials that are in line with principles of green chemistry and circular economy practices (Lionetto and Esposito Corcione 2021; Sienkiewicz, Dominic and Parameswaranpillai 2022).

Paper 3 reported on enhancing the mechanical and thermal properties of epoxy composites with fish scale-derived collagen (FSC). To assess the effectiveness of FSC in improving both the mechanical and thermal properties of epoxy, the composites were fabricated using different levels of FC concentrations (5, 10, 20, and 25 wt.%). The ratio of epoxy to hardener used for the samples was 1.4. The influence of collagen concentration on mechanical characteristics was investigated, uncovering intricate associations with tensile behaviour, flexural strength, hardness, and thermal behaviour. Mechanical tests, including tensile behaviour, flexural properties, Field Scanning Electron Microscopy (FSEM), and Thermogravimetric analysis, were employed to assess the mechanical, physical, surface morphology, and thermal behaviour of the composites.

As reported in Paper 3, the addition of FSC to epoxy resins, particularly 5 and 10 wt.%, resulted in significant improvement in the tensile strength and Young's modulus. This was associated with effective stress transfer within the composite, and possibly due to improved interfacial bonding and better load distribution. The finding supports Baldanov *et al.* (2022), who emphasise the reinforcement potential of collagen in composite materials. This phenomenon is further supported by SEM images, which showed a relatively uniform distribution of collagen particles, indicating good interfacial adhesion between the reinforcement and matrix. While the tensile and Young's modulus reduced with an increase in filler content, it was still higher than the unreinforced polymer, supporting the enhancement of epoxy resins with FSC. The gradual decrease in tensile strength with an increase in filler content was attributed to the aggregation of EFC particles. This might have led to stress concentration points and weaker interfaces. The assertion supports the literature, which shows that excessive filler content can lead to particle agglomeration, creating defects and reducing the overall strength of the composite (Leluk *et al.* 2020).

Paper 3 also reported the stress-strain relationship of the epoxy reinforced with different concentrations of FSC. The report suggests that the relationship between maximum stress and EFC filler content follows a linear trend. Like the tensile strength, it was observed that as the content of EFC filler in the epoxy increases, the strain of EFC composites decreases. This declining pattern in

composites is attributed to the rigid nature of EFCs, which renders the composites harder or stiffer and hence more resistant to straining under tensile loading. This is corroborated by Tamrakar et al. (2018), who found that an increase in strain correlates with an increase in the elasticity limit.

The flexural properties of the reinforced epoxy resins, however, exhibit unusual characteristics. It was observed that while the flexural strength decreases with an increase in filler content, the flexural modulus increases until 20 wt.% before decreasing. Although the unusual behaviour of the material was extensively explained in the paper, another plausible explanation may be due to overstretching of the neat (unreinforced) samples before fracture. Besides, the disruption of the matrix structure and the formation of filler agglomerates at higher filler loadings within the epoxy matrix may have also resulted in localised areas of weakness and ultimately reduced the overall flexural strength of the composite. This observation aligns with the findings of Mehdikhani *et al.* (2019), who noted that voids in composites increase with the rise in filler weight percentage, resulting in decreased flexural strength. Moreover, the increase in flexural modulus (FM), with an increase in filler content until 20 wt.%, supports Han, Jiang and Hu (2020), who observed a similar improvement in FM properties with the loading of fish scale bones up to a certain percentage. The improvement in FM is attributed to the strong physical interaction between the epoxy and the EFCs, as well as the good dispersion of EFCs into the epoxy, while the decrease beyond 20 wt.% may be connected to particle agglomeration.

The thermal properties of the composites were studied using thermogravimetry and differential thermal gravimetry analysis. The TGA analysis revealed that the introduction of EFC in the epoxy resin led to a decrease in the T_{onset} temperature from all EFC composites, suggesting that the inclusion of EFC could not retard the diffusion of heat into the epoxy resin. On the other hand, with the increasing degradation temperatures, the reinforced EFC composites displayed a slight increase in thermal stability, especially for the EFC5% and EFC10% composites. The study attributes this phenomenon to the formation of char at elevated temperatures. Similar observations were reported by Hani et al. (2021), who reported the improvement of T_{max} of composites, implying the thermal stability of composites.

Furthermore, the DSC curves suggest that incorporating EFC (5, 10, 15, and 20%) into the epoxy increased the T_g of the composites, while a further increase in filler content (25 wt.%) resulted in a decrease. The increase in T_g was associated with good adhesion between the epoxy and the EFC filler, implying that the incorporation of EFC delayed the movement of the molecular polymer. While the decrease in the T_g was attributed to the agglomeration of filler particles in the polymer and or poor dispersion of the filler. The result was supported by the SEM results.

Overall, Paper 3 demonstrates that FSC can significantly enhance epoxy resins, particularly at low-to-moderate concentrations (5–10 wt.%). The findings confirm that collagen reinforcement improves tensile and flexural stiffness while modestly increasing thermal stability. Yet, performance gains diminish at higher loadings due to agglomeration and interfacial defects. These observations not only advance the understanding of bio-derived fillers in thermosetting polymers but also highlight the practical importance of identifying concentration thresholds for optimal reinforcement.

4.5 CONCLUSION

The experimental findings provided strong evidence for the successful extraction and utilisation of collagen derived from fish scales, a waste material that is often discarded in its raw form by the fishing and food industries. This outcome is particularly significant in the South African context, where waste management challenges and environmental sustainability remain pressing concerns. By converting an underutilised biowaste into a value-adding material, this study contributes to reducing the environmental burden associated with fish scale disposal while aligning with broader goals of a circular and greener economy.

The research also demonstrated that both fish scale powder (MFS) and fish scale-derived collagen (FSC) can act as effective reinforcement agents for polymeric materials such as polyurethane and epoxy resins. These polymers are widely used across diverse sectors, from aerospace and automotive components to cushioning and bedding, where enhanced mechanical performance and thermal stability are critical. The incorporation of FSC and MFS into these polymers showed measurable improvements in tensile strength, flexibility, and, in some cases, flame resistance, thereby addressing common limitations such as brittleness and poor toughness in neat polymers.

By systematically testing both whole fish scale powder and extracted collagen, the experiments helped to identify the distinct and complementary reinforcing roles of the organic (collagen) and inorganic (mineral) fractions of fish scales. This not only addressed the knowledge gap on how waste fish scales interact within polymer matrices but also demonstrated a practical pathway for converting raw fish scale waste into sustainable, functional fillers for industrial applications. The next chapter presents the conclusions drawn from this study and outlines recommendations for future research, particularly in scaling production, optimising filler combinations, and further exploring the lifecycle benefits of fish scale-derived reinforcements.

Effect of milled fish scale powder reinforcement on physical properties of ether-based polyurethane foam composite

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Funding information

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Abstract

This study investigated the effects of reinforcing ether-based flexible polyurethane foam (PUF) with milled fish scale powder (MFSP) on the physical properties of the resulting composite under different temperature conditions. The composites were prepared with varying MFSP concentrations (0%, 0.5 wt%, 1 wt%) with the PUF matrix and then subjected to the resulting mixture in a mold and cured in an oven at 70 degrees for 15 min. The physical properties of the composites, namely tensile strength, elongation at break, tear resistance, density, color, and cell structure were characterized under different temperature conditions. The results showed that at standard temperature (23°C), the addition of 0.5 wt% MFSP resulted in an 18.8% increase in tensile strength and a 15.7% increase in elongation at break. Tear resistance exhibited significant improvements under heat aging, with a 22% increase (0.5 wt% MFSP) and a remarkable 37% increase (1 wt% MFSP). The cell structure revealed closed cells after the addition of MFSP to the PUF matrix. The salient feature of the study suggests that the use of MFSP as a reinforcing agent for PUF-based composites has the potential to improve their physical properties. This is critical in producing PUF at a reduced cost with less impact on the environment.

KEYWORDS

composite materials, milled fish, physical properties and temperature conditions, polyurethane foam, reinforcement

1 | INTRODUCTION

Flexible polyurethane foam (PUF) is a versatile material that is widely used in various industries due to its excellent mechanical properties, low cost, and lightweight.¹ Polyurethane materials are highly versatile due to their chemical composition, making them suitable for a wide range of applications where other materials may not be appropriate. For example, they are commonly used in

consumer bedding and cushioning products, such as furniture, automotive seats, and insulation.² While polyurethane materials have many practical applications, their production relies heavily on petrochemicals, which are now facing environmental concerns.^{2,3} The demand for a more sustainable environment has spurred the development of innovative green materials that rely on natural resources.⁴ Equally, PUF has some limitations, including poor thermal stability, and high flammability.⁵ To

overcome these limitations, researchers have focused on developing PUF-based composites by reinforcing PUF with different materials.⁶ Among these, natural fibers from agro-waste have been gaining significant attention due to their renewability, low cost, and environmental friendliness.^{6,7}

According to Kuranchie, Yaya,⁷ natural fibers used in flexible PUFs not only have the advantage of being renewable, but they also improve the properties of the composite foams compared to pure PUFs. Moreover, natural fibers offer a cost-efficient means of producing polymeric composites while retaining their intrinsic properties.⁷ Fish scales are one such natural fiber that has been gaining attention as a potential reinforcement for polymeric composites.⁸ Fish scales are composed of collagen fibers, which are known for their high strength, stiffness, and toughness.⁹ Additionally, fish scales are abundant and can be obtained as a by-product of the fish processing industry,¹⁰ making them a cost-effective and sustainable option for reinforcement.

While previous studies have investigated the reinforcement of PUF with natural fibers,¹¹ there is a gap in the literature regarding the effect of reinforcement milled fish scale powder (MFSP) on the physical properties of PUF-based composites under different temperature conditions. Most of the previous studies have focused on the use of other natural fibers such as walnut and hazelnuts shells,⁵ hemp fibers,¹² and coconut husk and corn cob.¹³ There is limited research on the use of MFSP. Moreover, the effect of temperature on the physical properties of PUF-based composites reinforced with MFSP has not been extensively studied. Temperature can have a significant impact on the properties of composite materials, including their mechanical and thermal properties.¹⁴ Therefore, there is a need to investigate the effect of temperature on the physical properties of PUF-based composites reinforced with MFSP. Thus, this study aims to address this gap in the literature by investigating the effect of MFSP on the physical properties of PUF-based composites under different temperature conditions. The purpose of this study is to provide insights into the potential of using MFSP as a reinforcement material for PUF-based composites and to contribute to the development of sustainable and eco-friendly composite materials with improved physical properties.

2 | MATERIALS AND METHODS

The materials used in this study include ether-based flexible PUF, reinforcement-MFSP, and other chemicals such as surfactants and crosslinking agents. The PUF was obtained from a commercial supplier, while the MFSP

was prepared in the laboratory via mechanochemistry. Fish scale wastes were collected from a local fish shop in Durban, South Africa. Fish scales were washed and disinfected thoroughly with distilled water and 5 mL household bleach and stored at -20°C until ready for use.

2.1 | Preparation of fish scales powder

The fish scales were treated with 0.1 N NaOH to remove non-collagenous proteins and pigments for 2 days. This process was followed by washing with distilled water and then sun dried for 2–3 weeks. The dried fish scales were dry milled in a 250 mL bowl at 500 rpm for 1 hour. Thirty stainless steel balls with a diameter of 10 mm were used in a planetary ball mill (Retsch® PM 100) during the milling process.

2.2 | Preparation of polyurethane-based composites

Table 1 highlights the general formula used for the preparation of the PUF-based composites. Flexible PUFs were prepared by mixing MFSP with the PUF using a mechanical stirrer. Different weight percentages of MFSP (ranging from 0 to 1 wt%) were added to the PUF to prepare composites with varying levels of reinforcement. The amounts of each chemical component were chosen to obtain a target density of $16\text{--}17\text{ kg/m}^3$. The experiment was conducted in a Foam Laboratory equipped with a manual mixing apparatus. The apparatus includes a stirrer with two operating speeds (600 and 1300 rpm) and is powered by a 7.5 HP motor, allowing for efficient mixing of the components. The chemical materials were measured and deposited into the large 3 L container by hand dosages. The materials used in this article comprehend a standard PUF recipe. The procedure for preparing PUF-based composites involved two steps. The first step entails weighing the polyol and all other ingredients such as methyl chloride, catalysts, other additives, and water to specific amounts in the 3 L container. Thereafter, MFSP was added as a filler and followed by stirring for 20–30 seconds. Then the second step follows by weighing out the toluene di-isocyanate (TDI) specific amounts separately in a 500 mL cup and adding to the stirred mixture of the polyol, water, and additives and stirring again for 10–15 s before it starts creaming in the container. This is immediately transferred into a $305 \times 235 \times 305$ mold where cream and rise times are recorded. After the polyurethane foam is at a full rise, it is transferred to a 70°C oven to cure for 10 minutes. This step is repeated five times with different amounts of filler swapped and added to the standard formulation. This is then cut using a band

TABLE 1 The general formula for the PUF-based composites

Materials	Sample groups by weight %		
	Std	Std + 0.5 g fish scale powder	Std + 1.0 g fish scale powder
POLYOL-1906	170.01	170.01	170.01
TDI-T80	123.79	123.79	123.79
WATER	9.81	9.81	9.81
CATALYSTMIX-TIN/MESAMOL	2.75	2.75	2.75
AMINE MIX-1906/B18	0.69	0.69	0.69
SILICONE-L620	3.11	3.11	3.11
BLOWING AGENT-METHYL CHLORIDE	29.01	29.01	29.01
COLOR STABILIZER-CS-15	3.01	3.01	3.01
FILLER-KULU POWDER	50.01	50.01	50.01
MILLED FISH SCALE POWDER	0	0.50	1.00

knife and clicking press for the correct dimensional for the physical property testing.

2.3 | Characterization of MFSP

Perkin Elmer Universal ATR was used to identify the functional groups present in the prepared MFSP. Prior to scanning, a background investigation was carried out. Afterwards, a small amount of the prepared sample powders was placed in a sample holder and scanned between 400 and 4500 cm^{-1} with a resolution of 4 cm^{-1} . An x-ray diffraction (XRD) analysis was carried out on the crystalline structure of the prepared MFSP. The D8 Advance BRUKER AXS instrument from Germany, using Cu-K α radiation ($\lambda_{\text{K}\alpha 1} = 1.5406$), was used to capture the patterns within the 10 to 80 (2 theta) range. The parameters used for the analysis included 40 kV of voltage, 40 mA of current, and 0.5 s of pass time. The surface morphology was studied using scanning electron microscopy (SEM) (field emission, Carl Zeiss) operating at 20 kV under controlled air conditions. A thin, electrically conductive gold coating was applied to the surface to prevent an electrostatic charge build-up before SEM observation.

2.4 | Physical properties of PUF-based composites

The physical properties of the PUF-based composites were

characterized using standard test methods. Tensile strength, elongation at break, and compression strength were measured using an Instron Universal Testing Machine. Physical testing properties are conducted under 3 temperatures:

room temperature humidity aging and heat aging. Four composite specimens were reinforced with different volume fraction percentages of fish scale powder and polyurethane polymer as shown in Table 1. The reinforced composites are then machined to obtain the mechanical test specimens. The reinforced and modified composites are then tested to obtain the physical results of the ether-based PUF.

2.4.1 | Tensile strength test

The tensile test was conducted as per SABS 640-1976 Section 6.8 standards using a 2kN Instron. The dimension of the specimen is 150 x 27 x 17 mm with a dog bone shape die. Using the die, three samples with a thickness of at least 12.5 mm were cut. During the test, the test piece is clamped in the jaws of the machine, making sure that the sample is vertical so that the tension will be distributed uniformly over the cross-section. The machine speed is set to 500 mm/min and the gauge length is set to 45 mm. The machine is operated until the sample ruptures and records the tensile strength results in kPa. The tensile strength was calculated using the formula:

$$\text{Tensile strength } \delta \text{ kPa} = \frac{\text{Max force applied } \delta \text{ N}}{\text{Thickness of sample } \delta \text{ mm}} \times \frac{1000}{13}$$

2.4.2 | Elongation at breakpoint

After conditioning, and using the die, 3 samples with thicknesses of at least 10 mm to 15 mm are cut without skin. The gauge lengths on the sample are marked. The test piece is clamped in the jaws of the machine, making

sure that the sample is vertical so that the tension is distributed uniformly over the cross-section. The machine speed is set to 500 mm/min and the gauge length is set to 45 mm. The machine is operated until the sample ruptures and records the elongation in %. Tensile strength is conducted simultaneously with the elongation at the break test. This test is conducted as per SANS 883:2009 refers to ISO 1798. The dimension of the specimen is the same as reported in Section 2.41. The elongation at break was calculated using the formula:

$$\text{Elongation at Break (\%)} = \frac{\text{Final gauge length} - \text{Original gauge length}}{\text{Original gauge length}} \times 100;$$

2.4.3 | Tear resistance test

The force needed to tear a piece of foam of standard thickness is measured. This test indicates the tear resistance of the foam in end-use. From the appropriate slab, three samples each of size 150 mm x 25 mm x 25 mm were cut longitudinally down the center of each specimen to form two tongues of length 40 mm. Thereafter, the tongue was clamped in the jaws of the machine. The machine speed was set to 100 mm/min. The machine was operated until the tear propagated through the center of the sample. The maximum force (N) was recorded during the test. This test is conducted as per SABS 640-1976 SECTION 6.9. The tear resistance was calculated using the formula:

$$\text{Tear resistance (N/mm)} = \frac{\text{Max. Force (N)}}{\text{Width of sample (mm)}};$$

2.4.4 | Density

Density was conducted using a sample size of 100 x 100 x 50 mm. The sample was measured with a steel ruler graduated in millimeters and accurate to 1 mm for length, breadth, and height. The sample was thereafter weighed for the mass of the sample using a balance having a sensitivity of 0.1 g. Density was kept standard as all samples were cut from the bottom of the rise profile. The mass per unit volume of a sample was measured and calculated as per the formula below.

$$D = \frac{M}{V} \times 1000,$$

where D = Density, Kg/m³, M = Mass, kg and V = Volume, m³.

2.4.5 | Color spectra light II lightbox

The Spectra Light II is a visual color evaluation system to identify color variation in material. The results of this visual test indicate color variation of samples compared to standards using the Macbeth Spectra Light II Lightbox. Test specimen and standard sample undergo 4 light sources to determine if there are any visual differences under different light conditions:

- daylight (D65)—(main visual test needed)
- horizon (early sunrise/afternoon sunset simulation)
- Illuminate A (incandescent home lighting)
- Cool white fluorescent (CWF)

2.5 | Microscopic assessment of PUF-based composites cell structure

Using a handheld instrument Dino Lite Digital microscope. Dino lite is a 1.3 Megapixel resolution with a 10–70x & 200x Magnification containing 8 LED white lights with an infrared cut filter of more than 650 nm. The DinoXcope (Mac OS) software is downloaded on the laptop connecting to the USB 2.0 device and laptop. Multiple pictures are taken as per the zoom magnification point chosen (50 and 225 are used in this study) this is repeated with all samples and labeled and saved on the computer after each individual analysis to compare cell structure, cell membrane, and cell size change with the addition of MFSP.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of MFSP

FTIR was used to identify the major components present in the powder, such as proteins, lipids, and minerals. The FTIR spectrum of MFSP shows several peaks that correspond to different functional groups. For example, peaks in the range of 3000–2800 cm⁻¹ are associated with the stretching vibrations of C–H bonds in organic compounds such as lipids and proteins.¹⁵ The presence of peaks in the 1700–1245 cm⁻¹ range indicates the presence of amide I, amide II, and amide III bands, which are characteristic of type I collagen.¹⁶ The peaks appearing at approximately 2970 cm⁻¹ and 2930 cm⁻¹ are attributed to the asymmetrical and symmetrical stretching vibration of C–H bonds, respectively.¹⁷ In addition, the O–H stretching vibration in the 3200–3600 cm⁻¹ corresponds to the presence of hydroxyl groups (OH) or water molecules (H₂O). The absorption peaks in the region between 3300 and 3480 cm⁻¹ correspond to the NH stretching

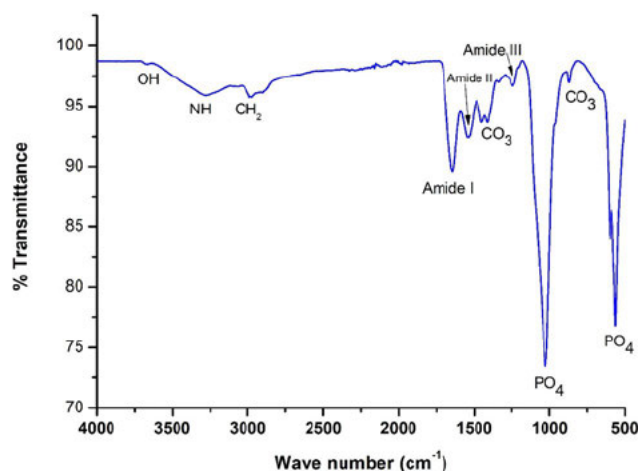


FIGURE 1 FTIR spectra of milled fish scale powder. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

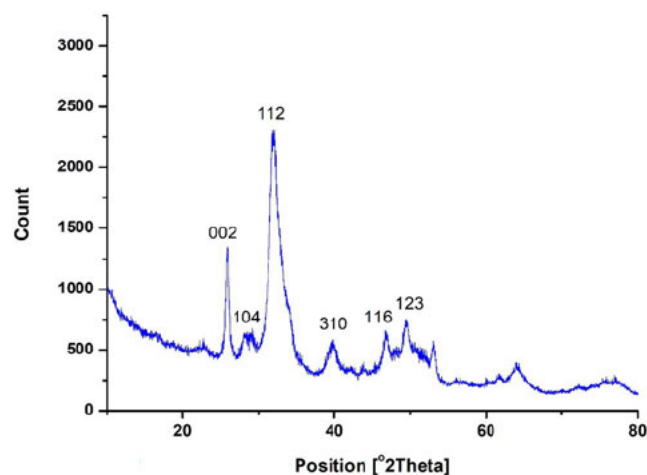


FIGURE 2 X-ray diffraction (XRD) pattern of milled fish scale powder. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

frequency.¹⁶ The CO stretching vibration in the 1439–864 cm^{-1} range is associated with carbonate groups incorporated into the apatite structure.¹⁶ The absorption bands at 1060 and 576 cm^{-1} are associated with the vibrational mode of the phosphate ions in the hydroxyapatite framework.^{18,19} The findings suggested that nanocomposites, which contain collagen type I, carbonate ions, and calcium-deficient apatite, can be produced from fish scales. (Figure 1)

XRD was used to study the crystal structure of MFSP. The XRD spectrum of MFSP typically shows sharp peaks at specific angles that correspond to the crystal structure of the calcium phosphate minerals present. The intensity and position of these peaks can provide information about the crystal structure, crystallite size, and degree of crystallinity of the sample.²⁰ The sharp peaks around 25.9° (002), 31.8° (121), 32.2° (112), 32.9° (030), 39.8° (310), 46.7° (222), 49.5° (123), 53.2° (004) correspond to hydroxyapatite (Reference code #96-230-0274), while the peaks around 18.2° (001), 34.4° (011) 51.2° (110), corresponds to portlandite (Reference code #96-900-6833). According to the XRD spectra, the sharp peak at 32.9 2 theta, originates more probably from optimum reinforcement content and is dispersed well within the matrix due to proper particle size. In addition, the peak of calcite (Reference code #96-101-0963) was found around angles of 15.6° (003), 21.2° (101), 29.5° (104), 48.6° (116). The finding is consistent with those reported by Thammahiwes, Riyajan.²¹ (Figure 2)

The mineralogical content, crystal structure, and d-spacing in the MFSP obtained from the XRD analysis are further represented in Table 2. The crystallinity value of MFSP was 67.22%, which suggests a semi-crystalline material. Similar semi-amorphous characteristics were

observed by Safronova, Vorobyov²² after grinding and heat treatment of fish scale powder.

The SEM image of MFSP in Figure 3 shows the surface morphology of the powder at a high magnification. In the image, the fish scale particles appear angular, rod-like and irregularly shaped fragments with varying sizes and surface features. The surface of the particles is rough and appears to be covered with small protuberances and ridges. These features may be attributed to the presence of mineralized tissues, such as hydroxyapatite and collagen,²¹ which are known to contribute to the mechanical properties of fish scales. This result was also supported by the FTIR spectra in Figure 1. The SEM image also reveals the presence of pores and cracks on the surface of the particles, which could be due to the mechanical processing of the fish scales during milling. These pores and cracks may provide sites for chemical reactions and enhance the interfacial bonding between the fish scale powder and the polyurethane matrix in composite materials.

3.2 | Physical properties of PUF-based composites

3.2.1 | Tensile strength at breaking point

Figure 4 provides the data on the tensile strength at the breaking point of PUF-based composites at different temperatures. The data indicates that the addition of MFSP to the PUF-based composites resulted in improved physical properties, as evidenced by the higher values observed for each property compared to the standard sample At standard temperature (23°C), for example, the addition of

TABLE 2 Mineral content of MFSP, as determined by XRD analysis

Visible	Ref. code	Score	Compound name	Displacement ($^{\circ}2\theta$)	Scale factor	Chemical formula	Crystal structure
*	96-230-0274	70	Hydroxyapatite	0.006	1.012	Ca _{10.00} P _{6.00} O _{28.00}	Hexagonal
*	96-101-0963	10	Calcite	0.282	0.060	Ca _{6.00} C _{3.00} O _{18.00}	Trigonal
*	96-900-6833	36	Portlandite	-0.955	0.250	Ca _{1.00} O _{2.00}	Hexagonal

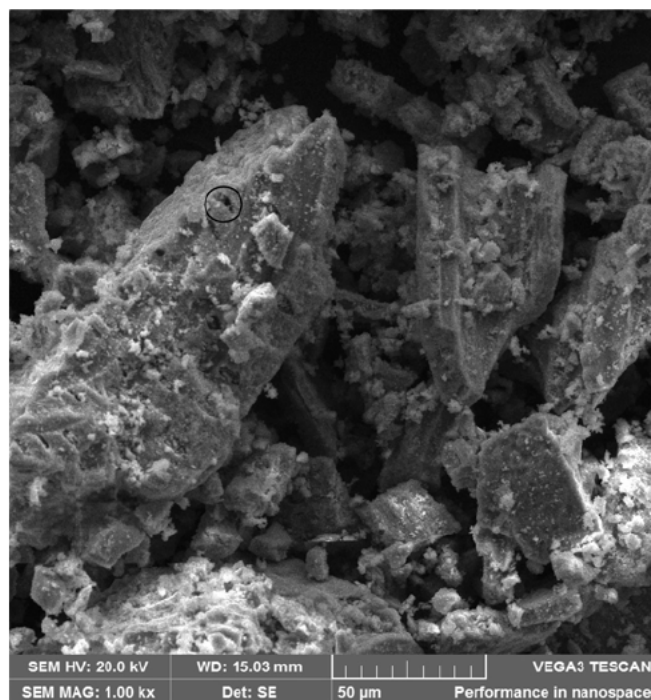


FIGURE 3 Scanning electron microscopy (SEM) images of milled fish scale powder.

0.5 grams of MFSP resulted in a 18.8% increase in the physical property (56.37 ± 9.8 kpa), while the addition of 1 gram of MFSP resulted in a 31.97% increase (62.6 ± 7.4 kpa). At heat aging (140°C @22 hours), the addition of 0.5 grams of MFSP resulted in a slight increase (64.68 ± 9.6 kpa), while the addition of 1 gram of MFSP resulted in a 7.81% increase (68.35 ± 13.7 kpa). However, at humidity aging (-4°C @22 hours), the results were more variable, with the addition of 0.5 grams of MFSP resulting in a decrease in the physical property compared to the standard sample (52.72 ± 2.8 kpa), while the addition of 1 gram of MFSP resulted in a 4.97% decrease (58.82 ± 3.3 kpa).

Fish scale powder is composed of collagen fibers, which are known for their high strength, stiffness, and toughness.⁹ According to Wang, Zhang²³ polyurethane foam materials generally show a significant reduction in strength as the temperature increases. Previous studies such as Horak, Dvorak²⁴ and Song, Lu²⁵ revealed that an

increase in temperature caused a loss of strength and modulus. However, the results of this study suggest that the addition of MFSP can improve the physical properties of PUF-based composites, particularly at standard temperature and heat aging conditions. This is consistent with previous research that has shown that the incorporation of natural fillers, such as fish scale powder, can improve the mechanical and thermal properties of polymer composites.²⁶ The observed improvements in physical properties with the addition of MFSP to the PUF-based composites may be due to several factors. One possible explanation is that the fish scale powder acts as a reinforcing filler, increasing the stiffness and strength of the composite material. Also, with increasing filler content an increase in physical properties is observed. This might be influenced by good dispersion of the filler which nucleated well with the polymer chains. Those influenced by humidity might be absorbed water resulting in plasticization thereby decreasing the physical properties.

3.2.2 | Elongation at break (%) of PUF-based composites

The values for elongation at break (%) of PUF-based composites with different amounts of MFSP under different temperature conditions are given in Figure 5. It was observed that the addition of MFSP has a positive effect on the elongation at break of the PUF-based composites, as evidenced by the higher values observed for each sample compared to the standard sample. At standard temperature (23°C), the addition of 0.5 grams of MFSP resulted in a 15.7% increase in elongation at break ($228.43 \pm 28\%$), while the addition of 1 gram of MFSP resulted in a 43.2% increase ($282.14 \pm 26.9\%$). At heat aging (140°C @22 hours), the addition of 0.5 grams of MFSP resulted in a slight decrease in elongation at break ($241.4 \pm 25.7\%$), while the addition of 1 gram of MFSP resulted in a slight increase ($258.68 \pm 6.5\%$).

Under humidity aging (-4°C @22 hours), the results were more variable, with the addition of 0.5 grams of MFSP resulting in a slight increase in elongation at break ($258.68 \pm 36.9\%$), while the addition of 1 gram of MFSP resulted in a significant increase ($265.47 \pm 16.7\%$).

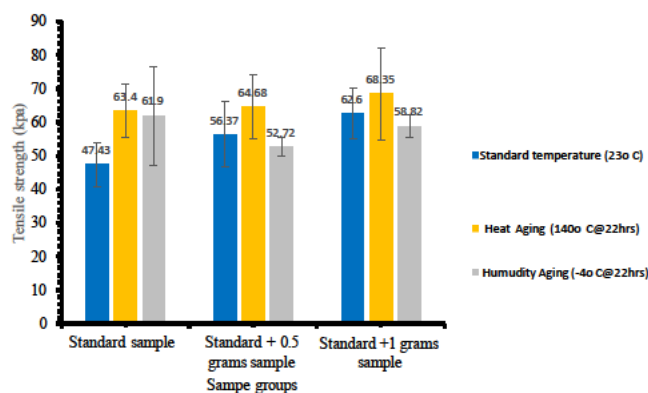


FIGURE 4 Tensile strength of polyurethane foam (PUF)-based composites at different temperatures. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

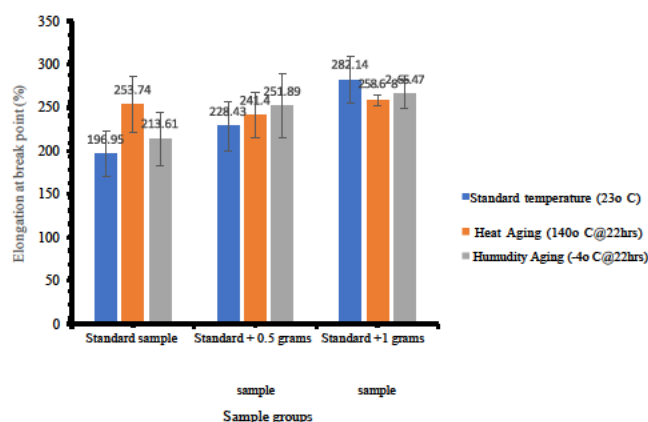


FIGURE 5 Elongation of polyurethane foam (PUF)-based composites at different temperatures. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

The finding suggests that the effects of fish scale powder on elongation at break may vary under different temperature and humidity conditions. While the results suggest that the addition of MFSP can improve the elongation at break of PUF-based composites, particularly at standard temperature and humidity aging conditions, however, the effects may be less consistent under heat aging conditions. In practical terms, the use of fish scale powder as a filler material could be beneficial for the production of flexible and durable PUF-based composites, particularly in applications where the material is subjected to moderate temperatures and humid conditions.²⁷

3.2.3 | Tear resistance of PUF-based composites

The tear resistance measured for PUF-based composites at

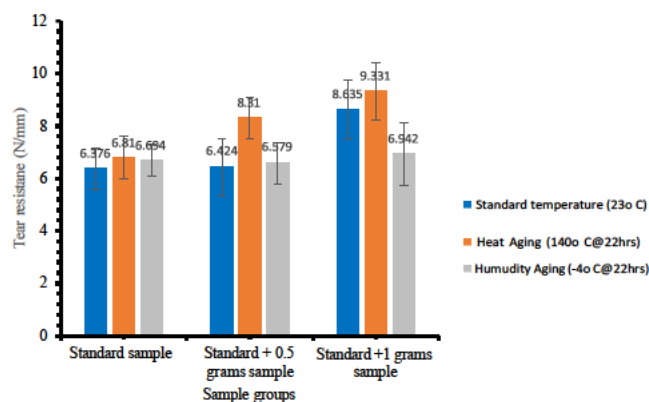


FIGURE 6 Tear resistance of polyurethane foam (PUF)-based composites at different temperatures. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

of the tear strength test on PUF-based composites with different amounts of MFSP indicate that the addition of fish scale powder can significantly improve the tear strength of the composites under both heat aging and humidity aging conditions. It was observed that the addition of 0.5 grams and 1 gram of reinforcement fish scale powder improved the tear resistance of the polyurethane foam composites under heat aging and standard conditions. The tear resistance of the composites with 0.5 grams and 1 gram of fish scale powder showed an

increase of up to 22% (8.31 ± 0.8 N/mm) and 37% (9.31 ± 1.1 N/mm), respectively, compared to the standard different temperatures is given in Figure 6. The results

sample under heat aging (6.81 ± 0.8 N/mm). Similarly, under standard conditions, the tear resistance of the composites with 0.5 and 1 gram of fish scale powder increased up to 0.753% (6.424 ± 1.1 N/mm) and 35.47% (8.635 ± 1.1 N/mm) respectively, compared to the standard sample (6.376 ± 0.8 N/mm). There was also a 3.7% increase under humidity aging with 1 gram of fish scale (6.942 ± 1.2 N/mm). This improvement in tear resistance could be attributed to the reinforcing effect of fish scale powder in the polyurethane matrix. For instance, the improvement of tear resistance under heat aging may be attributable to the presence of collagen fibers and minerals, which could help bridge and distribute stress within the material, preventing the propagation of tears. While temperature conditions can affect material properties,²³ the result suggests that the addition of 1 gram of fish scale powder may help mitigate the negative effects of moisture on tear resistance.

The results of this study are consistent with previous research that has investigated the use of natural materials as reinforcements in polyurethane foam composites. For example, a study by Ghasemi, Amini²⁸ reported that the addition of cellulose nanocrystals to polyurethane foam composites resulted in improved tear strength and other

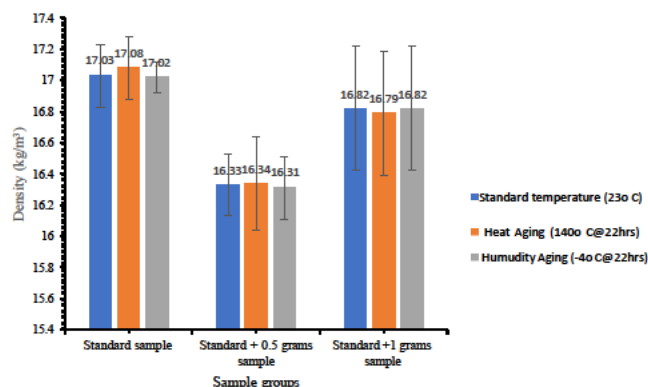


FIGURE 7 Density of polyurethane foam (PUF)-based composites at different temperatures. [Color figure can be viewed at wileyonlinelibrary.com]

mechanical properties. Another study by Uzoma, Ogbobe²⁹ investigated the use of Bambara nutshell as a reinforcement in polyurethane foam composites and reported improvements in both tear strength and tensile strength.

3.2.4 | Density of PUF-based composites

The density of the reinforced PUF-based composites at different temperature conditions is given in Figure 7.

It was observed that the addition of 0.5 grams and 1 gram of MFSP to the standard sample resulted in a decrease in density compared to the standard without reinforcement. At standard temperature, for instance, the density of the sample was $17.03 \pm 0.2 \text{ kg/m}^3$. The addition of 0.5 grams and 1 gram of the component resulted in densities of $16.33 \pm 0.2 \text{ kg/m}^3$ and $16.82 \pm 0.4 \text{ kg/m}^3$, respectively. These values indicate a slight reduction in density compared to the standard sample without reinforcement. Similarly, under heat aging and humidity aging conditions, the densities of the samples with the added MFSP remained slightly lower than the standard sample, although the differences are minimal. The findings suggest that the addition of fish scale powder caused a reduction in the mass of the material without significantly affecting its volume. This is a desirable outcome as it suggests that the addition of fish scale powder can be used to enhance the mechanical properties of the composite material without significantly affecting its weight. The slight decrease in density observed with the addition of MFSP can have certain implications. A lower-density material can offer advantages such as improved lightweight properties,³⁰ making it easier to handle and transport. This is important in applications where weight is a critical factor, such as in the automotive and aerospace industries.³¹ It may also contribute to better thermal insulation

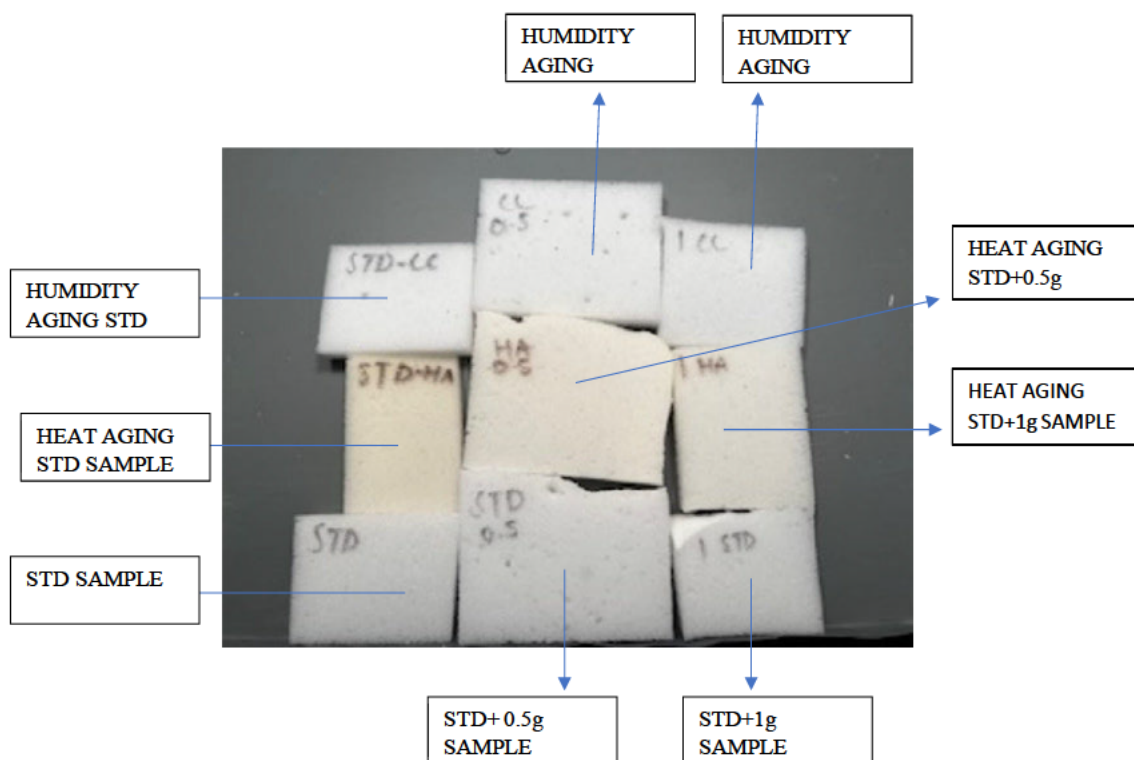


FIGURE 8 Scorching test. [Color figure can be viewed at wileyonlinelibrary.com]

characteristics, as materials with lower density often exhibit increased porosity, which enhances their insulation performance.³²

3.2.5 | Color spectra light II lightbox

The resistance of PUF-based composites at different temperatures to discoloration is given in Figure 8. The results showed that heat aging caused discoloration and yellowing in the polyurethane foam samples, which is commonly referred to as scorching in the foam industry.³³ A study by Agrawal, Kaur³⁴ has shown that the addition of natural filler materials to polyurethane foam enhances the mechanical, thermal, and flame-retardant behaviors. Consistent with this, the finding suggests that fish scale powder may improve the thermal stability of the composite by

providing a heat sink, which can help dissipate heat and reduce scorching. This may be due to the unique properties of fish scales, such as their high protein content (Figure 1). These properties may help to reduce the thermal reaction that occurs during heat aging and prevent the foam from discoloring to a significant extent. This is evidenced by the fact that there was no significant color difference observed between the standard and the samples subjected to humidity aging in the climatic chamber.

3.3 | Microscopic assessment of the cell structure

The cell structure of the PUF-based composites at different temperatures is given in Figure 9a–c. Based on the images obtained, the study suggests that thermal exposure under

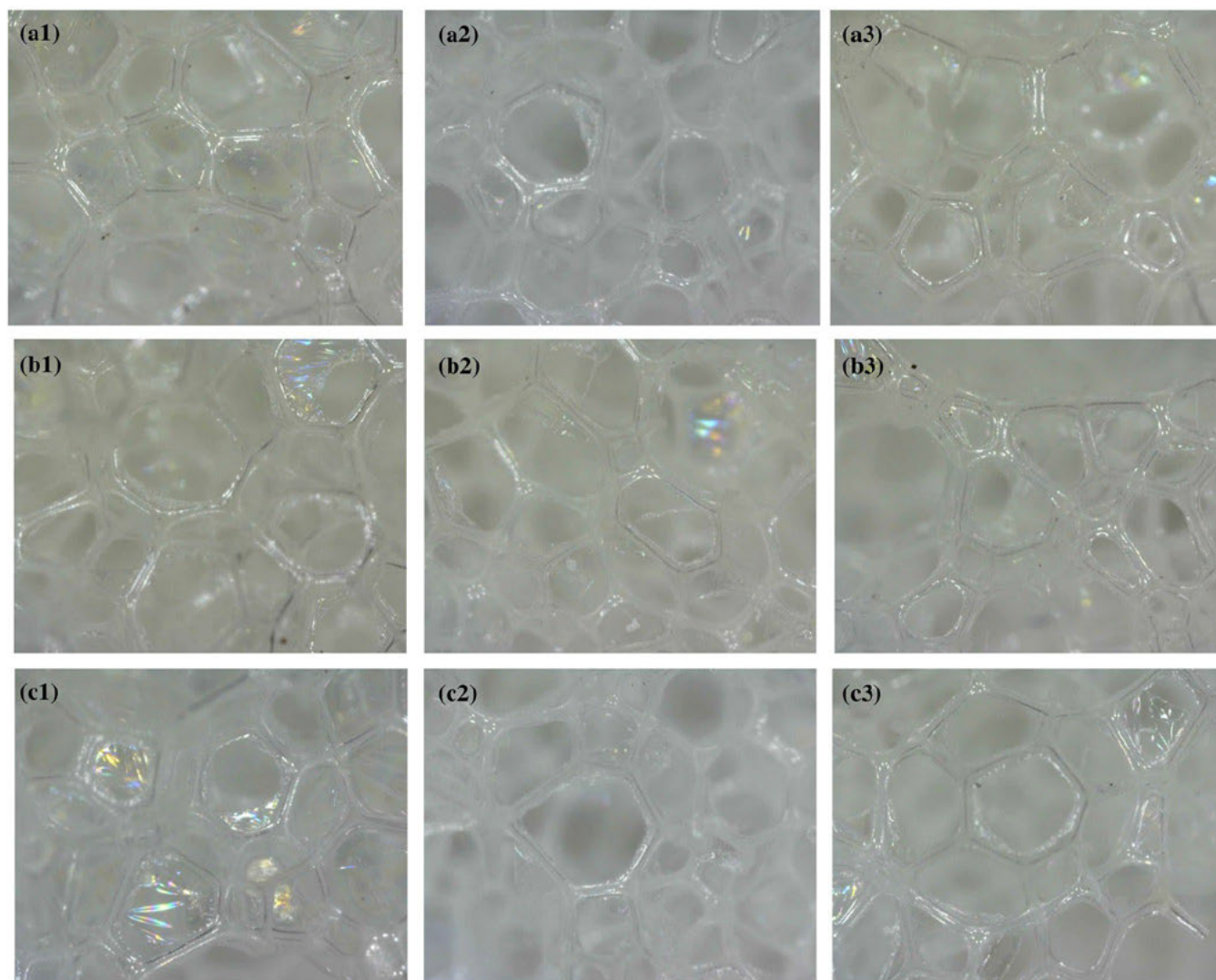


FIGURE 9 Image of polyurethane foam (PUF)-based composites at different temperatures (a) standard room temperature; (b) heat aging; (c) humidity (magnification x225). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/app.54735)]

three different temperatures (heat aging, humidity aging, and standard temperature) does not cause any noticeable changes in the foam's cell morphology. However, the study observed the effect of adding MFSP to polyurethane foam to identify changes in the foam's cell count, cell size, cell packing, and cell membrane characteristics of the overall cell structure. Based on the observations, it appears that increasing the amount of MFSP leads to visual differences in the cell structure compared to the standard sample. For instance, when no MFSP is added (standard sample), the cell structures appear uniform, with polyhedron walls and evenly distributed struts. However, as the MFSP content increases, several changes become noticeable. Firstly, the cell size of each cell wall reduces, but there is an increase in the number of cells packed closely together. This suggests that the addition of MFSP affects cell morphology and arrangement.

Furthermore, the polyhedron walls of the cells become irregular in shape and differ in size. The struts connecting the cell walls also appear to be pulled apart, indicating a disruption in the cellular structure. Additionally, the cell membrane, which is semi-closed and thin in the standard sample, becomes open in the presence of MFSP. This change in the cell membrane's properties suggests that the addition of MFSP affects the cell's integrity and stability.

Research has shown that the cell structure of polyurethane foam plays a crucial role in determining its mechanical properties, such as its compression and tensile strength.³⁵ Closed-cell structures have been found to have higher compression and tensile strength than open-cell structures.³⁶ This suggests that the semi-rigid foam created by the addition of fish scale powder may have increased strength compared to the standard flexible foam. In terms of potential applications, the semi-rigid foam created by the addition of fish scale powder may be suitable for use in insulation or packaging materials that require increased strength and durability. However, the reduced flexibility and breathability may limit its use in other applications, such as cushioning or bedding materials.

4 | CONCLUSIONS

This study investigated the use of MFSP as a reinforcing agent for ether-based flexible polyurethane foam (PUF) composites. The physical properties of the resulting composites were characterized under different temperature conditions, and the microstructure was analyzed using a microscope. The study's quantitative results demonstrated the positive impact of MFSP reinforcement on PUF composites. At standard temperature (23°C), the addition of 0.5 wt% MFSP resulted in an 18.8% increase in tensile

strength and a 15.7% increase in elongation at break. Further addition to 1 wt% MFSP led to a 31.9% increase in tensile strength and a substantial 43.2% increase in elongation at break. Tear resistance exhibited significant improvements under heat aging, with a 22% increase (0.5 wt% MFSP) and a remarkable 37% increase (1 wt% MFSP). Density reduction was observed with the addition of 0.5 wt% (16.33 kg/m³) and 1 wt% (16.82 kg/m³) MFSP, suggesting lightweight enhancements. Furthermore, the study noted limited color variation under humidity aging, signifying potential thermal stability improvements due to MFSP's heat sink effect. Microscopic analysis showcased closer cell structures and increased strength due to MFSP, enhancing the material's potential for applications demanding durability and strength.

Additionally, while the findings suggest that the use of PUF-based composites in high-temperature applications may be limited by their reduced mechanical properties at elevated temperatures, however, the use of MFSP as a reinforcing agent for PUF-based composites has the potential to improve their physical properties and reduce their cost and environmental impact. Overall, the results of this study suggest that the use of MFSP as a reinforcing agent for PUF-based composites has potential practical applications in the development of cost-effective and environmentally friendly materials for various industries, including packaging, automotive, and construction.

AUTHOR CONTRIBUTIONS

Stanley Chibuzor Onwubu: Conceptualization (equal); formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). Deneshree Naidoo: Data curation (equal); methodology (equal); writing – original draft (lead); writing – review and editing (equal). Thabang Hendrica Mokhothu: Supervision (lead); writing – review and editing (equal). Phumlane Selby Mdluli: Conceptualization (equal); supervision (equal); writing – review and editing (equal). Ajay Kumar Mishra: Supervision (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

On reasonable request, the corresponding author will provide the datasets generated during and/or analyzed during the current study.

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Research Article

Effectiveness of Fish Scale-Derived Collagen as an Alternative Filler Material in the Fabrication of Polyurethane Foam Composites

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This study is based on the utilization of fish scale-derived collagen (FSC) as a potential filler material in polyurethane foam (PUF) composites. The composites were prepared with varying FSC concentrations (2.5%, 5 wt%, and 10 wt%) with the standard PUF matrix, while calcium carbonates in the standard sample (STD) were completely substituted with 50 wt% of collagen. When examining the effects of collagen concentration on mechanical characteristics, complex correlations emerge between tensile strength, elongation, tear resistance, and ductility. The results reveal that the addition of 2.5 wt% FSC increased tensile strength by 12.66% during heat aging, while the addition of 5 wt% at standard temperature increased elongation by 6.65%. Under normal conditions, collagen significantly enhanced the material's resistance to tearing, demonstrating its potential for long-term durability. Under typical conditions, tear resistance showed notable gains, increasing by 84.85% (50 wt% FSC) and 33% (10 wt% FSC), respectively. The tear resistance, however, diminishes under heat aging for all concentrations. Morphological assessments indicate a consistent closed cell structure across all samples, with collagen potentially contributing to reinforcement. The study supports the sustainable use of fish scale-derived collagen as a filler, addressing waste management challenges and aligning with principles of environmentally conscious material development.

1. Introduction

Polyurethane foams (PUFs), widely used in various industries, are versatile materials known for their lightweight nature, insulation properties, and affordability [1, 2]. Polyurethanes (PU) are commonly used in engineering due to their adjustable mechanical properties and biocompatibility [3]. The composition and configuration of their constituent parts can be modified to tailor their properties. Traditionally, petrochemicals have been used to produce PU foams, but environmental concerns have recently emerged regarding their use [4, 5]. As a result, there is a growing movement toward the development and application of bio-based polyurethane (PU), which is derived from renewable sources

such as soybeans [3]. While bio-based PU foams can be produced with different functionalities and hydroxyl group concentrations, their mechanical properties generally do not match those of petroleum-based PU [3, 6]. Alyamaç and Teke propose that adding reinforcing agents [3] can enhance soy-based PU formulations. Bio-based PU foams have been reinforced in the literature using a variety of micro- and nanoscale fillers, including hydroxyapatite [7, 8], calcium carbonates [9], wood fiber [10], sawdust [11], graphene oxide [12], magnesium hydroxide [13], montmorillonite [14], halloysite nanoclay [15], eggshells [16], and chitosan [17]. In particular, Zhang and Hu [8] looked at how hydroxyapatite nanofillers affected PU composites. According to their findings, the PU composite's surface wettability rose with the

addition of hydroxyapatite nanofillers, improving its biocompatibility [8]. Additionally, PU composites with CaCO_3 fillers showed improved mechanical properties, according to a study by Choe and Lee [9].

In recent years, there have been an increasing demand for sustainable and ecofriendly materials that align with the principles of green chemistry and circular economy practices [18]. Fish scale-derived collagen emerges as a potential candidate for replacing traditional fillers in PU foam formulations. Naidoo et al. [19] investigated the effects of eggshells as a filler material in PU on different environmental conditions. The authors reported an improved mechanical property, which they attribute to the collagen constituents of the eggshell fillers [19]. Collagen, a fibrous protein abundant in animal tissues, particularly in fish scales [18], exhibits unique properties that make it an attractive additive in PU fabrications. Due to its biocompatibility, nontoxicity, and biodegradability, collagen is widely used in a variety of sectors [3]. However, little research has been done on the possible application of fish scale-derived collagen (FSC) as a support material in traditional biomaterials. FSC is a byproduct of the seafood business that is frequently regarded as waste and causes problems for the environment. Utilizing this collagen as a filler not only addresses the issue of waste management but also aligns with the principles of sustainable development [20].

Recognizing the enormous potential and critical role of collagen in spectrum of applications, we aim to assess its effectiveness in enhancing the mechanical properties of PU composite in comparison with commonly used filler material in the industry (calcium carbonates). The study investigated the fabrication of PU foams with the addition of different weight concentrations of collagen and temperature conditions. Temperature reportedly has a significant impact on the properties of PU composites, such as their mechanical and thermal properties [19, 21].

Consequently, it is imperative to examine how temperature affects the mechanical characteristics of PU-based composites reinforced with FSC. Tensile, shear, elongation, and FSEM tests were used to describe the mechanical and physical characteristics of the PU-based composites. Tensile, shear, elongation, and FSEM tests were used to describe the mechanical and physical characteristics of the PU-based composites. The composites were prepared with varying concentrations of FSC (2.5%, 5 wt%, and 10 wt%) in a standard PUF matrix, while the standard sample (STD) had 50 wt% collagen as a complete substitute for calcium carbonates. The concentration-dependent effects of collagen on mechanical properties were examined, revealing nuanced relationships with tensile strength, elongation, tear resistance, and ductility.

2. Methodology

The PUF chemicals such silicone L620 and color stabilizer CS-15 were purchased from Momentive. Methylene chloride 99.9% was sourced from local supplier in South Africa. Mesamoll was supplied by Lanxess (China), while desmodur

T80 toluene-2,4-diisocyanate (TDI) and polyol Desmophen 1906 were supplied by Covestro. Catalyst Kosmo T9 was sourced from Evonik, and calcium carbonate called kulu powder was sourced from Idwala. Sodium hydroxide (NaOH, 0.1 M) and acetic acid (0.5 M) and sodium chloride (NaCl, 9% m/v) were sourced from local Laboratory Analytical Supplies in Durban South Africa. Tris-glycine buffer ($\times 10$ concentrated, pH = 8.8) was sourced from Shalom Laboratory Supplies and a dialysis membrane (MWCO 12–14 kDa, 30 mm diameter) from Lichro Chemicals. All chemicals and reagents were of analytical grade and ready to use.

2.1. Fish Scale Collagen (FSC) Preparation. Fish scale waste was collected from a local fish shop in Durban, South Africa. The scales were thoroughly washed and disinfected using distilled water and 5 mL of household bleach. To remove bacteria, the fish scales were defrosted to room temperature and then air-dried for 2 days to remove excess water. To obtain fish scale collagen (FSC), a well-established protocol involving demineralization and acid hydrolysis was used [18]. For 2 days, the scales were treated with 0.1 N NaOH to get rid of pigments and noncollagenous proteins. After washing and drying for 2–3 weeks, collagen extraction was carried out using various parameters. To extract collagen from the demineralized fish scales, 100 g of the scales was soaked in 0.5 M acetic acid for 48 hr at 4°C with occasional stirring. The solution was then centrifuged at 3,000 rpm for 30 min at 4°C. The supernatants were salted out by adding 9 % NaCl and automatically stirred for 1 day. They were then suspended in a tris-glycine buffer. Pellets were collected by centrifugation, washed with distilled water, and dialyzed using a dialysis membrane (MWCO 12–14 kDa) to remove residual salts. The resulting collagen was freeze-dried at -80°C under vacuum conditions. The amino acid composition of the resulting collagen was used for characterization.

2.2. Fabrication of Polyurethane-Based Composites. The general formula utilized to prepare the PUF-based composites is highlighted in Table 1. Following a standardized process similar to that described by Naidoo and Onwubu [19], the PU foam samples were manufactured, and their qualities were methodically assessed. In this process, a mechanical stirrer was used to combine FSC and PUF to make PUFs. To create composites with various reinforcement levels, different weight percentages of FSC (2.5, 5, and 10 g) were added to the conventional PUF mix. In addition, 50 g of FSC (swap sample) was used in place of Kulu powder, or calcium carbonate, in the usual formula. This was done with the aim of assessing the possibilities of replacing the commonly used filler material in the foam industry. In order to get the target density of $16\text{--}17\text{ kg/m}^3$, the composition was changed. In the experimental setting, a manual mixing device was included in the foam laboratory. Weighing the polyol, additives, and additional ingredients, such as kulu powder, to precise proportions in a 3 L container was the process for making PUF-based composites. FSC in varying concentrations were then added as a substitute and an additional filler, followed by stirring. In a separate cup, toluene di-isocyanate (TDI) with an index value of 113.6 was weighed and added to

TABLE 1: Formulation details for producing foams made of PU composites using fillers made of FSC and CaCO_3 .

Materials	Std	Swap out filler (Kulu powder) with fish scale collagen	Std + 2.5 g fish scale collagen	Std + 5g fish scale collagen	Std + 10 g fish scale collagen
Polyol	170.01	170.01	170.01	170.01	170.01
Tdi	123.79	123.79	123.79	123.79	123.79
Water	9.81	9.81	9.81	9.81	9.81
Catalyst mix	2.75	2.75	2.75	2.75	2.75
Amine mix	0.69	0.69	0.69	0.69	0.69
Silicone	3.11	3.11	3.11	3.11	3.11
Blowing agent	29.01	29.01	29.01	29.01	29.01
Color stabilizer	3.01	3.01	3.01	3.01	3.01
Filler—Kulu powder (CaCO_3)	50.01	0	50.01	50.01	50.01
Fish scale collagen	0	50	2.5	5	10
Cream time	10	12	11	10	12
Rise time	115	129	139	144	148

Note. Std = standard sample; all preparations were in weight.

the stirred mixture of polyol, water, and additives. The mixture was stirred again before being transferred into a mold for creaming and rise time recording. The foam was dried for 10 min at 70°C in an oven following a full rise. Three trials of this procedure were conducted, one with a standard sample and two with varying FSC doses. Before being subjected to physical property testing, the foam blocks were sliced using a band cutter and clicking press to ensure dimensional correctness. Correctly sized cut samples were subjected to two different temperatures: 24°C in a controlled laboratory setting and 140°C in an oven for heat aging.

2.3. Calculating the Amino Acid Content of FSC. The Kjeldahl method was utilized to ascertain the amino acid content of the extracted FSC, while Stegmann method 19 was employed to evaluate the hydroxyproline concentration. An automatic amino acid analyzer was used to get the results of the amino acid composition tests for FSC, which are shown in Table 2. Amino acids are the building blocks of proteins, and their distribution is crucial in determining the overall characteristics of the protein. The composition of the amino acids in 100 g revealed 17 different types of amino acids. The high percentages of glycine (20.98%), proline (15.43%), and hydroxyproline (11.51%) suggest a significant presence of structural proteins like collagen. According to Gauza-Włodarczyk and Kubisz [22], glycine determines the regular structure of the collagen chain and allows for the formation of stabilizing bonds between chains.

2.4. Fourier Transform Infrared Evaluation of FSC. To examine the structural alterations in the PU composite, the Perkin Elmer Universal ATR was employed. An investigation into the background was done prior to scanning. Subsequently, every sample that had been cut was put in a sample holder and scanned at a resolution of 4 cm^{-1} between 400 and $4,500\text{ cm}^{-1}$.

2.5. Microscopic Evaluation of the FSC. Using a Carl Zeiss scanning electron microscope (SEM) with field emission, the surface morphology of PU composites was examined. The

TABLE 2: Amino acid composition of the extracted FSC.

Type of amino acid	Composition (%) (100 g)
Histidine	1.19
Arginine	6.69
Serine	3.04
Glycine	20.98
Asparagine	6.63
Glutamic	10.19
Threonine	2.58
Alanine	9.81
Proline	15.43
Lysine	4.53
Tyrosine	0.24
Methionine	2.85
Valine	1.92
Isoleucine	1.51
Leucine	2.59
Phenylalanine	1.55
Hydroxyproline	11.51

SEM was run in controlled air conditions at 20 kV. Before SEM inspection, a small layer of electrically conductive gold was put to the surface to prevent an accumulation of electrostatic charge.

2.6. Mechanical Testing. Standard test procedures were used to characterize the PUF-based composites' physical characteristics. An Instron Universal Testing Machine was used to assess the tensile, elongation, and tear resistance. Two distinct temperatures were used to examine the samples: room temperature and heat aging.

A 2 kN Instron was utilized for the tensile test, which was conducted in accordance with SABS 640–1976 Section 6.8 regulations. The SANS 883 : 2009 was followed for conducting the elongation at break test. The item was shaped like a dog bone and was $150 \times 27 \times 17\text{ mm}$. The gauge length was

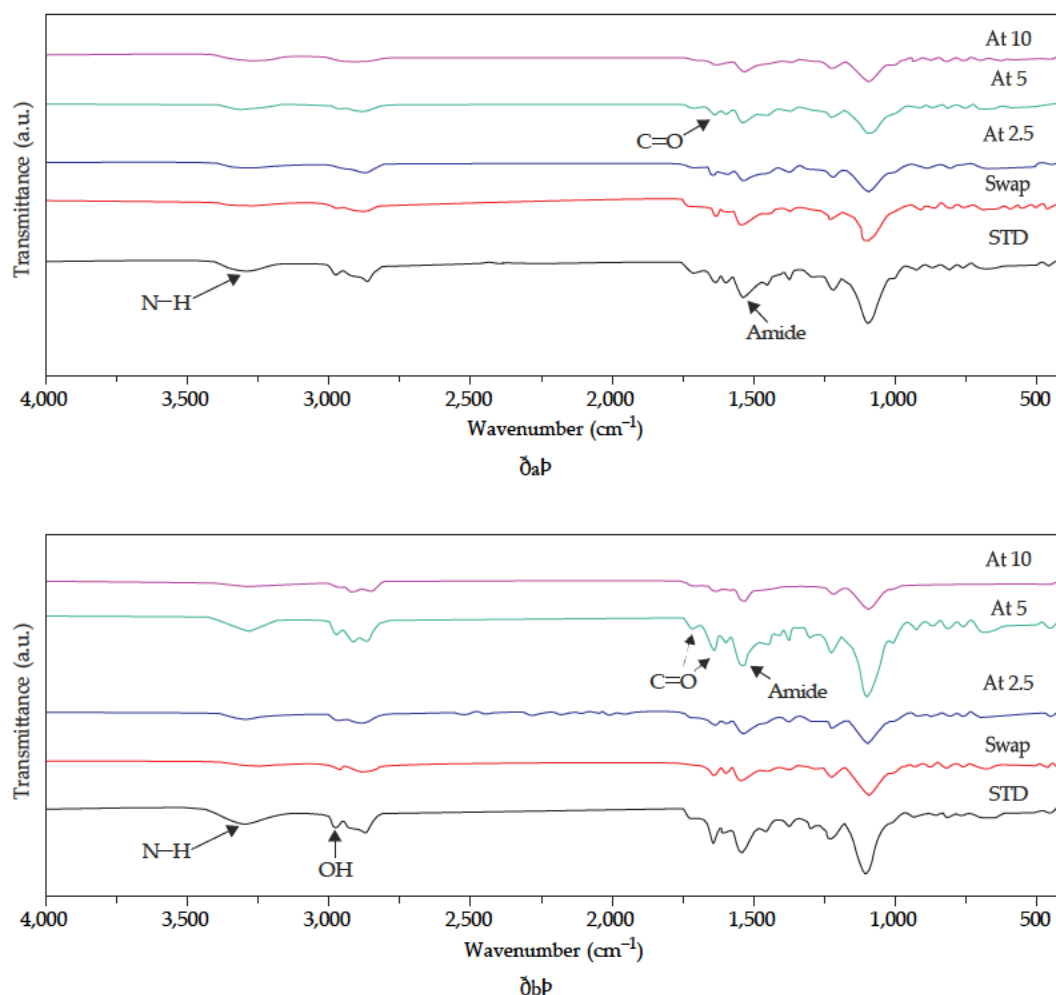


FIGURE 1: FTIR spectrum of PU samples under (a) standard temperature and (b) heat aging (STD contains only Kulu powder as filler; swap contains only collagen as filler; 2.5–10 g are concentrations of collagen in the STD). *Notes.* STD (PU without fish scale collagen; swap standard sample (50 g of fish scale collagen); at 2.5 (2.6 g of fish scale collagen + standard sample); at 5 (5 g of fish scale collagen + standard sample); and at 10 (10 g of fish scale collagen + standard sample).

set at 45 mm, and the machine speed was set to 500 mm/min. Tensile strength findings were recorded in kPa, and the test was maintained until the sample burst. To get an average value for statistical analysis, five samples underwent tensile and elongation testing.

The SABS 640–1976 was followed in conducting the tear resistance test. The test was run with the machine speed set to 100 mm/min until the tear spread to the center of the sample. The highest force (N) measured throughout the experiment.

The sample size used for the density measurement was $100 \times 100 \times 50$ mm. A steel ruler with a millimeter grade and a precision of 1 mm was used to measure the dimensions of the object's length, width, and height. The mass of the sample was then ascertained by weighing it on a scale that had a sensitivity of 0.1 g.

2.7. Statistical Analysis. Using statistical packaged for social science (SPSS, IBM, version 29), one-way analysis of variance (ANOVA) was applied to test the mean differences in tensile

strength, elongation at breaking point, and tear strength at $\alpha.05$.

3. Results and Discussion

3.1. FTIR Characterization. FTIR analysis of PU foam reinforced with standard sample (calcium carbonates filler) and collagen under standard temperature and heat aging conditions are presented in Figure 1. The FTIR provides a detailed understanding of the molecular changes occurring in the composite materials. In the FTIR spectrum of standard sample (STD) at standard temperature (Figure 1(a), characteristic peaks associated with polyurethane are observed. Peaks around $3,300 \text{ cm}^{-1}$ indicate the presence of N–H stretching vibrations, while peaks in the region of $1,720\text{--}1,650 \text{ cm}^{-1}$ correspond to C=O stretching vibrations in urethane groups [6, 23]. The peaks around $1,400\text{--}1,600 \text{ cm}^{-1}$ indicate the presence of calcium carbonate in the STD [24]. The peaks around $1,650\text{--}1,550 \text{ cm}^{-1}$ related to amide groups associated with incorporation of collagen into the polyurethane matrix

[25]. As shown in Figures 1(a) and 1(b), with addition of collagen, the relative intensity difference between bonded C=O and free C=O ($1,720\text{ cm}^{-1}$ and $1,650\text{ cm}^{-1}$) decreased with the increase in filler contents. The finding is consistent with Han et al. [26]. The intensity and position of these amide peaks were consistent both in standard temperature and heat aging, indicating the stability of PU foams under different temperature conditions.

Under heat aging conditions (Figure 1(b)), the FTIR spectrum of standard sample PU foam exhibits subtle changes. An increase in peak intensity around $3,000\text{ cm}^{-1}$ indicates potential changes in hydrogen bonding or the presence of degradation products.

3.2. Morphological Evaluation of the Cell Structure. Polyurethane foam can exist in two primary microstructure configurations: open-cell and closed-cell. These terms refer to the arrangement of voids or cells within the foam structure. Figure 2 shows the FESEM image of PUF-based foam cell structure at 27x magnification at standard temperature (Figure 2(a)–(e)) and at heat aging conditions (Figure 2(a1)–(e1)). Based on the observations, the standard sample showed a close-cell structure (Figure 2(a)–(a1)). Because of the increased dispersion of calcium carbonate throughout the entire surface of PU foams, the cells were likely adjacent to one another. On the other hand, it seems that a higher proportion of FSC causes visible variations in the cell structure. As an illustration, the addition of 10 g results in both an increase in the quantity of densely packed cells and a decrease in cell size (Figure 2(e)). In comparison to the typical PU foams, the structure's shape was likewise neater and more consistent (Figure 2(a)). Comparable patterns were noted when 50 g of collagen was substituted for calcium carbonate in the swap sample, as Figure 2(b)(b1)) illustrates. This could be explained by the bulk viscosity being raised to withstand cell expansion as the concentration of inorganic fillers rose [27]. In order to attain the desired qualities of PU composite foams, it is crucial to take into account the kind of suitable fillers.

Research has revealed that the cellular makeup of polyurethane foam is pivotal in determining its mechanical properties, including compression and tensile strength [28]. Closed-cell structures have been observed to possess greater compression and tensile strength compared to open-cell structures [29]. The findings suggest that all samples maintain a closed-cell structure, regardless of standard temperature or heat aging, implying that temperature conditions do not impact the morphology of the PU foams. It is also well-known that fillers increase cell density and decrease cell size. This may be attributable to the fact that closed-cell foam is impermeable to air and moisture due to its sealed cell structure. Overall, the pores exhibit greater closure in fish scale collagen (FSC)–PU composites compared to unfilled PU. The greater closure of pores in FSC–PU composites compared to unfilled PU can be attributed to enhanced filler–polymer interaction increased crosslinking density, optimized filler dispersion, and/or the physical barrier effect provided by FSC particles. For instance, proper dispersion of FSC particles within the polyurethane matrix ensures uniform distribution and close packing of filler particles, leading to efficient space

utilization and reduced pore size. Studies have indicated that well-dispersed fillers can act as nucleation sites for polymer crystallization, resulting in finer cell structures and improved pore closure [30, 31]. It is envisaged that by affecting the macroscopic cell in this way, collagen acts as reinforcement materials in polyurethane foam composite.

3.3. Mechanical Characteristics of Composites Based on PUF

3.3.1. Tensile Strength of Composites Based on PUF. Figure 3 provides a comprehensive analysis of tensile strength across various sample groups under standard temperature and heat aging conditions. The results reveal distinct patterns influenced by composition and environmental factors. At standard temperature, no significant difference in tensile strength was observed among sample groups ($P = 0.805$), suggesting that variations in composition, including collagen addition and calcium carbonate substitution (swap), do not substantially affect tensile strength under normal operating conditions. This implies that FSC could potentially substitute kulu powder in the foam industry, offering environmental benefits by utilizing fish scale waste [32]. However, in the “swap” condition where calcium carbonate was substituted, a decrease in tensile strength ($65.0 \pm 5.6\text{ kPa}$) occurred compared to the standard (73.3 ± 8.91). The observed decrease may be related to how the different fillers interact with the polymer matrix. Despite this reduction, repositioning FSC for value-added products could be environmentally beneficial, considering the environmental, health, and economic damage associated with fish scale waste disposal [32].

The addition of collagen at different concentrations demonstrated a concentration-dependent effect on tensile strength. The most significant decrease (62.7 ± 6.97) occurred with 2.5 g collagen, indicating a substantial adverse effect with a percentage change of approximately -14.47% . Similar trends were observed with 5 and 10 g collagen, suggesting that increasing collagen content tends to decrease tensile strength compared to standard samples. These observations align with existing literature highlighting the sensitivity of material properties to changes in composition, particularly when introducing foreign elements like collagen [3].

Under heat aging conditions, tensile strength significantly differed among sample groups ($P = 0.023$). The substitution of calcium carbonate with 50 g of collagen in the “swap” sample led to a substantial decrease in tensile strength (65.0 ± 5.6), consistent with studies indicating that PU may become more detrimental under elevated temperatures [33]. Surprisingly, the addition of 2.5 g collagen resulted in a remarkable increase in tensile strength (86.9 ± 8.1), approximately 12.66% higher than the STD. This suggests a positive influence of this collagen concentration under heat aging conditions, possibly attributed to its reinforcing properties [34]. This finding contrasts with studies suggesting a loss of strength and modulus with increased temperature [35, 36]. Conditions with 5 and 10 g collagen exhibited decreases in tensile strength (71.1 ± 7.4 vs. 71.49 ± 9.5), albeit to a lesser extent than observed at standard temperature (77.1 ± 5.74). These variations indicate a nuanced relationship between collagen content and tensile strength,

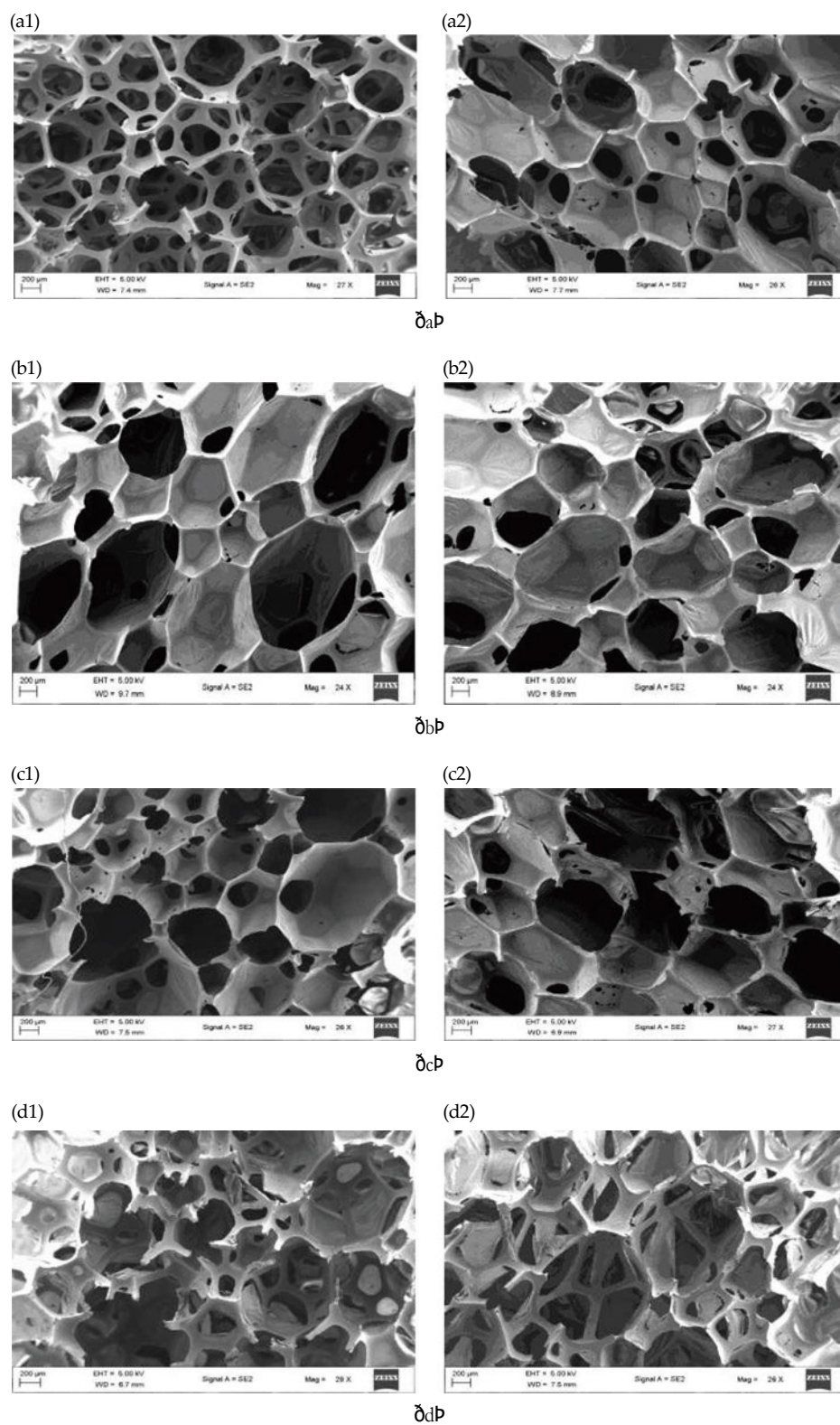


FIGURE 2: Continued.

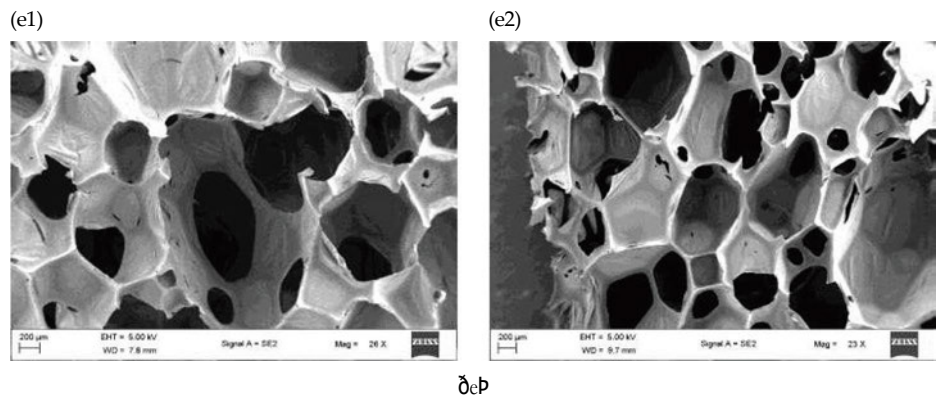


FIGURE 2: FESEM image of PUF-based composites at different temperatures; (a) STD (PU without fish) scale collagen; (b) swap standard sample (50 g of fish scale collagen); (c) 2.5 g of fish scale collagen + standard sample; (d) 5 g of fish scale collagen + standard sample; (e) 10 g of fish scale collagen + standard sample; (a–e) standard room temperature; (a1–e1) heat aging (magnification $\times 27$).

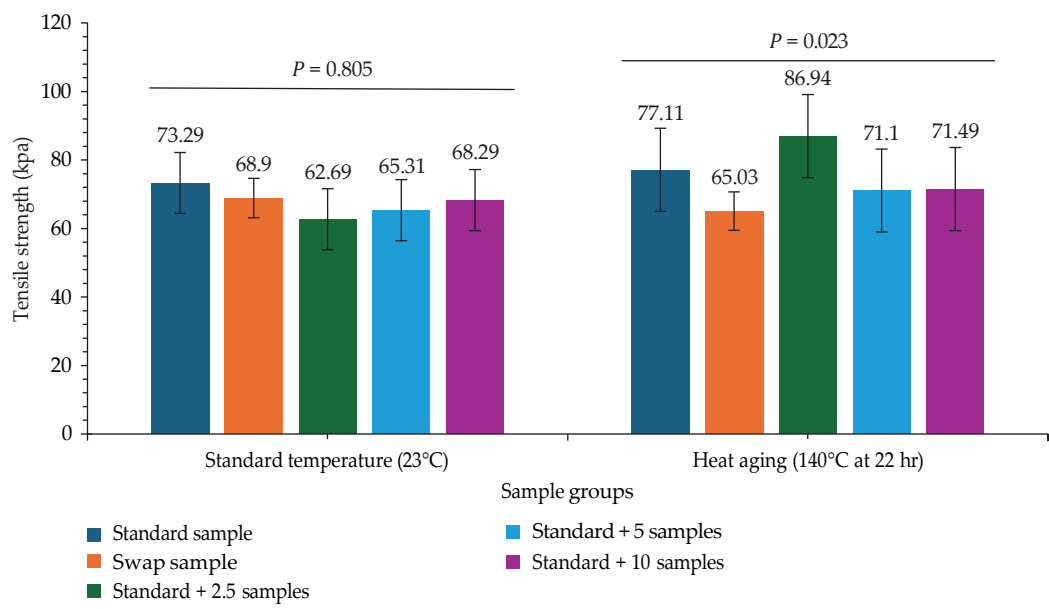


FIGURE 3: Tensile strength of the reinforced composites at different environmental conditions (STD contains only Kulu powder as filler; swap contains only collagen as filler; 2.5–10 g are concentrations of collagen in the STD). **Notes.** Standard sample (PU without fish scale collagen); swap sample (50 g of fish scale collagen); standard + 2.5 samples (2.6 g of fish scale collagen + standard sample); standard + 5 samples (5 g of fish scale collagen + standard sample); standard + 10 samples (10 g of fish scale collagen + standard sample).

emphasizing the importance of optimizing composition for specific environmental conditions [9].

3.3.2. Elongation at Break of Composites Based on PUF. Figure 4 presents a detailed analysis of mean elongation, standard deviation, and P values for various PU sample groups under different environmental conditions, shedding light on the material's flexibility and ductility. At standard temperature, standard samples demonstrated an elongation at the breaking point of $96.1\% \pm 2.10\%$, with comparable behavior across sample groups ($P = 0.376$). The substitution of calcium carbonate in the “swap” condition led to a nonsignificant increase ($101.1\% \pm 19.0\%$) of approximately 5.20% in elongation, suggesting a potential influence on ductility. The addition of 2.5 g collagen resulted in a notable decrease ($83.9\% \pm 9.77\%$) of approximately -12.64% ,

indicating a stiffening effect. In contrast, 5 g collagen exhibited a moderate, nonsignificant increase ($102.5\% \pm 15.8\%$) of approximately 6.65%, enhancing material flexibility. The inclusion of 10 g collagen yielded a subtle, nonsignificant improvement (100.0 ± 3.6) of approximately 4.05% in ductility. This aligns with the general rule that the mechanical strength of PU composites increases with higher filler contents, as seen in other studies [9]. The probable increase in elongation of PU foam at higher concentration of the collagen filler may be attributed to the ability to resist compression load, thus enhancing the durability of the composite during service.

Under heat aging conditions, standard samples displayed a significant decrease in elongation ($77.1\% \pm 5.74\%$, $P = 0.015$), indicating altered material properties. The “swap” condition with calcium carbonate substitution resulted in a

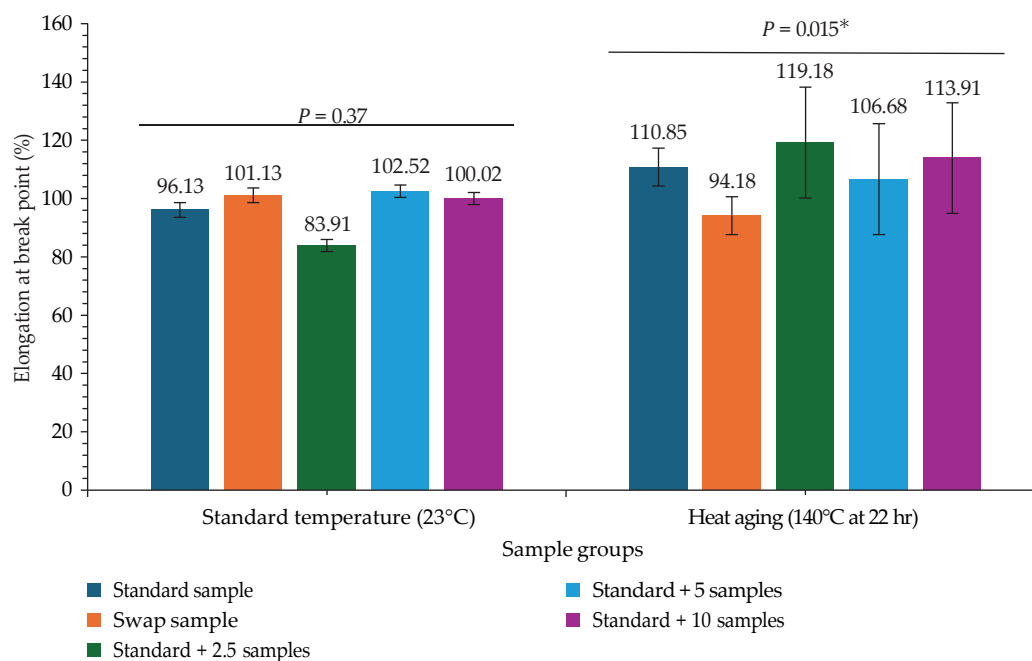


FIGURE 4: Elongation of the reinforced composites at different environmental conditions (STD contains only Kulu powder as filler; swap contains only collagen as filler; 2.5–10 g are concentrations of collagen in the STD). **Notes.** Standard sample (PU without fish scale collagen); swap sample (50 g of fish scale collagen); standard + 2.5 samples (2.6 g of fish scale collagen + standard sample); standard + 5 samples (5 g of fish scale collagen + standard sample); standard + 10 samples (10 g of fish scale collagen + standard sample).

substantial, significant decrease (65.0 ± 5.6) of approximately -15.63% , negatively impacting flexibility and ductility. Conversely, the addition of 2.5 g collagen led to a significant increase (86.9 ± 8.1) of approximately 12.66% , emphasizing a positive influence on material flexibility and ductility despite elevated temperatures. However, both 5 and 10 g collagen concentrations resulted in significant decreases (71.1 ± 7.4 vs. 71.49 ± 9.5) of approximately -7.77% and -7.27% , respectively, indicating a negative influence on material ductility under heat aging conditions.

These findings underscore the intricate interplay between PU filler contents and environmental conditions in determining elongation at the breaking point. While calcium carbonate and collagen concentrations influence material behavior, their effects vary under standard and heat aging conditions. According to Moo-Tun et al. [27], the use of FSC as a filler material may be advantageous in the real world for creating flexible and long-lasting polyurethane-based composites, especially in applications that are exposed to moderate temperatures.

3.3.3. Tear Resistance of Composites Based on PUF. Figure 5 provides a detailed analysis of tear resistance across various sample groups at standard temperature and under heat aging conditions. Tear resistance is a critical mechanical property, indicative of a material's ability to resist tearing forces. At standard temperature, the standard samples exhibited a mean tear resistance of 0.2093 ± 0.01 N/mm, showcasing a significant difference compared to other sample groups ($p \leq 0.001$). Notably, the substitution of calcium carbonate in the “swap” with 50 g of collagen condition resulted in a substantial and statistically

significant increase (0.3867 ± 0.06), marking an 84.85% enhancement in tear resistance compared to the standard. The addition of 2.5 g collagen significantly contributed to tear resistance (0.2567 ± 0.02), reflecting an increase of approximately 22.60% . Conditions with 5 and 10 g collagen displayed significant increases of approximately 25.50% and 33.00% , respectively, emphasizing collagen's effectiveness in enhancing tear resistance under normal conditions. These results align with previous research, such as Naidoo et al. [19], Ghasemi et al. [37], and Uzoma et al. [38], which investigated natural materials as reinforcements in polyurethane foam composites, reporting improvements in tear strength.

Under heat aging conditions, standard samples exhibited a mean tear resistance of 0.3757 ± 0.02 N/mm, with a nonsignificant P value of 0.362 . The substitution of calcium carbonate in the “swap” with 50 g of collagen led to a nonsignificant decrease (0.3453 ± 0.02) of approximately -8.09% in tear resistance compared to the standard. This reduction suggests a potential negative influence of collagen on tear resistance under elevated temperatures. Similarly, the addition of 2.5 g collagen resulted in a nonsignificant decrease (0.3383 ± 0.03) of approximately -9.93% , indicating a potential adverse effect on tear resistance. Conditions with 5 and 10 g collagen showed nonsignificant decreases of approximately -10.85% and -6.63% , respectively, hinting at a potential compromise in tear resistance with collagen additions under heat aging conditions. This decrease aligns with literature suggesting that temperature conditions can indeed impact material properties [33].

Tear resistance, tensile strength, and elongation at break point are all critical mechanical properties of polymer

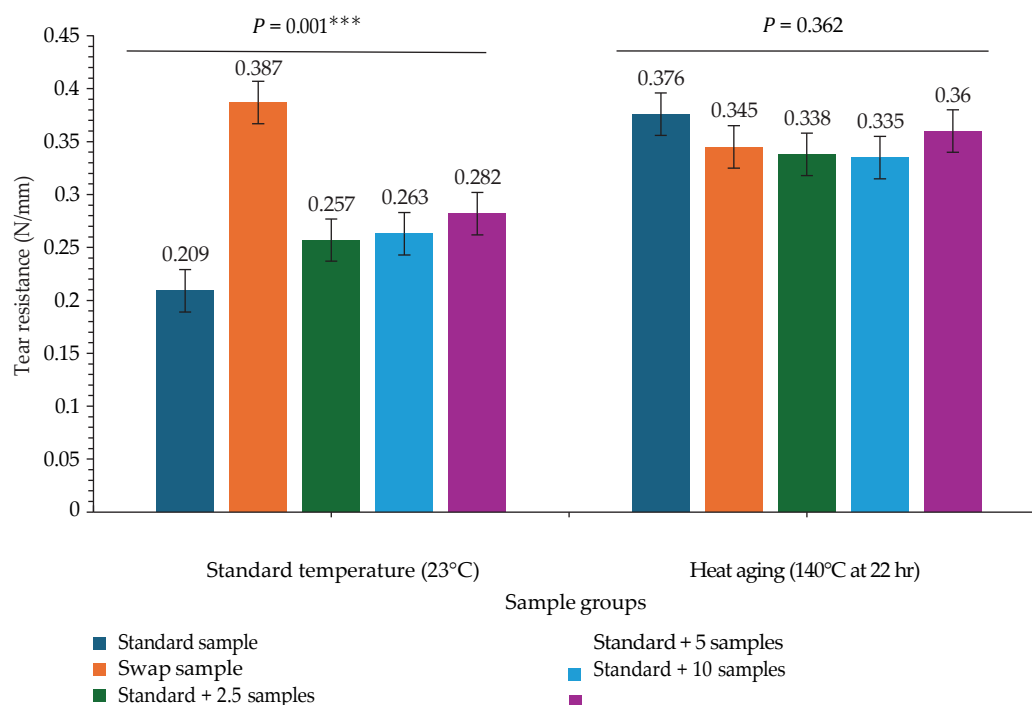


FIGURE 5: Tear resistance of the reinforced composites at different environmental conditions (STD contains only Kulu powder as filler; swap contains only collagen as filler; 2.5–10 g are concentrations of collagen in the STD). **Notes.** Standard sample (PU without fish scale collagen); swap sample (50 g of fish scale collagen); standard + 2.5 samples (2.6 g of fish scale collagen + standard sample); standard + 5 samples (5 g of fish scale collagen + standard sample); standard + 10 samples (10 g of fish scale collagen + standard sample).

composites, but they measure different aspects of material performance and respond differently to compositional changes and environmental conditions. The differences in tear resistance (Figure 5), tensile strength (Figure 3), and elongation at break (Figure 4) arise from the distinct ways these properties interact with the material's microstructure and their response to stress and environmental conditions. Tear resistance, being a localized property, is more sensitive to the material's ability to resist crack propagation, which can be significantly influenced by filler content and environmental conditions [39]. In contrast, tensile strength and elongation at break measure the material's overall ability to withstand deformation and stress under more uniform conditions [40]. These factors could have contributed to the subtle differences observed in the tear resistance, tensile strength, and elongation at break point.

3.3.4. Density of Composites Based on PUF. Figure 6 presents the density of PU foam at different temperature conditions. PUF-based composites, as investigated in this study, reveal a nuanced relationship between composition and density under both standard temperature (23°C) and heat aging conditions (140°C at 22 hr). The baseline standard sample, characterized by a mean density of $18.97 \pm 0.06 \text{ kg/m}^3$ at standard temperature, serves as a reference point for understanding the impact of introduced variations. At standard temperature, the substitution of calcium carbonate in the "swap" sample with 50 g of collagen yielded a substantial increase in density, registering at $20.37 \pm 0.75 \text{ kg/m}^3$ —an

augmentation of approximately 7.37% compared to the standard sample. This finding suggests that higher collagen filler content contributes to enhanced material density. Conversely, the addition of a modest 2.5 g of collagen resulted in a marginal decrease in density ($18.87 \pm 0.01 \text{ kg/m}^3$) by approximately -0.53%. The delicate balance in material composition is evident, emphasizing the intricate relationship between components and resulting density.

Further variations in collagen concentrations underscored the material's versatility. Incorporating 5 and 10 g of collagen led to significant increases in density— $20.35 \pm 0.17 \text{ kg/m}^3$ (7.27%) and $20.67 \pm 0.25 \text{ kg/m}^3$ (8.97%), respectively—compared to the standard sample at standard temperature. These results underscore collagen's positive influence on material density, emphasizing its potential for optimization in composite materials.

Under heat aging conditions (140°C at 22 hr), the standard sample exhibited a mean density of $18.99 \pm 0.05 \text{ kg/m}^3$. Notably, the substitution of calcium carbonate in the "swap" sample induced a substantial and statistically significant increase in density ($20.38 \pm 0.76 \text{ kg/m}^3$) by approximately 7.31%. This suggests a crucial role for collagen in enhancing material density under prolonged heat exposure. In contrast, under heat aging conditions, the addition of 2.5 g of collagen led to a slight decrease in density ($18.88 \pm 0.01 \text{ kg/m}^3$) compared to the standard sample by roughly -0.58%. This subtle variation highlights the delicate interplay between composition and density, emphasizing the need for precision in material formulation. Conversely, the inclusion of 5 and 10 g of collagen, even under elevated temperature

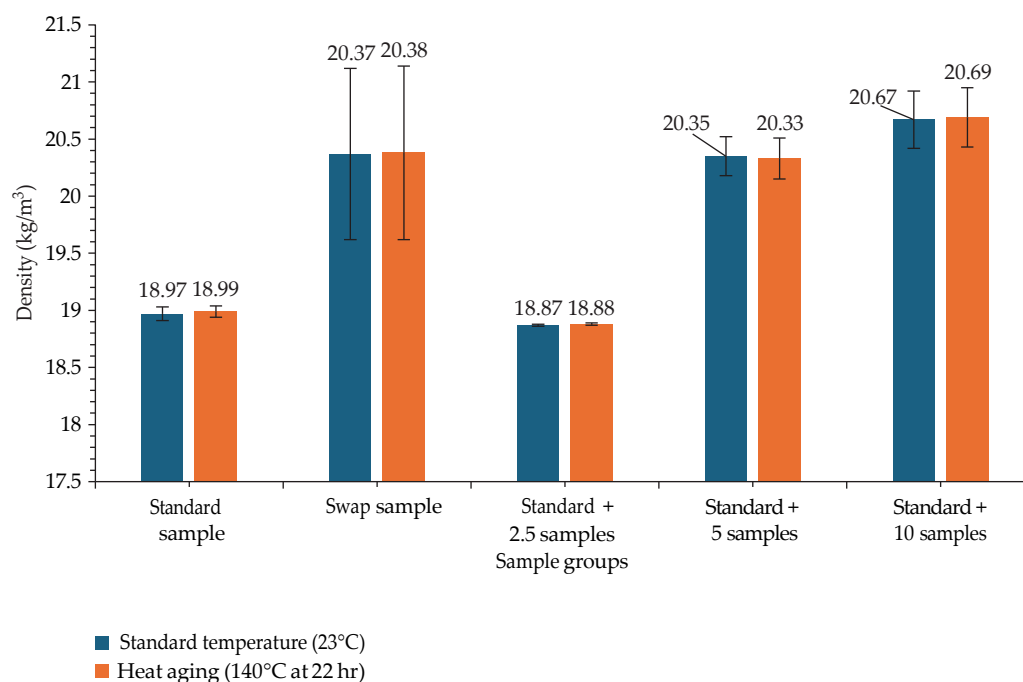


FIGURE 6: Density of the reinforced composites at different environmental conditions (STD contains only Kulu powder as filler; swap contains only collagen as filler; 2.5–10 g are concentrations of collagen in the STD). **Notes.** Standard sample (PU without fish scale collagen); swap sample (50 g of fish scale collagen); standard + 2.5 samples (2.6 g of fish scale collagen + standard sample); standard + 5 sample (5 g of fish scale collagen + standard sample); standard + 10 samples (10 g of fish scale collagen + standard sample).

conditions, demonstrated significant increases in density— $20.33 \pm 0.18 \text{ kg/m}^3$ (7.06%) and $20.69 \pm 0.26 \text{ kg/m}^3$ (8.95%), respectively, compared to the standard sample.

Overall, the findings showed that density measured is within the range obtainable for a flexible PU foam. The practical implications of these findings extend to industries where material density is a critical factor. Lower-density materials, for example, offer advantages such as improved lightweight properties, facilitating handling and transportation. This is particularly relevant in applications like automotive and aerospace, aligning with previous research emphasizing the importance of weight considerations in these industries [41, 42]. Additionally, lower-density materials may contribute to enhanced thermal insulation characteristics due to increased porosity, as indicated by prior studies [43].

4. Conclusions

In this study, collagen, derived from fish scales, was explored as a sustainable and environmentally friendly alternative to traditional fillers like calcium carbonate in PUF composites. The concentration-dependent effects of collagen on tensile strength were evident, with a remarkable increase (12.66%) observed with 2.5 g collagen under heat aging conditions. This result signifies a positive influence of collagen, especially in environments with elevated temperatures. While higher collagen concentrations exhibited a decrease in tensile strength under standard conditions, the nuanced relationship suggests the need for careful optimization based on application-specific requirements. The study demonstrated varying effects on elongation at break, emphasizing the intricate relationship between collagen content

and material flexibility. Higher concentrations of collagen, particularly 5 g, exhibited promising improvements (6.65%) in ductility under standard temperature conditions, suggesting potential advantages in applications where flexibility is crucial.

Furthermore, collagen emerged as a significant enhancer of tear resistance under standard temperature conditions, showcasing its potential for reinforcing PUF composites. The substantial increase (84.85%) in tear resistance with collagen substitution in the “swap” condition indicates its efficacy in improving material durability. Collagen positively influenced material density, showcasing its potential benefits in applications where specific densities are critical. The observed increases in density with collagen additions, even under heat aging conditions, highlight its role in enhancing material compactness.

The morphological assessment using FESEM revealed a consistent closed-cell structure across all samples, indicating the stability of PU foams. The ability of collagen to maintain a closed-cell structure, irrespective of temperature conditions, suggests its resilience and suitability for diverse environmental settings. Beyond mechanical properties, the utilization of fish scale-derived collagen addresses waste management challenges associated with the seafood industry. By repurposing collagen, a byproduct often considered waste, the study aligns with the principles of sustainable development, contributing to environmentally conscious material practices.

Data Availability

The corresponding author will make the datasets created and/or analyzed during the current study available upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Research Article

Enhancing Mechanical and Thermal Properties of Epoxy Composites with Fish Scale-Derived Collagen Reinforcement

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Epoxy polymer composites reinforced with fish scale-derived collagen (EFC) have garnered significant interest due to their potential for enhancing mechanical properties and environmental sustainability. In this study, we investigated the mechanical, thermal, and morphological characteristics of epoxy composites reinforced with varying concentrations of EFC (5%, 10%, 15%, 20%, and 25% wt). Tensile strength testing revealed an initial increase in Young's modulus with 5% and 10% EFC concentrations, followed by a decrease at higher concentrations, attributed to agglomeration effects. Flexural strength (FS) exhibited a decreasing trend with increasing EFC content, while flexural modulus (FM) showed improvement up to 20% EFC loading. Scanning electron microscopy (SEM) analysis highlighted the distribution of collagen particles, with agglomeration observed at higher concentrations. Fourier-transform infrared spectroscopy (FTIR) spectroscopy indicated alterations in hydrogen bonding with the addition of EFC. Thermal analysis revealed a reduction at onset degradation temperature with EFC incorporation, attributed to poor dispersion and agglomeration effects, alongside a slight enhancement in thermal stability at higher concentrations. The study supports the sustainable use of EFC as a filler, by offering a renewable and eco-friendly alternative to reinforcing polymer composites.

1. Introduction

Epoxy polymers stand out as popular polymer matrices for composite applications owing to their exceptional mechanical properties, chemical resistance, and thermal stability [1, 2]. Despite these strengths, epoxy polymers exhibit limitations such as low-impact resistance, poor toughness, and susceptibility to crack propagation [3]. Additionally, the brittleness of cured epoxy resins due to extensive cross-linking diminishes their impact strengths [4]. To address these drawbacks, epoxy polymers are often reinforced with other materials to enhance their mechanical properties [2]. Reinforcement materials significantly augment the strength, stiffness, and toughness of epoxy polymers, rendering them suitable for diverse applications spanning aerospace, automotive, marine, construction, and civil engineering sectors [5, 6, 7].

However, conventional reinforcement agents like carbon, glass, and Kevlar fibers, while effective, pose environmental

concerns due to their nondegradability [8, 9]. This has led to an escalating demand for sustainable alternatives in line with principles of green chemistry and circular economy practices [10, 11]. Natural fillers have emerged as promising and eco-friendly alternatives for reinforcing polymer matrices across various industries [12]. These natural fillers, derived from sources such as plant fibers and waste materials, offer cost reduction, processing enhancement, property improvement, and environmental friendliness [11]. Despite extensive research on natural reinforcements, there remains a notable gap concerning the systematic utilization of fish scale-derived collagen (EFC) as a reinforcement in epoxy composites. Previous studies primarily focus on natural fibers from plants [11], with limited attention given to the unique properties of fish scale collagen (FC) and its potential to enhance epoxy composite performance.

FC, sourced from waste in the fishing industry, presents a renewable and eco-friendly alternative for reinforcing composite

materials. Collagen, a predominant structural protein found in various biological tissues, exhibits remarkable mechanical attributes, as evidenced by the hierarchical arrangement of collagen fibers within fish scales [10, 13]. This suggests that collagen extracted from fish scales could effectively reinforce synthetic materials like epoxy resin, thereby advancing composite technology. More particularly, it is suggested in the literature that by selecting the appropriate reinforcement material and adjusting the volume fraction of the reinforcement, it is possible to tailor the properties of the composite material to meet the specific requirements of a given application [14]. By systematically varying concentrations of EFC in epoxy resin matrices, it is hypothesized that the mechanical properties of the resulting composites, particularly tensile strength, will increase proportionally.

This study aims to investigate the impact of EFC reinforcement on epoxy composites and explore its potential for enhancing mechanical performance. The composites were fabricated using different levels of FC concentrations (5, 5, 10, 20, and 25 wt%) in an epoxy matrix. The influence of collagen concentration on mechanical characteristics was investigated, uncovering intricate associations with tensile behavior, flexural strength (FS), hardness, and thermal behavior. Mechanical tests including tensile behavior, flexural properties, field scanning electron microscopy (FSEM), and thermogravimetric analysis were employed to assess the mechanical, physical, surface morphology, and thermal behavior of the composites.

2. Materials and Methods

Commercially available epoxy resin serves as the primary matrix material for composite fabrication. The materials used in preparing the laminate composite include Ampreg 30 Resin (epoxy resin), Ampreg 3X Slow Hardener (hardener), and extracted FC. The epoxy and hardener were supplied by AMT Composites, Durban, South Africa.

2.1. Preparation of Fish Scale-Derived Collagen (FC). Fish scale waste was acquired from a local fish shop in Durban, South Africa, and underwent thorough washing and disinfection using distilled water and 5 ml of household bleach. Following defrosting to room temperature to facilitate bacteria removal, the scales were air-dried for 2 days to eliminate excess water. FSC was extracted according to a well-established protocol involving demineralization and acid hydrolysis [15]. The scales were treated with 0.1 N NaOH for 2 days to eliminate noncollagenous proteins and pigments. After washing and drying for 2–3 weeks, collagen extraction was carried out using varied parameters. Collagen extraction involved soaking 100 g of scales in 0.5 M acetic acid for 48 hr at 4°C with intermittent stirring. The solution underwent centrifugation at 3,000 rpm for 30 min at 4°C. Supernatants were salted out by adding 9% NaCl, stirred automatically for 1 day, and suspended in a tris-glycine buffer. Pellets were collected via centrifugation, washed with distilled water, and subjected to dialysis using a dialysis membrane (molecular weight cutoff (MWCO) 12–14 kDa) to eliminate residual salts. The resulting material was freeze-dried at –80°C under vacuum conditions. Characterization of the

resulting collagen was conducted using different analytical techniques (Figure 1).

2.2. Preparation of Epoxy-Fish Scales Collagen Laminate. The epoxy laminate composite was prepared through a conventional resin casting technique. The epoxy resin is hand-mixed with the FC, then mixed with the hardener, and poured into the glass sheets. The ratio of epoxy (84 g) to hardener (316 g) used for the samples were 1 : 4. A rectangular glass sheet (300 mm × 300 mm × 4 mm) serves as a mold during the casting process, ensuring homogeneity and consistency in the laminate formation. A 4 mm rubber was used in between the glass sheets to determine the thickness and tightened with bolts and screws till cured for 24 hr. The epoxy laminate sample after 24 hr of curing at room temperature was removed from glass sheets and placed into an oven at 50°C for 16 hr for postcuring. Five composite specimens ($n = 5$) were fabricated with different volume fraction percentages of FC and epoxy polymer as shown (Table 1). The reinforced composites were then machined to obtain mechanical test specimens. The reinforced and modified composites were tested to obtain the mechanical results of the epoxy resin.

2.3. Mechanical Properties of Epoxy Resin Composites. The physical properties of the epoxy resin composites were assessed using standardized test methods. Tensile strength was evaluated according to ISO 527-2 standards, employing a 2 kN Instron apparatus known for its standardized methodology. This test aimed to characterize key mechanical properties, including tensile strength, elastic modulus, and elongation at the break. Dog bone-shaped specimens with dimensions conforming to ASTM D638 (20 mm width, 4 mm thickness, and 165 mm length) were utilized to ensure consistency with industry norms. To ensure statistical reliability, five specimens were tested to obtain mean values and deviations. Careful attention was given to sample clamping to ensure uniform tension distribution. Operational parameters such as a speed of 1 mm/min and a load cell size of 100 kN were meticulously controlled to ensure reproducibility.

The Barcol hardness of cured epoxy resin laminates was evaluated in accordance with ASTM D2583 standards. This method involved indenting the material's surface with a sharp steel point and measuring the resulting hardness, providing insights into the laminate's strength and suitability for various applications. Flexural properties were assessed following ISO 178 standards. Specimens with dimensions of 80 mm × 10 mm × 4 mm were tested using a Lloyds tensile tester. The test aimed to determine FS and modulus, crucial parameters indicative of material stiffness and deformation resistance. The experimental setup included a specified crosshead speed of 2.2 mm/min, a span of 68 mm, and a load cell size of 30 kN. This testing configuration ensured adherence to standardized procedures and facilitated comparisons with existing literature.

2.4. Microscopic Assessment of Epoxy Fish Scale-Derived Collagen Composites (EFC). The fractured sample of EFC composites was analyzed via scanning electron microscopy (SEM) using a field emission microscope from Carl Zeiss. The SEM was operated at 20 kV under controlled air conditions. Prior to observation, a fine layer of electrically conductive

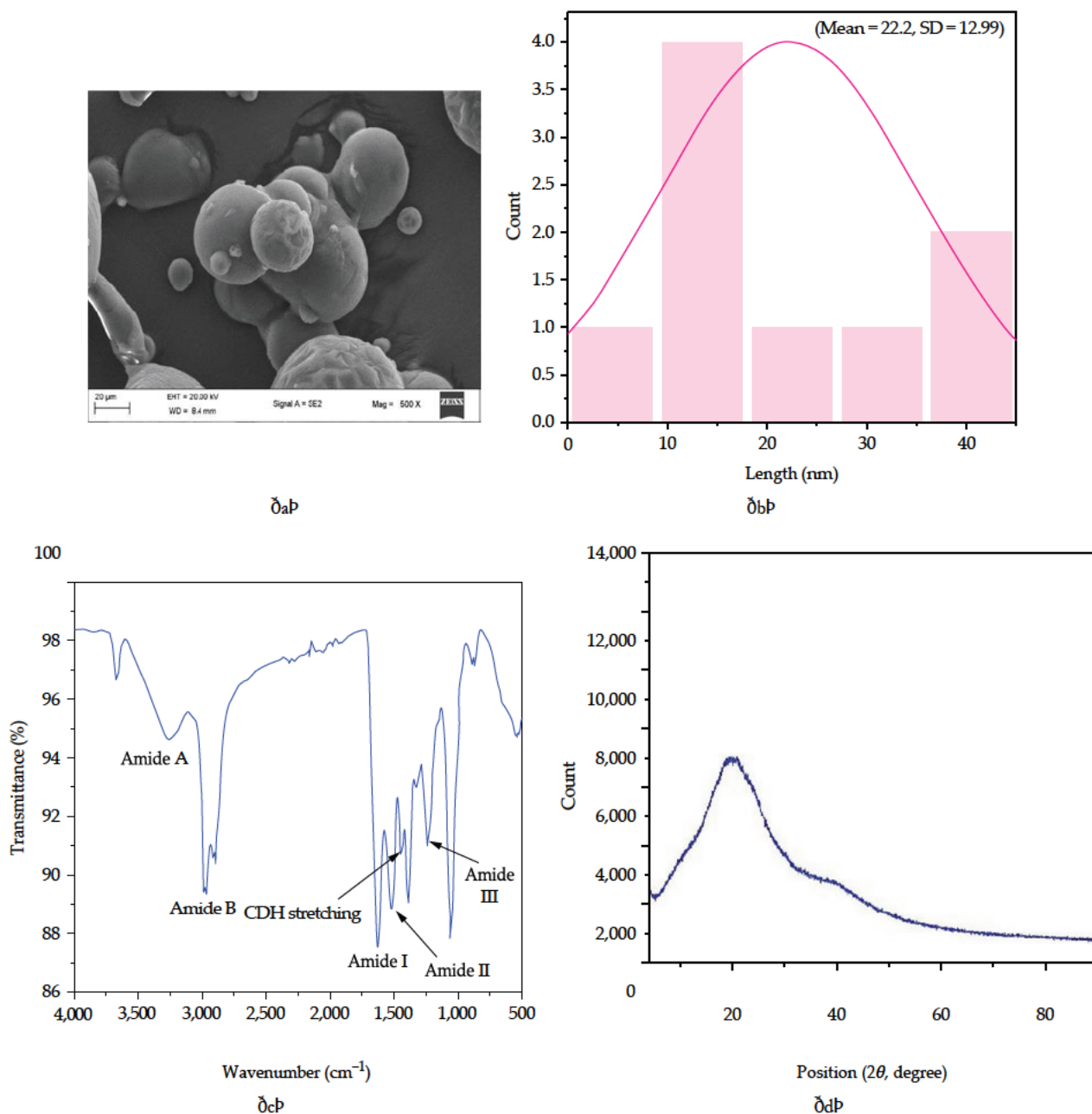


FIGURE 1: Characterization of FSC (a) SEM images; (b) particle size; (c) FTIR spectrum; and (d) XRD pattern.

gold coating was deposited on the surface to mitigate the risk of electrostatic charge accumulation. The surface morphology of the EFC was also studied using the same technique. In addition, the average particle size of the EFC was analyzed using ImageJ software (National Institute of Health USA, <http://imagej.nih.gov/ij/>).

2.5. Method of Characterization. Perkin Elmer Universal ATR was used to identify the changes in the epoxy composite's structure. Prior to scanning, a background investigation was carried out. Afterward, each of the cut samples was placed in a sample holder and scanned between 400 and 4,500 cm⁻¹ with a resolution of 4 cm⁻¹. Similar techniques were also used to identify the functional groups' presence in the EFC. An X-ray diffraction (XRD) analysis was carried out on the crystal-line structure of the prepared EFC. The D8 Advance BRUKER

AXS instrument from Germany, using Cu-Kα radiation (Kα1 = 1.5406), was used to capture the patterns within the 10–80 (2θ) range. The parameters used for the analysis included 40 kV of voltage, 40 mA of current, and 0.5 s of pass time.

2.6. Testing of the Thermal Properties. For thermal properties, analysis of the samples was performed using thermogravimetric analysis (TGA). TGA testing employed small quantities of samples (20 mg) in platinum crucibles. The temperature range was fixed between 30 and 600°C, with nitrogen flow rate and heating rate maintained at 30 ml min⁻¹ and 20°C/min, respectively, during testing. Calorimetry analysis was performed by a differential scanning calorimeter (DSC), Mettler Toledo (model: TAGC 10, model: DSC 822e). DSC testing took few amounts of samples (20 mg). A platinum crucible was used to carry the samples. The temperature ranges were

TABLE 1: Sample groups.

Sample codes	Epoxy resin (g)	Hardener (g)	Fish scale collagen (g)	Fish scale collagen (%)
Neat	84	316	0	0
EFC5%	84	316	20	5
EFC10%	84	316	40	10
EFC15%	84	316	60	15
EFC20%	84	316	80	20
EFC25%	84	316	100	25

kept fixed in between 30 and 600°C. Nitrogen flow rate and heating rate were maintained at 30 ml min⁻¹ and 20°C/min, respectively, during testing.

3. Results and Discussion

3.1. Characterization of FSC. Figure 1 shows the characterization of the extracted FSC. The SEM image in Figure 1(a) reveals that FSC consists of predominantly spherical particles. The surfaces of these spherical particles appear relatively smooth with an average particle size of around 22.2 ± 12.9 nm (Figure 1(b)). Some particles show minor surface irregularities, which could be due to the inherent structure of collagen

or the processing method used. The image in Figure 1(c) shows the fourier-transform infrared spectroscopy (FTIR) spectrum of FSC. The FTIR shows several peaks that corre-

spond to different characteristic amide bands (A, B, I, II, and III). This confirms the presence of collagen, as these are typical of the peptide bonds in protein structures [16]. For example, the amide A band was observed around 3,293 cm⁻¹. This peak corresponds to N—H stretching vibrations. It is characteristic of the peptide bonds in proteins, such as collagen. The amide B band was observed around 2,927 cm⁻¹. This peak is associated with asymmetrical N—H stretching vibrations, also part of the peptide linkage in collagen. The amide I band was observed around 1,611 cm⁻¹. This is one of the most important bands for protein analysis, primarily associated with C=O stretching vibrations in the peptide bond. It provides information about the secondary structure of the protein. The amide II band was observed around 1,523 cm⁻¹. This band is related to N—H bending and C—N stretching vibrations. The amide III band was observed around 1,300 cm⁻¹. This peak corresponds to complex vibrations involving C—N stretching and N—H bending, giving additional information about the protein's conformation. In addition, C—H deformation (CDH) peaks around 1,451 cm⁻¹ are attributed to C—H stretching vibrations, which can arise from various side chains in the collagen molecule [16].

The XRD pattern of FSC is given in Figure 1(d). The image shows broad peak at approximately $2\theta = 20^\circ$, accompanied by a few smaller, sharper peaks at higher angles ($2\theta = 40^\circ$). The broad peak reflects the amorphous nature of collagen and is associated with the arrangement of collagen fibers [17].

3.2. Tensile Strength

3.2.1. Tensile Strength Behavior. Figure 2 shows the influence of varying concentrations of EFC on the tensile strength and

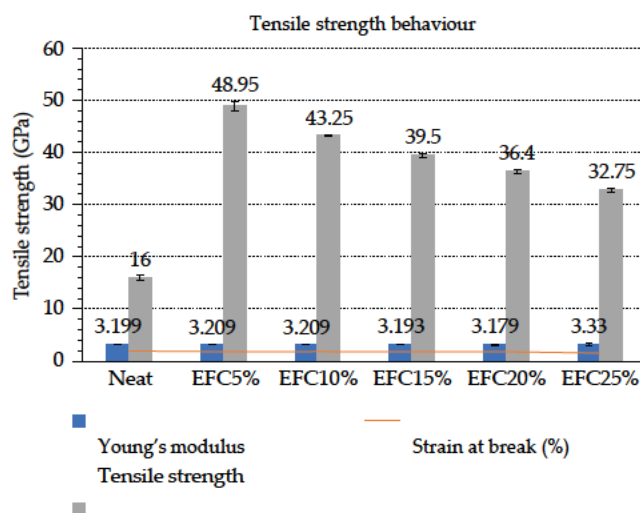


FIGURE 2: Tensile strength behavior of the reinforced epoxy resin composites.

modulus behavior of epoxy composites. Tensile strength significantly increases with the addition of EFC up to 5% and then gradually decreases as EFC content increases further. The significant increase in tensile strength at EFC5% (48.95 ± 0.9 GPa) suggests effective stress transfer within the composite. The addition of EFC particles enhances the tensile strength, possibly due to improved interfacial bonding and better load distribution. The gradual decrease in tensile strength beyond EFC5% could be due to the aggregation of EFC particles, leading to stress concentration points and weaker interfaces. Excessive filler content can lead to particle agglomeration, creating defects and reducing the overall strength of the composite [18].

Furthermore, the results indicate an increase in tensile modulus after adding 5% FC as exhibited by a 0.3% increase in Young's modulus and a 6.9% reduction in strain at break. This improvement in tensile modulus aligns with findings by Baldanov et al. [19], which emphasize the reinforcement potential of collagen in composite materials. No difference in Young's Modulus was observed between composites containing 5% and 10% EFC as they have the same Young's modulus value (5.5%) reduction in strain at break, suggesting a reduction in material ductility. These results resonate with a study by Zhang et al. [20], which emphasizes the influence of reinforcement concentration on mechanical properties.

The addition of 15% and 20% EFC resulted in a decrease in tensile modulus accompanied by reductions of 0.19% and 0.63% in Young's modulus, respectively. However, strain at break decreased by 6% and 6.7%, respectively, indicating an

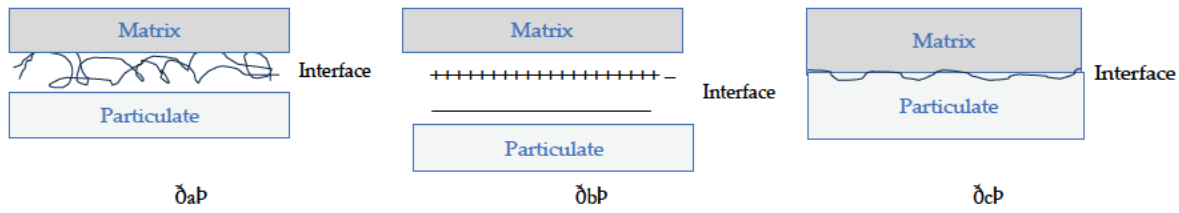


FIGURE 3: Schematic illustration of interfacial bonding mechanisms for EFC: (a) molecular entanglement following interdiffusion, (b) electrostatic adhesion, and (c) mechanical interlocking.

increase in toughness. This phenomenon suggests that while the overall strength may decrease, the material's ability to withstand deformation without fracture improves, enhancing toughness. Nevertheless, the slight variations in Young's modulus suggest that the stiffness of the composite remains mostly unaffected by the EFC content. The slight increase in Young's modulus (4.1%) at EFC25% might indicate a minor reinforcement effect, despite the lower tensile strength (32.75 $\pm$ 0.4 GPa). The finding is consistent with literature that suggests modulus can slightly increase due to the higher filler content [21]. Additionally, the strain at break decreased significantly to 21%, indicating increased stiffness. These findings are consistent with research by Naidoo et al. [13], which highlights the potential of collagen reinforcement to enhance the mechanical properties of composite materials.

The initial increase in the tensile strength and modulus in

the composite is due to the efficient stress transfer from the matrix to the filler, which indicates good interfacial bonding and molecular interactions between the matrix and filler. SEM images showed a relatively uniform distribution of collagen particles, indicating good interfacial adhesion between

the reinforcement and matrix. Hence, the filler bears a significant portion of the load, leading to improved mechanical properties compared to the pure matrix. The efficiency of the load transfer is determined by the molecular interaction at the interface along with the thickness and strength of the interfacial region formed [22, 23]. Interfacial bonding mechanisms in general include interdiffusion, electrostatic adhesion, and mechanical interlocking, which were presented in Figure 3.

3.2.2. Stress and Strain Relationship. The impact of EFC concentration on maximum stress and strain is illustrated in Figure 4. The relationship between maximum stress and EFC filler content follows a linear trend. Figure 4 demonstrates that as the content of EFC filler in the epoxy increases, the strain of EFC composites decreases. This declining pattern in composites is attributed to the rigid nature of EFCs, which renders the composites harder or stiff and hence more resistant to straining under tensile loading. Additionally, Tamrakar et al. [24] found that an increase in strain correlates with an increase in the elasticity limit.

Table 2 details the effects of different concentrations of EFC on the Young's Modulus (YM) of epoxy composites. The results indicate that treatments with 5% and 10% EFC led to substantial increases in YM, with a 26% increment observed for both concentrations. Similarly, treatments with 15% and 20% EFC resulted in a 19% increment in YM, while the 25% treatment exhibited a 17% increment. Notably,

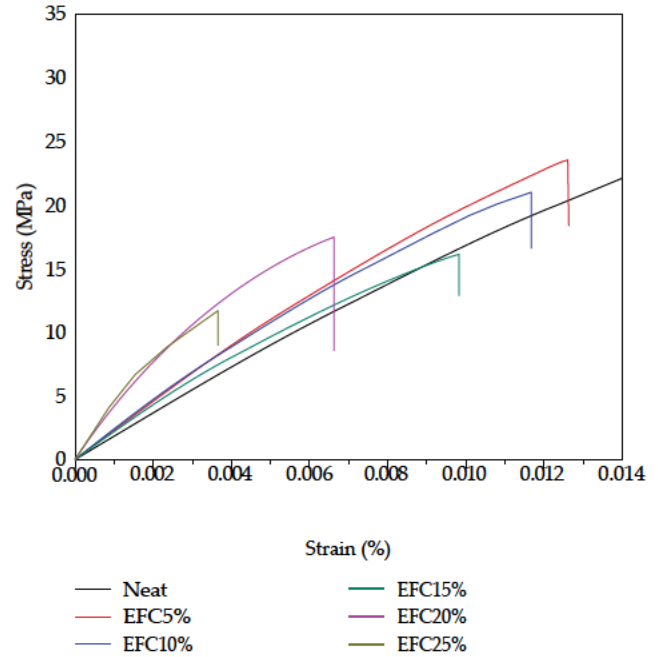


FIGURE 4: Stress and strain relationship of the reinforced epoxy resin composites.

TABLE 2: Effect of EFCs content on young modulus.

Sample	Young's modulus (MPa)	Change (%)
Neat	1,846 $\pm$ 6.1	0
EFC5%	2,327 $\pm$ 7.2	26.05634
EFC10%	2,328 $\pm$ 6.2	26.11051
EFC15%	2,196 $\pm$ 5.5	18.95991
EFC20%	2,196 $\pm$ 2.9	18.95991
EFC25%	2,166 $\pm$ 4.4	17.33478

concentrations beyond 10% showed a reduction in YM. The observed increase in YM with 5%, 10%, 15%, and 20% EFC treatments suggests effective reinforcement of the epoxy matrix by collagen.

However, treatments beyond 10% resulted in a reduction in YM, indicating a saturation effect or inadequate dispersion of collagen within the epoxy matrix. These findings highlight the importance of optimizing the concentration of EFC to achieve the desired mechanical properties. This is particularly useful as the mechanical properties of composite materials are dependent on filler content [25, 26].

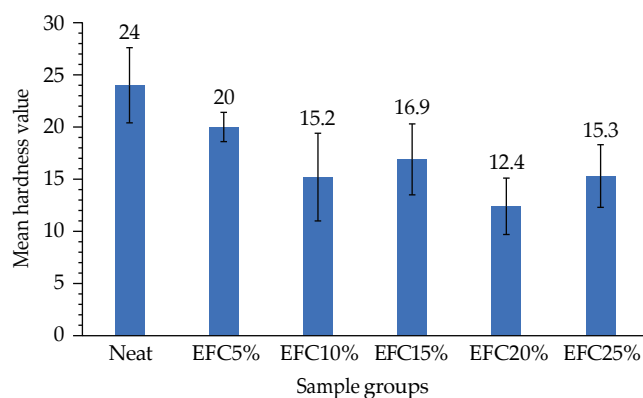


FIGURE 5: Hardness of the reinforced epoxy resin composite.

3.3. Hardness Properties. The hardness of composite materials is an important mechanical property that influences their suitability for various applications, including wear resistance, surface durability, and load-bearing capacity [5, 6]. Figure 5 presents the hardness measurements of the EFC composites, conducted using a Barcol hardness tester. The neat epoxy composite exhibits the highest mean hardness value of 24 ± 3.6 , serving as the baseline hardness in the absence of any reinforcement. However, as the concentration of EFC increases from 5% to 25%, a consistent trend of decreasing hardness is observed. Specifically, the EFC10% composite demonstrates a significant reduction in hardness compared to the neat epoxy, with a mean value of 15.2 ± 4.2 . Subsequent increases in EFC concentration led to a gradual decline in hardness, culminating in the EFC20% composite displaying the lowest mean value of 12.4 ± 2.7 .

Similar trends of decreasing hardness with increasing filler content have been reported in prior studies investigating composite materials reinforced with natural fillers [11, 12]. The decline in hardness with escalating FC concentration suggests that the incorporation of EFC influences the material's resistance to indentation or scratching. This effect may stem from alterations in the microstructure and mechanical properties of the composite material [2]. While EFC offers reinforcement potential, it may also introduce structural irregularities or weaken interfacial bonding within the epoxy matrix, thereby reducing hardness values [1]. Moreover, the observed decline in hardness beyond certain threshold concentrations (e.g., EFC20% and EFC25%) may indicate saturation effects or inadequate dispersion of the reinforcement material, resulting in diminished reinforcement effectiveness [4].

3.4. Flexural Strength. Figure 5 illustrates the flexural properties of the composites, with the FS of neat epoxy recorded at 95.54 ± 3.5 MPa. However, the FS values of the EFC5%, EFC10%, EFC15%, EFC20%, and EFC25% composites decrease to 78.15 ± 2.9 , 72.48 ± 4.6 , 57.39 ± 2.5 , 54.30 ± 2.6 , and 48.75 ± 1.2 MPa, respectively, as the filler content increases. This decreasing trend in FS of epoxy composites with increasing filler content can be attributed to several factors.

One primary reason is the disruption of the matrix structure and the formation of filler agglomerates at higher filler loadings. As the concentration of EFC increases, the

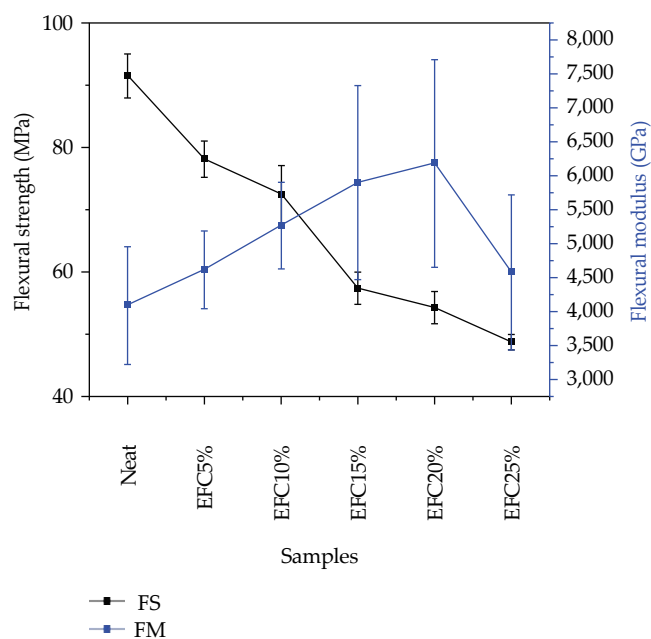


FIGURE 6: Flexural properties of the reinforced epoxy resin composite.

likelihood of particle clustering or agglomeration within the epoxy matrix rises, leading to localized areas of weakness and ultimately reducing the overall FS of the composite. This observation aligns with the findings of Mehdikhani et al. [27], who noted that voids in composites increase with the rise in filler weight percentage, resulting in decreased FS.

Furthermore, Figure 6 demonstrates the response in flexural modulus (FM) of EFC composites for various percentages of EFC loading into the epoxy. The FM values for neat, EFC5%, EFC10%, EFC15%, EFC20%, and EFC25% composites were measured at 4,095.69, 4,617.74, 5,269.8, 5,901.43, 6,187.47, and 4,578.52 GPa, respectively. The results indicate that FM continues to increase with the loading amount of FC until 20 wt%. Further incorporation of FC into epoxy results in a decrease in FM of the epoxy/FC composites. The largest incremental increase in FM was observed with the addition of 20 wt% of FC. This trend is consistent with the findings of Han et al. [28], who observed a similar improvement in FM properties with the loading of fish scale bones up to a certain percentage.

The enhancement in FM properties can be attributed to the strong physical interaction between the epoxy and the EFCs, as well as the good dispersion of EFCs into the epoxy. According to Han et al. [28], good filler dispersion improves the flexural properties of composites by enhancing load transfer capacity from matrix to fillers, likely resulting in the improved FM of the composites with the addition of FC into the epoxy matrix. However, further loading of EFC particles beyond 20 wt% leads to a decrease in the FM of the composites, attributed to agglomeration and disruption of the interface between the EFCs and the epoxy [29]. Overall, the findings from Figure 4 suggest that as FS decreases with an increase in EFC filler content, FM increases with the increase in EFC filler content until 20 wt%. This observed trend can be attributed to various factors related to the mechanical behavior and microstructure of the epoxy

composites, including filler dispersion, interfacial bonding, and mechanical compatibility [30].

3.5. Surface Morphology. The microstructure analysis based on SEM images provides valuable insights into the morphology and distribution of collagen reinforcement within epoxy composites. SEM images of neat epoxy and epoxy composites reinforced with various weight percentages (wt) of collagen (5%, 10%, 15%, 20%, and 25%) were examined to understand the impact of reinforcement on the composite's internal structure. In the SEM images of the neat epoxy composite (Figure 7(a)), a homogeneous matrix with minimal internal features is observed. The absence of distinct features indicates a uniform distribution of epoxy resin throughout the material. However, upon the addition of collagen reinforcement, notable changes in the microstructure are observed. At lower collagen concentrations (5% and 10% wt), SEM images (Figures 7(b) and 7(c)) reveal the presence of dispersed collagen particles within the epoxy matrix. These images show a relatively uniform distribution of collagen particles, indicating good interfacial adhesion between the reinforcement and matrix. This observation is consistent with studies by Giménez et al. [2], which emphasize the importance of interfacial bonding for effective reinforcement in composite materials.

As the collagen concentration increases to 15% and 20% wt, SEM images (Figures 7(d) and 7(e)) display a higher density of collagen particles within the epoxy matrix. However, some clustering of collagen particles is observed, suggesting the onset of agglomeration effects. This phenomenon could be attributed to insufficient dispersion of collagen at higher concentrations, leading to localized areas of reinforcement. Similar observations of particle clustering have been reported in studies on composite materials by Saba and Jawaid [3], highlighting the challenges associated with achieving uni-

form dispersion at higher filler loadings.

At the highest collagen concentration (25% wt), SEM images (Figure 7(f)) reveal significant agglomeration of collagen particles within the epoxy matrix. The presence of large clusters or agglomerates indicates poor dispersion and uneven distribution of reinforcement, which can adversely affect mechanical properties. These findings underscore the importance of optimizing filler loading to maintain uniform dispersion and maximize reinforcement effectiveness.

3.6. Fourier Transform Infrared Spectrophotometry. The FTIR spectra of the samples are given in Figure 8. The epoxide groups of the epoxy gave the stretching vibration in the range of 823–750 cm^{-1} [31]. The absorption peak in this region corresponds to the stretching vibration of the C—O—C bond in the epoxy ring. Bands associated with bending vibrations of the epoxy ring (COC) appear in the region of 1,360 cm^{-1} . It was found that the stretching of C—C bonds of the epoxy gives a peak at around 1,260 and 1,300 cm^{-1} [32]. Peaks in the region of 2,841–2,914 cm^{-1} are associated with C—H stretching vibrations. The stretching vibration of the phosphate gave a peak at 1,090 cm^{-1} [33]. The strong stretching vibration at 1,455 cm^{-1} is attributable to the C—N and N—H bonding of the protein compounds in collagen. The peak around the

range of 1,550–1,600 cm^{-1} is related to amide groups (amide I and amide II bands) associated with the incorporation of collagen into the epoxy matrix [34]. Peaks around 3,386 cm^{-1} indicate the presence of N—H stretching vibrations or hydrogen bonding. The rise in peak intensity at approximately 3,386 cm^{-1} with an increase in filler content suggests possible alterations in hydrogen bonding or the existence of degradation by-products. Furthermore, with the addition of collagen, the relative intensity difference between bonded C=O around 1,739 cm^{-1} decreased with the increase in filler contents, particularly for the EFC25%. The decrease in the intensity of the peaks could indicate interactions or changes in the chemical structure of the epoxy due to the reinforcement. This finding is consistent with Han et al. [28].

3.7. Thermal Properties. The thermogravimetry and differential thermal gravimetry results of neat epoxy and its reinforced EFC composite samples (EFC5%, EFC10%, EFC15%, EFC20%, and EFC25%) are reported in Figures 9(a) and 9(b). The thermal stability was characterized by temperatures taken at the onset (T_{onset}), maximum peak (T_{max}), and at 50% mass loss ($T_{50\%}$), which are shown in Table 3. The neat sample and its EFC composites exhibited a single degradation step which is related to the main degradation of epoxy at 365°C, associated with the decomposition of collagen molecules [35]. The introduction of EFC in the epoxy resin led to a decrease in the T_{onset} temperature from all EFC composites, thereby indicating that the inclusion of EFC could not retard the heat to diffuse into the epoxy resin (Figure 8 and Table 3). This could be attributed to the clustering of collagen particles in the epoxy resin observed in SEM, which suggested the effect of agglomeration. On the other hand, with increasing degradation temperatures, the reinforced EFC composites displayed a slight increase in thermal stability, especially for the EFC5% and EFC10% composites that exhibited

$T_{50\%}$ of 368°C (Table 3), and were observed from derivative

thermogravimetry (DTG) curves (Figure 9(b)). This may be attributed to the formation of char at elevated temperatures resulting in reduced polymer chain mobility and which retarded the diffusion of volatile products from the sample. Similar observations were reported by Hani et al. [36], who reported that the improvement of T_{max} of composites meant that it took a higher temperature to decompose the molecules of composites, which was an indication of higher thermal stability of the epoxy/fishbone particle (FBP) composites.

The DSC curves of neat epoxy and its reinforced EFC composite samples (EFC5%, EFC10%, EFC15%, EFC20%, and EFC25%) are reported in Figure 10. The glass transition temperature (T_g) and crystalline temperature (T_c) of the neat epoxy and its EFC composites were measured from the DSC curves. The neat epoxy and EFC25% showed a lower T_g of 60°C among the composites (Table 3). The T_g of the EFC5%, EFC10%, EFC15%, and EFC20% composites were measured at 75, 70, 60, and 75°C, respectively. Incorporating EFC (5%, 10%, 15%, and 20%) into the epoxy increased the T_g of the composites, due to good adhesion between the epoxy and the EFC filler. This implies that the incorporation of EFC delayed the movement of the molecular polymer, hence increasing

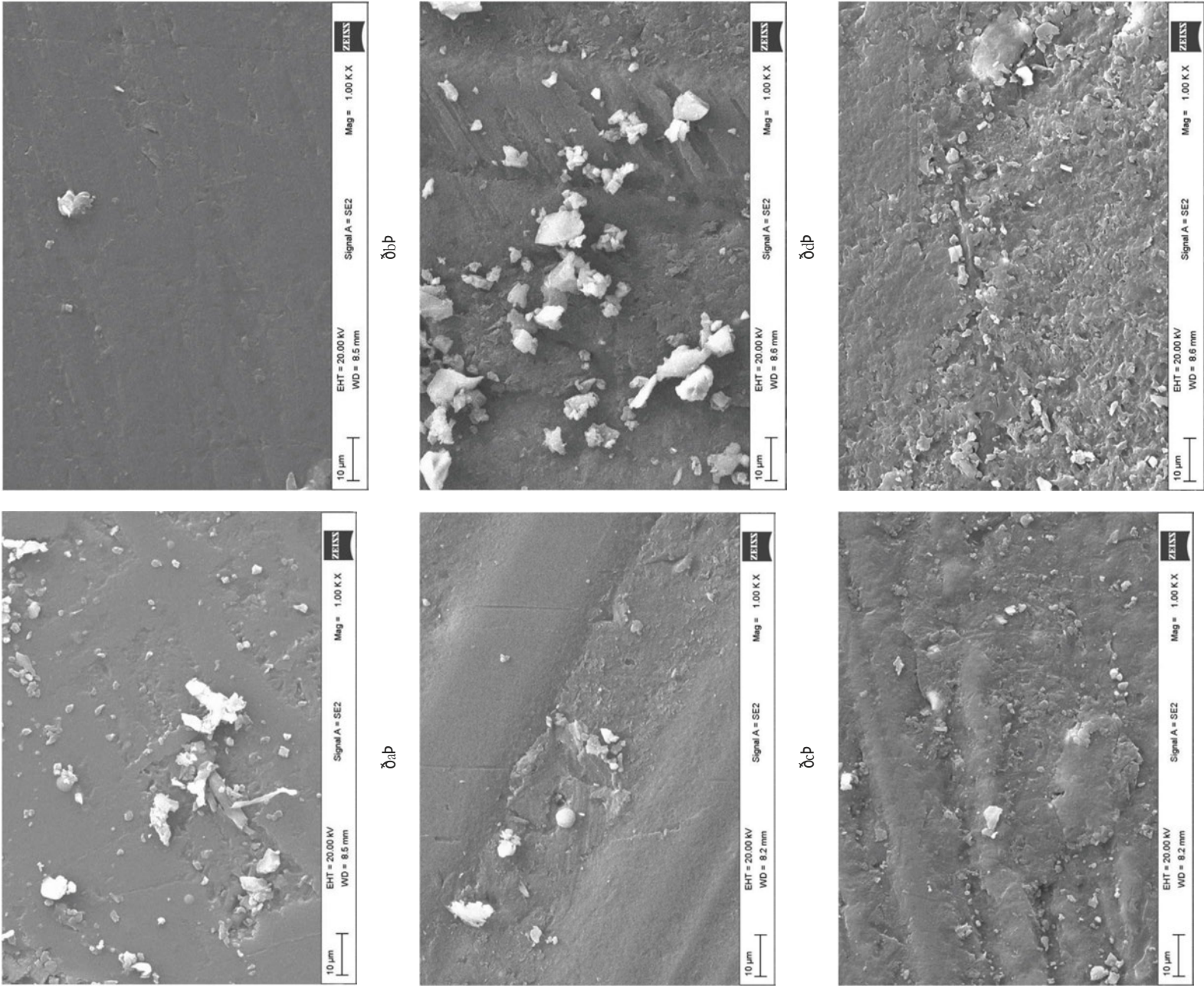


FIGURE 7: FSEM images of the reinforced resins composite: (a) neat; (b) 5 wt%; (c) 10 wt%; (d) 15 wt%; (e) 20 wt%; and (f) 25 wt% (magnification 1,000x).

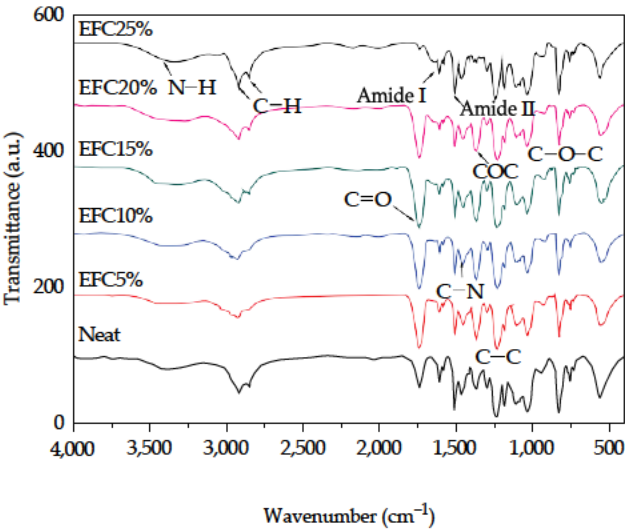


FIGURE 8: FTIR spectrum of the reinforced composite resin.

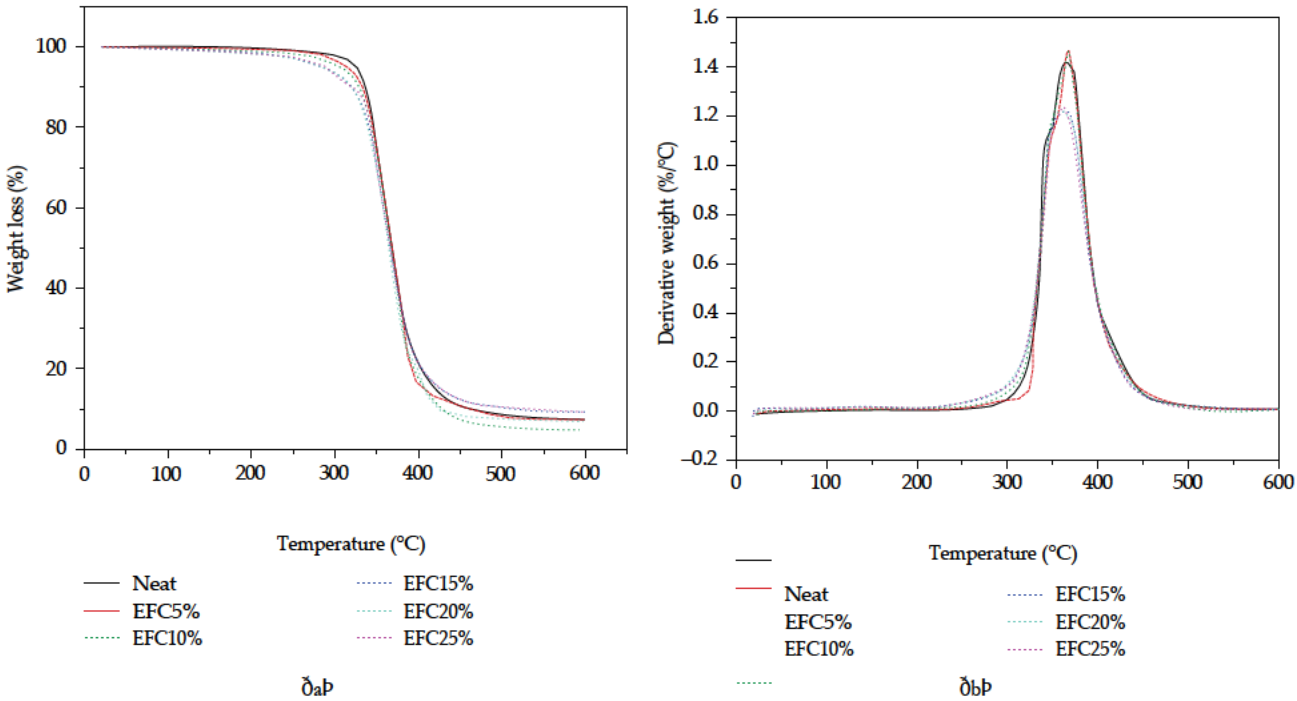


FIGURE 9: (a) TGA and (b) DTG curves of neat epoxy and its reinforced EFC composites.

TABLE 3: Summary of TGA results for neat epoxy and EFC composites.

Sample	T_{onset} (°C)	T_{max} (°C)	$T_{50\%}$ (°C)	T_g (°C)	T_c (°C)	Residue weight loss (%)
Neat	352	365	367	60	340	4.97
EFC5%	315	365	368	75	340	3.73
EFC10%	305	370	368	70	335	4.66
EFC15%	285	360	367	75	345	5.91
EFC20%	280	360	362	75	345	6.2
EFC25%	270	360	366	60	345	5.29

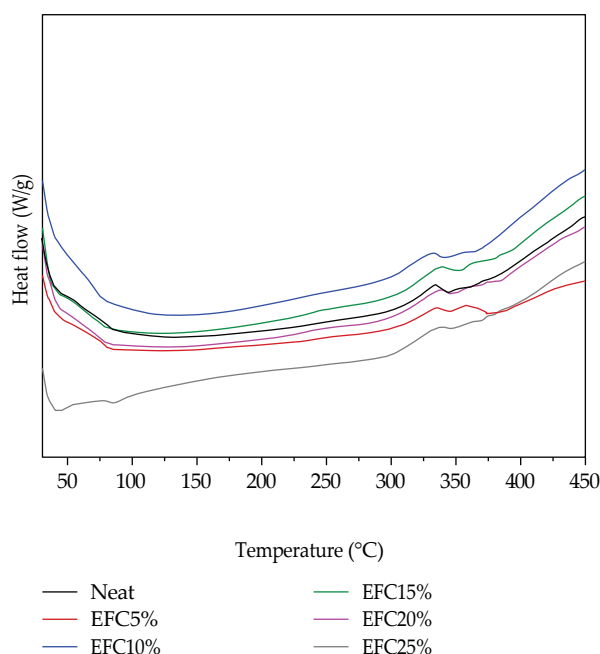


FIGURE 10: DSC curves of neat epoxy and its reinforced EFC composites.

the T_g . On the other hand, the decrease in T_g for the EFC25% composite is a result of the agglomeration of filler particles in the polymer, which leads to poor dispersion of the filler as observed from the SEM results. In the case of the crystalline temperature (T_c), a slight increase in T_c was observed for EFC10%, EFC15%, EFC20%, and EFC25% composites except for EFC10% and no change for EFC5% (Table 1). The increase suggests that the EFC caused epitaxial crystallization and crystal growth on the EFC surface which probably influenced the crystallization of the polymer. In the case of EFC10%, the decrease might be due to reduced crystal size and amount of crystalline domains [37]. Similar observations were reported by Hani et al. [36], who reported an improvement in T_g for 10 and 15 wt% although the 5 wt% composite did not improve the T_g . Furthermore, an increase in T_c was also reported for FBP composites.

4. Conclusions

In this study, epoxy composites reinforced with EFC were systematically investigated to evaluate their mechanical, thermal, and morphological properties. Key findings revealed an intricate relationship between EFC concentration and composite performance. Tensile strength initially increased with 5% and 10% EFC loading before decreasing at higher concentrations, indicating a balance between reinforcement and agglomeration effects. FS exhibited a declining trend with increasing EFC content, while FM showed improvement up to 20% loading, suggesting optimal reinforcement at this concentration. SEM analysis highlighted the distribution of collagen particles, with agglomeration observed at higher concentrations, affecting composite morphology. FTIR spectroscopy revealed alterations in hydrogen bonding and chemical structure with EFC addition, indicating potential interactions between collagen and epoxy. The thermal analysis demonstrated a reduction in onset

degradation temperature with EFC incorporation, attributed to poor dispersion and agglomeration effects, alongside a slight enhancement in thermal stability at higher concentrations.

These findings support the importance of optimizing EFC concentration to achieve the desired mechanical and thermal properties in epoxy composites. Furthermore, the observed agglomeration effects highlight the need for improved dispersion techniques to maximize reinforcement effectiveness. The implications of this study extend to various industries seeking sustainable reinforcement materials for composite applications, with EFC showing promise as a renewable and eco-friendly alternative. Future research directions may involve exploring advanced dispersion methods and investigating the long-term durability and environmental impact of EFC-reinforced composites.

Data Availability

The corresponding author will make the datasets created and/or analyzed during the current study available upon reasonable request.

Additional Points

Highlights. Epoxy composites reinforced with EFC were systematically investigated. Tensile strength initially increased with 5% and 10% EFC loading before decreasing at higher concentrations. FS exhibited a declining trend with increasing EFC content, while FM improved up to 20% loading. SEM analysis revealed the distribution of collagen particles, with agglomeration observed at higher concentrations, affecting composite morphology. FTIR spectroscopy indicated alterations in hydrogen bonding and chemical structure with EFC addition. Thermal analysis demonstrated a reduction in onset degradation temperature with EFC incorporation, alongside a slight enhancement in thermal stability at higher concentrations.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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CHAPTER FIVE – CONCLUSIONS AND RECOMMENDATIONS

Polymeric materials such as epoxy and polyurethane are widely utilized in various industries and household applications, ranging from bedding and cushions to aerospace components, due to their versatility and favourable properties. However, despite these advantages, they exhibit certain limitations. For instance, while epoxy resins are renowned for their excellent chemical resistance and mechanical strength, their inherent brittleness significantly limits their impact resistance (Saba and Jawaid 2017; Zhao *et al.* 2022). Similarly, polyurethane (PUF) materials are valued for their flexibility and biocompatibility, yet they are predominantly derived from petrochemicals, raising environmental concerns (Gama, Ferreira and Barros-Timmons 2018). Furthermore, natural biobased PUF foams have been reported to exhibit relatively low mechanical strength (Barczewski *et al.* 2020). These environmental and mechanical limitations necessitate the reinforcement and enhancement of polymer properties.

Natural fillers, particularly those derived from agro-waste such as fish scales, have garnered significant attention as environmentally friendly materials for enhancing polymer properties. Collagen, a natural component of fish scales, has attracted research interest due to its rough texture and high tensile strength, making it a promising candidate for reinforcement. The focus of this study was to extract collagen from natural waste and evaluate its potential as a reinforcing agent for two polymeric materials: polyurethane and epoxy. To achieve this, the study employed a quantitative research approach and an experimental research design to assess the reinforcing capabilities of collagen derived from fish scales. This chapter concludes by discussing the research objectives and offering recommendations based on the study's findings, providing a pathway for future exploration in enhancing polymeric materials with natural biobased fillers.

5.1 REVISITING THE RESEARCH OBJECTIVES

The research objective was achieved in three phases.

Objective One: Preparation and optimisation of collagen extraction from fish scales biowaste.

The sub-objectives are to:

1. Prepare milled fish scales (FS) using alkaline hydrolysis and the mechanochemical method
2. Extract collagen (FSC) from fish scale biowaste using acid hydrolysis and tris glycine buffer.
3. Optimise the extraction conditions (Concentration of the acid, volume of the acid, soaking time, and buffer) using design software.

The findings of this study confirmed that both FS and FSC were successfully extracted from fish scale bio-waste materials and optimised (Paper I, Paper 2, Paper 3, and Addendum 1). It was found that under optimal conditions (0.5M of acetic acid, volume of acids of 100mL, soaking time of 120 min, and Tris-Glycine buffer of 10mL), the results demonstrate that temperature and buffer concentration significantly influence collagen yield, with a collagen purity of 17.14 ± 0.05 mg/g. An R^2 value of 73.84% was obtained for the mathematical model (Addendum 1).

Objective Two: Physicochemical characterisation and quantification of FS and FSC.

The sub-objectives are to:

1. Characterise the physicochemical characteristics of FS and FSC using different analytical techniques.

The study established that FS contains collagen type I (amide I, amide II, and amide III), carbonate ions, and calcium-deficient apatite (Paper 1). The surface morphology of the FS shows that the particles appear as angular, rod-like, and irregularly shaped fragments with varying sizes and surface features (Paper 1). The XRD analysis of MFSP (modified fish scale powder) revealed key insights into its crystal structure and mineralogical composition. Sharp peaks observed in the XRD spectrum correspond to various minerals such as hydroxyapatite, portlandite, and calcite. The prominent peak at 32.9° 2theta is attributed to optimal reinforcement content and effective dispersion of particles within the matrix, suggesting well-maintained particle size. The crystallinity of MFSP was determined to be 67.22%, indicating a semi-crystalline nature.

On the other hand, the study findings reveal that FSC consists of predominantly spherical particles. The surfaces of these spherical particles appear relatively smooth with an average particle size of around 22.2 nm (Paper 3). The study confirmed the presence of collagen with several peaks that correspond to different characteristic amide bands (A, B, I, II, and III). This confirms the presence of collagen, as these are typical of the peptide bonds in protein structures (Paper 3). The XRD pattern observed for the FSC suggests the amorphous nature of collagen (Paper 3).

2. Quantify and determine the amount and type of collagen extracted using amino acid analysis.

The SDS-PAGE analysis revealed a protein banding pattern consistent with the molecular weight of collagen (Addendum 1). The amino acid analysis revealed 17 different types of amino acids (Paper 2). The high percentages of glycine, proline, and hydroxyproline confirmed the presence of structural proteins like collagen (Paper 2).

Objective Three: Assess the Mechanical, Physical, and Thermal Properties of the reinforced Composites.

The sub-objectives are to:

- Determine the mechanical Properties of Epoxy Resin Composite, such as the tensile strength, flexural properties, and hardness, reinforced with varying concentrations of FSC (5, 10, 15, 20, and 25 wt.%).
- Determine the tensile strength, elongation, tear resistance, and density of PUF foams reinforced with varying concentrations of fish scales (0.5 and 1 wt.%) and FSC (2.5, 5, and 10 wt.%) at different temperature conditions.
- Determine the cell count and visual colour differences of PUF foams under temperature conditions.
- Microscopically assess the microstructure of Epoxy Fish Scale-Derived Collagen Composites (EFC) as well as evaluate the cell structure of PUF foams

The findings of this study confirmed that incorporating milled fish scale powder (FS) into PUF composites enhances mechanical properties, particularly tensile strength and tear resistance (Paper 1). It was demonstrated that the improved mechanical properties were temperature and filler-content-dependent (Paper 1). Higher temperatures positively affected the mechanical properties of PUF composites, increasing stiffness and strength due to better dispersion of FS in the matrix. Adding FS reduced the mass without significantly affecting volume, resulting in lightweight materials desirable in automotive and aerospace applications. FS-enhanced PUF exhibited improved flame-retardant properties, attributed to the high protein content in the fish scales (Paper 1).

The study confirmed that FSC could potentially replace calcium carbonate in PUF, with no significant difference in tensile strength at standard temperature, but variations under heat aging (Paper 2). The study shows that FSC improved PUF flexibility and tear resistance, particularly at 2.5 wt.%. However, complete substitution with FSC negatively impacted flexibility. While temperature did not affect density, it influenced tear resistance and tensile strength. The study confirmed that FSC provided better performance in higher temperatures, making it suitable for applications exposed to moderate heat (Paper 2). It was found that FSC increased cell closure, resulting in semi-rigid foam with enhanced durability (Paper 2).

The study confirmed that the addition of FSC, particularly at 5 and 10 wt.%, significantly improved the tensile strength and Young's modulus of the composites (Paper 3). This was due to effective stress transfer, improved interfacial bonding, and better load distribution. However, excessive filler content

(above 10 wt.%) led to a reduction in these properties due to particle aggregation, creating stress concentration points and weaker interfaces. It was found that the maximum stress decreased with increasing FSC content, and strain decreased with higher FSC concentrations due to the rigid nature of the filler, which made the composites stiffer and less prone to strain under tensile loading (Paper 3). An unusual trend was observed where flexural strength decreased with increasing FSC content, while the flexural modulus increased up to 20 wt.% before declining. This was attributed to filler agglomeration and disruption of the matrix structure at higher filler loadings, leading to localized weakness (Paper 3).

Additionally, FSC composites showed a decrease in the onset temperature (T_{onset}), indicating reduced heat resistance. However, the maximum degradation temperature (T_{max}) increased slightly, especially for 5 and 10 wt.% FSC, suggesting improved thermal stability due to char formation at elevated temperatures (Paper 3). The glass transition temperature (T_g) increased with FSC content up to 20 wt.%, signifying improved molecular adhesion and delayed polymer movement. However, at 25 wt.%, T_g decreased due to filler agglomeration and poor dispersion (Paper 3).

Surface morphology images showed a uniform distribution of collagen particles at lower filler concentrations (5 and 10 wt.%), indicating good interfacial adhesion. At higher concentrations, aggregation of FSC particles was observed, leading to defects that contributed to the reduced mechanical performance (Paper 3).

5.2 LIMITATION

While both FS and FSC extracted from fish scales waste were promising as filler material in polymeric materials, they did not fully determine the optimal filler content for maximizing both mechanical and thermal properties. The focus on specific weight percentages (5, 10, 15, 20, and 25 wt.%) for FSC and 0.5 and 1 wt.% for FS leaves room for further exploration of intermediate concentrations to identify more precise performance thresholds.

5.3 RECOMMENDATIONS

Considering the study findings and the research objectives, the researcher proposes the following recommendations:

5.3.1 OPTIMISE FILLER CONTENT TO ENHANCE MECHANICAL PROPERTIES

Based on the study's findings, the tensile strength and Young's modulus of the epoxy composites were significantly improved at lower FSC concentrations (5 and 10 wt.%), while higher filler content (above 10 wt.%) led to particle aggregation and reduced mechanical performance. Therefore, it is recommended to optimise the FSC filler content to around 5 and 10 wt.% to maximize tensile and flexural properties. This concentration range ensures effective stress transfer within the composite, promotes interfacial bonding, and avoids the negative impact of particle agglomeration.

5.3.2 EXPLORE SURFACE TREATMENTS TO IMPROVE FILLER DISPERSION

The SEM images revealed a uniform distribution of FSC particles at lower concentrations, while higher concentrations led to aggregation. To improve the dispersion of FSC at higher concentrations (above 10 wt.%), it is recommended to explore surface treatments or modifications of the filler. Surface treatments can improve the interfacial bonding between the collagen filler and epoxy matrix, thereby enhancing mechanical properties even at higher filler loadings.

5.3.3 INVESTIGATE LONG-TERM THERMAL STABILITY FOR HIGH-TEMPERATURE APPLICATIONS

The study showed a decrease in thermal onset temperature (T_{onset}) with the inclusion of FSC, which suggests a limitation in heat resistance. However, thermal stability improved for 5 and 10 wt.% FSC composites. To ensure broader industrial application, it is recommended to further investigate the long-term thermal stability of FSC-reinforced epoxy composites, particularly for high-temperature environments. This includes exploring methods to enhance the thermal properties, such as combining FSC with other heat-resistant additives.

5.3.4 EXPAND MECHANICAL TESTING TO INCLUDE IMPACT RESISTANCE AND TOUGHNESS

Although the study focused on tensile and flexural properties, other critical mechanical characteristics, such as impact resistance, fracture toughness, and fatigue behaviour, were not evaluated. It is recommended to expand future testing to include these properties to provide a more comprehensive understanding of the composite's performance. These additional tests would offer insights into the material's potential use in demanding applications where toughness and durability are crucial.

5.3.5 FOCUS ON TEMPERATURE-DEPENDENT APPLICATIONS

Paper 1 and Paper 2 both highlighted that PUF composites reinforced with fish scale fillers exhibit enhanced mechanical properties under elevated temperatures, particularly in terms of tear resistance and tensile strength. Therefore, it is recommended to explore these materials for applications requiring exposure to moderate or high temperatures, such as insulation, automotive interiors, and packaging materials. The flame-retardant and heat-resistant properties observed with fish scale additives make them particularly suitable for temperature-sensitive environments.

5.3.6 LEVERAGE THE ENVIRONMENTAL BENEFITS OF NATURAL FILLERS

With rising environmental concerns surrounding the petrochemical-based production of traditional PUF, both papers emphasise the sustainable advantages of using fish scale-derived fillers. Industries should capitalise on the eco-friendly, bio-based characteristics of FSC and MFS for greener manufacturing practices. Incorporating these materials into PUF foams can reduce reliance on non-renewable resources and address waste management challenges by utilising biowaste, making the composites more sustainable for various industries, including packaging, construction, and transportation.

5.4 CONCLUSION

In summary, the study, which aims to extract collagen from fish and assess its reinforcing properties on polymeric composite with special emphasis on epoxy and polyurethane, was achieved as illustrated by the study findings through Publications (Papers 1,2, and 3). The published papers demonstrate that fish scale-derived materials, including MFS and FSC, are viable, eco-friendly alternatives for reinforcing PUF composites. They offer mechanical, thermal, and flame-retardant benefits, particularly at higher temperatures, while addressing sustainability and waste management challenges. Similarly, the study found that FSC, particularly at moderate concentrations, can enhance the mechanical and thermal properties of epoxy composites. However, excessive FSC leads to particle agglomeration, reducing the beneficial effects. The study provides a benchmark for the use of FS and FSC extracted from bio-waste as a filler material in polymeric composites. The findings suggest that both FS and FSC hold promises for developing sustainable and eco-friendly composite polymers, in line with green chemistry and circular economy principles. Nonetheless, optimising filler content and improving filler dispersion is critical for achieving consistent enhancements in both mechanical and thermal properties.

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Addendum 1

It is worth mentioning that the results in addendum 1 were published as Makgobole, M.U., Onwubu, S.C., Baruwa, A., Mpofana, N., Obiechefu, Z., Naidoo, D., Khathi, A., and Mkhwanazi, B., 2024. *Optimization of Collagen Extraction from Fish Scales Using Tris-Glycine Buffer: A Taguchi Methodological Approach. Marine Drugs*, 22(12), p.562

SDS-PAGE Characterisations of Collagen

Figure 1 displays the SDS-PAGE analysis of Type I collagen extracted from marine samples. Lane 1 represents the molecular weight marker (MW), while Lanes 2-4 show the extracted Type I collagen using various substrates, including 25 mM Tris-HCl buffer (pH 8.0), 2% SDS, 2% dithiothreitol, 20% glycerol, and 0.02% bromophenol blue. The SDS-PAGE reveals three distinct bands characteristic of Type I collagen, corresponding to its structural components. These include the monomeric α -1 and α -2 chains and the typical collagen secondary structure motifs: the β -band and γ -band. The α chains are observed at approximately 100-130 kDa, while the β -band is around 250 kDa

Experimental factors affecting the extraction of collagen

Table 1 presents the results of a Taguchi method experiment examining four factors influencing collagen extraction. The Delta values indicate the difference between the highest and lowest responses for each factor, while the Rank reflects the relative importance of each factor based on its impact on collagen yield.

Acid concentration: This factor has the most significant effect, with the highest Delta value of 17.7105. Collagen yield decreases sharply as acid concentration increases, with the highest yield achieved at Level 1 (lower concentration). Higher acid concentrations reduce extraction efficiency.

Acid volume: This factor has a moderate effect, with the highest collagen yield observed at Level 1 (100 mL). Increasing the acid volume beyond this point (Levels 2 and 3) does not significantly improve yield, likely due to solution saturation, where additional acid volume provides no further benefit.

Soaking time: This has a smaller impact compared to acid concentration and buffer volume. The optimal soaking time is Level 2 (60 minutes), offering a slight improvement over Level 1 (30

minutes). However, prolonged soaking at Level 3 (120 minutes) reduces yield, likely due to collagen degradation caused by extended exposure to acidic conditions.

Buffer volume: This factor has the second-largest impact on collagen yield. The highest yield is observed at Level 3 (10 mL), indicating that larger buffer volumes enhance extraction efficiency.

This is likely due to pH stabilization during the process, which protects collagen from degradation.

Table 1: Ranking of extraction parameters

Level	Conc of acid	Volume of acidic acid (mL)	Time of soaking (minutes)	Buffer (mL)
1	18.9100	8.1615	5.1312	1.5647
2	4.0194	0.0000	6.3310	4.5850
3	1.1995	0.0000	0.3480	13.7750
Delta	17.7105	8.1615	5.9830	12.2103
Rank	1	3	4	2

Response modelling.

The mathematical relationships between the responses and the factors studied are represented by equations provided in Table 2. The model accounts for a significant portion of the data variance ($R^2 = 73.84\%$) and indicates acceptable multicollinearity levels among the variables (based on VIF values). The concentration of acetic acid and buffer volume emerge as the most influential factors affecting collagen yield. Specifically, acetic acid concentration has a negative impact on yield (-1.504), while buffer volume positively influences it (1.152). The volume of acetic acid moderately reduces yield (-0.0133), whereas soaking time shows an insignificant effect within the tested range (0.0127). These results align with prior research emphasizing the importance of optimizing acid concentration and ensuring buffer stability to maximize collagen extraction efficiency.

Table 2: Response modelling

Response	The obtain model	Factors	Coef	SE Coef	R2	T-value	P-value	VIF
Yield (%)	6,17 - 1,504 Concentration of acetic acid - 0,01330 Volume of acetic acid (ml) + 0,0127 Time of soaking fish + 1,152 Buffer (mL)	Constant	6.17	3.88	73.84	1.59	0.122	
		Conc of acetic acid	-1.504	0.425		-3.54	0.001	1.41
		Volume of acetic acid	-0.01330	0.00619		-2.15	0.039	1.61
		Time of soaking	0.0127	0.0260		0.49	0.629	1.18
		Buffer	1.152	0.330		3.49	0.001	2.22