ISOLATION AND CHARACTERIZATION OF CASSAVA FIBRE FOR
TISSUE ENGINEERING SCAFFOLD APPLICATION

BY

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DECLARATION

I, Emmanuel Diabor, do hereby declare that except for the references which have been duly cited, the entire work presented in this thesis, titled “Isolation and characterization of cassava fibre for tissue engineering scaffold application” was solely conducted and written by me, and that, this thesis has never been presented either in part or in whole for any degree in this University or elsewhere.

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This thesis has been submitted for examination with our approval as supervisors.

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ABSTRACT

Cassava bagasse and its extracted cellulose fibres have seen frequent application mostly in the packaging industry as reinforcement material in plastic composites development. However, the material properties such as the mechanical properties of the single elementary cassava cellulose fibres have not been examined and reviewed literature does not show its potential use in the development of tissue engineering scaffolds for cell culture. The study, therefore, characterized the mechanical properties, physicochemical, morphological and microstructural characteristics and thermal degradation profiles of single elementary cellulose fibre as well as the central vascular fibre (“thick-core fibre) isolated from three genotypes of cassava (tagged in this study as ID4, ID6 and AF). Additionally, the study examined the effect of incorporating cassava cellulose microfibres as reinforcement on the mechanical properties and microstructure characteristics of three-dimensional gelatin scaffolds. Non-treated isolated cassava fibres were tested according to ASTM C1557. Three-dimensional cassava microfibre/gelatin scaffolds with different fibre weight fractions were fabricated using phase separation and freeze-drying methods. Tensile test results showed that there was no significant difference (p > 0.05) in mechanical properties recorded between the single elementary fibre and vascular fibre (thick-core) for the three cassava genotypes. Different genotypes of cassava fibre showed significant differences (p < 0.05) in tensile strength and Young’s modulus, with ID4 fibre recording the highest average tensile strength of 7.567 ± 3.844 MPa and highest elastic modulus of 336.485 ±130.803 MPa. XRD analysis showed similar diffraction pattern with minimal variation in signal intensities for both single and thick-core fibres for all cassava genotypes suggesting nonsignificant differences in crystalline structure between them. TGA analysis showed that cassava fibre is thermally stable between the
temperatures of 100 °C – 200 °C. The cassava cellulose microfibre/gelatin scaffolds fabricated showed rough surfaces compared to pure gelatin scaffolds and were highly porous with surface porosity ranging between 84 and 90%, and had interconnected pores of average size 36 ±12 µm. Gelatin scaffolds containing up to 7% cassava cellulose microfibre load recorded a maximum compressive strength of 0.29±0.02 MPa, about eight (8) times higher than that for the pure gelatin scaffolds and average Young’s modulus of 1.31 ±0.03 MPa, about four times higher than pure gelatin scaffolds. Preliminary theoretical modelling using Halpin-Tsai model could accurately explain the variabilities in the compression modulus of the gelatin composite scaffolds. In all, the results showed that cassava fibre has considerable mechanical strength and stiffness and can be used as reinforcement filler to improve the mechanical integrity of tissue engineering polymer scaffolds. The cassava fibre/gelatin scaffolds showed surface architecture that could improve cell–matrix adhesion and efficient cell seeding and diffusion of nutrients during cell culture.
DEDICATION

I dedicate this thesis to the Almighty God, my family and my mentors.
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I am most grateful to God for his strength and wisdom throughout my graduate study. My deepest gratitude goes to my supervisors, Dr. Elsie Kaufmann (University of Ghana, Legon) and Prof. Paul Funkenbusch (University of Rochester, USA) for their invaluable support, encouragement, mentorship, and making time to carefully read through my thesis countless times for errors, and their insightful contributions to finalize my thesis.

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<tbody>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>XRD</td>
<td>X-Ray Diffraction</td>
</tr>
<tr>
<td>MPa</td>
<td>Mega Pascal</td>
</tr>
<tr>
<td>GPa</td>
<td>Giga Pascal</td>
</tr>
<tr>
<td>KPa</td>
<td>Kilo Pascal</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>Cr.I</td>
<td>Crystallinity Index</td>
</tr>
<tr>
<td>K-S</td>
<td>Kolmogorov-Smirnov</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>MATLAB</td>
<td>Matrix Laboratory</td>
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CHAPTER ONE

INTRODUCTION

1.0 Background

Cassava, biologically known as *Manihot esculenta* Crantz, is a fibrous root crop that grows very well on marginal lands. It is known to be one of the major popular and cheap staple foods in Ghana (IFAD, 2005 and Ennin et al., 2009). Many people living in the tropical areas of Africa, Latin America and Asia consider cassava as the third most significant source of food after cereal crops (Food FAO Stat, 2009). World production of cassava in 2014 was estimated at 291.3 million tonnes, of which 48% (138.9 million tonnes) is produced in sub-Saharan Africa (Food FAO Outlook, 2014). Ghana is the sixth largest producer of cassava globally and third in Africa, after Nigeria and the Democratic Republic of Congo with an annual production of nearly 14.755 million tonnes, representing about 10.63 % of the total cassava production in Sub-Saharan Africa (Food FAO Outlook, 2014). Studies conducted by Ennin et al. (2009) indicated that cassava production in Ghana contributed substantially (22 percent) to Ghana’s Agricultural Gross Domestic Product (AGDP). Most Ghanaians use cassava tuber to produce different kinds of traditional foods such as “fufu” (pounded cassava), “kokonte” (dried chips), “gari” (roasted fermented cassava), and other forms of use including flour and liquid paste. Cassava peels serve as food for livestock, such as goats and sheep, and the leaves contain bioactive compounds and proteins, which makes them potentially suitable for medical use.

Cassava root tuber is mainly a starch-rich material (containing more than 80% starch (dry wt.)), and contains proteins, lipids, lignocellulosic fibres, sugars, vitamin C, carotenoids, and minerals (Tonukari, 2004; Sapuan and Jawaid, 2015). The central
portion of the tuber contains xylem bundle (also known as central vascular fibre) (Egbeocha et al., 2016) that is usually thick and strong and will be referred to in this study as a “thick-core fibre”. Different bio-products of cassava have found widespread industrial application based on their desired properties which includes their low cost, availability, thermoplasticity, biodegradability, and good mechanical properties (Sapuan and Jawaid, 2015). For instance, in the food industry, cassava starch is used as a thickening agent in foods that are not subject to rigorous processing conditions (Nyerhovwo, 2004). The textile industry uses cassava starch in dyeing and sizing to increase the brightness and weight of cloth (Nyerhovwo, 2004). Cassava starch serves as the major raw material in the glue and adhesive industries. Cassava starch and flour have also found diverse application in the development of starch-based biodegradable polymer blends (Lu et al., 2009) and bio-composites (Kim et al., 2011) and also used in the fabrication of tissue scaffolds (Sunthornvarabhas et al., 2011).

Cassava bagasse is a solid residue, which is usually produced in the industry after processing of cassava starch and soluble sugars. Although, the biological source of the cassava and the processing method can determine the composition of the bagasse, research indicates that cassava bagasse is mainly composed of moisture (5-11 %), residual starch (40-60 %), lignocellulose fibre (15-50%) of the total solid residue (g/100g dry weight basis), as well as small amounts of lipids and proteins (Pandey et al., 2000 and Matsui et al., 2004). This solid residue is usually left to go waste and eventually causes environmental pollution (Teixeira et al., 2009; Hermiati et al., 2012). However, cassava bagasse and its extracted cellulose fibres have seen application, mostly in the packaging industry, as reinforcement materials for biodegradable plastics and disposables for home use (Sriroth and Sangseethong, 2006) as well as reinforcing
nanofibrils or fillers in thermoplastic starch matrices, to improve the mechanical strength and hydrophobic character of the matrix (Teixeira et al., 2009). Work done by Pasquini et al. (2010) shows that the inclusion of extracted cassava cellulose whiskers from cassava bagasse as fillers in nanocomposite films alongside a natural rubber matrix led to a considerable improvement in the dynamic storage modulus. Wicaksono et al. (2013) applied cellulose nanofibres from cassava bagasse as reinforcement fillers to tapioca film to improve its mechanical properties. Edhirej et al. (2017) investigated the influence of cassava bagasse reinforcement on the physical, thermal, mechanical and structural properties of thermoplastic cassava starch films. They found that the modulus and maximum tensile strength of composite films that contained bigger size and a higher concentration of cassava bagasse were increased from 69.03 to 581.68 MPa and 4.7 to 10.78 MPa respectively. These studies have demonstrated how the incorporation of cassava bagasse and its isolated nanocellulose fibres as reinforcement material improved the mechanical properties of plastic composites. However, none of them examined the mechanical properties of the single elementary cassava cellulose fibres. This lack of detailed characterization may be limiting consideration of cassava fibre for use in more specialized and demanding applications, such as using cassava fibre as reinforcement material to improve the mechanical properties of tissue engineering scaffolds. Limited research has been done on cassava fibre in Ghana and around the globe in relation to its possible biomedical applications. Larbie et al. (2012) performed a preliminary cytotoxicity test and immersion studies of de-starched cassava fibres in simulated body fluid. Their results showed that small concentrations of cassava fibre granules and powder did not significantly (p<0.05) induce the discharge of high levels of Lactate Dehydrogenase (LDH) when compared to the positive control (Labie et al., 2012). They concluded based on their preliminary results that cassava fibre had
no significant cytotoxic effect on human peripheral blood mononuclear cells (PBMC). This demonstrates that further investigation on the material properties of the cassava fibres will be significant concerning its application for biomedical engineering research.

Tissue engineering (TE) is an emerging field that applies principles and knowledge of engineering and life sciences towards the development of biological substitutes that restores, maintain, or enhance tissue function (Vacanti and Lager, 1999; Huang & Ingber, 2000). One major component in TE is the use of polymeric scaffolds from natural and synthetic biomaterials to serve as support for cells to promote cellular activity and tissue regeneration (Asghari et al., 2016). One of the critical requirements for TE scaffolds is to have sufficient mechanical properties comparable to the native tissue which it is intended to regenerate to prevent possible implant failure and improve scaffold implant stability (Loh and Choong, 2013). Recent research outcomes have shown that mechanical properties, such as strength, stiffness and elasticity, are critical factors that highly affect the ability of cell adhesion, proliferation and differentiation (Courtney et al., 2006; Zhu et al., 2011; Grover et al., 2012).

Gelatin polymer is a degenerated collagen which has been employed widely as a biomaterial for scaffolds in the tissue engineering field (Asghari et al., 2017; Pei et al., 2017). Gelatin was used as a polymer matrix in the development of tissue engineering three-dimensional composite scaffolds for soft tissue engineering due to its desirable properties such as biodegradability, biocompatibility, low cost and non-immunogenic properties (Zhao et al., 2006; Xing et al 2010). However, gelatin hydrogel has a poor mechanical stability which undermines the characteristics needed for tissue engineering scaffolds (Xing et al., 2013). Therefore, the use of cellulose fibre-reinforced composite
scaffolds has recently become an interesting target for biomedical engineers and biomaterials researchers for TE applications. This is because natural cellulose fibres possess interesting properties such as, availability of reactive surfaces for protein binding, good mechanical integrity, potential biocompatibility and biodegradability (Entcheva et al., 2004). Cellulose has densely-packed glucan chain structure which provides them the considerable mechanical strength to support cellular structures (Ko and Iwata, 2001) and its potential to be removed from a culture substrate when no longer required has also been shown (Ko and Iwata, 2001). Xing et al. (2009) showed that the use of hard wood cellulose fibres improved the mechanical properties of gelatin scaffolds for soft tissue engineering. Therefore, to explore and develop other sources of cellulose fibre such as cassava fibre as a potential biomaterial for use, for instance in the development of tissue engineering scaffolds, the material properties of the individual cassava fibres should be determined. Thus, this research characterized the material properties (mechanical properties, physicochemical, morphological and microstructural characteristics and thermal degradation behaviour) of both isolated single elementary and thick-core cassava fibres of different genotypes and additionally, used cassava microfibres as reinforcement material combined with cross-linked gelatin to improve the mechanical properties of porous gelatin scaffolds for possible tissue engineering application.

1.1 Problem statement

The industrial exploitation of cassava starch yields larger quantities of cassava bagasse (solid residue) which contains considerable amount of starch and lignocellulosic fibres. Cassava bagasse and its extracted cellulose fibres have gained significant attention by many material science researchers due to its desirable properties such as low cost,
availability, low density and good mechanical properties (Célino et al., 2014). Majority of these researchers (Teixeira et al., 2009; Pasquini et al., 2010; Aripin et al., 2013; Farias et al., 2014) have applied cassava bagasse and its extracted cellulose fibres as reinforcement material in the development of plastic composites and disposables in the paper and plastics packaging industries to improve the mechanical properties of the composites, however, none of them examined the mechanical properties of the cassava cellulose elementary fibre and reviewed works of literature also do not show its potential application in the development of tissue engineering scaffold for cell culture. The lack of this essential information, regarding the mechanical properties of individual cassava cellulose elementary fibres could deter many biomedical and biomaterial engineers from considering cassava fibres as a potential biomaterial for possible biomedical application during the evaluation of new materials at the research and development levels.

1.2 Significance of study

Biomedical Engineering in Ghana and Africa is taking a good shape, and further research in local materials is expected to contribute significantly to the overall growth of the field. Characterization of various properties such as the structural, microstructure and morphological, mechanical and biocompatibility properties of novel biomaterials are very critical before their application. This essential information is not only useful for evaluation of new biomaterials at the research and development levels, but also it allows biomedical engineers to develop mathematical models and computer simulations to predict the behaviour of the biomaterial before in vivo application. The findings of this study provide basic scientific knowledge regarding the mechanical, thermal degradation, physicochemical, morphological and microstructural
characteristics of cassava fibre from different cassava genotypes and evaluate its potential application in tissue engineering scaffold development. The study promotes cassava fibre as a potential biomaterial that can be applied in different research areas in biomedical engineering, such as tissue engineering scaffold applications, drug delivery carriers, and hydrogels. Therefore, the material property characterization and development of cassava fibre as a potential reinforcement biomaterial for tissue engineering scaffold application not only add high value to the material, but promote the production of cassava and, also helps prevent environmental pollution.

1.3 Research questions

1. What are the material properties of cassava fibre?
2. Do different genotypes of cassava yield fibres with different properties?
3. Are there any microstructural and morphological differences between the single elementary and ‘thick-core cassava fibres’?
4. What is the effect of incorporating cassava fibres in a polymer composite scaffold?

1.4 Hypothesis

(a) Different botanical sources of cassava would yield different fibres with different properties.
(b) Different amount of cassava fibres in gelatin composite scaffolds will have significant effect on the mechanical properties of the scaffold.
1.5 Aim and objectives of the study

This research aims to develop cassava fibre as a potential biomaterial for possible biomedical applications, for example in the fabrication of 3D polymer composite (cassava fibre/gelatin) scaffolds for cellular activity and tissue regeneration.

1.5.1 The objectives of the study are as follows:

a) Compare the mechanical properties of cassava fibres of different genotypes by determining tensile strength, stiffness and elongation to failure.

b) Investigate the crystallinity and thermal degradation characteristics of cassava fibres of different genotypes.

c) Fabricate 3-D gelatin-cassava fibre composite scaffolds using the cassava genotype with higher mechanical properties as a reinforcement biomaterial.

d) Examine the morphological and surface architecture (thickness, density, porosity, and pore interconnectivity) of cassava fibre-gelatin composite scaffolds.

e) Determine the effect of incorporating different cassava microfibre loads on the mechanical, morphological and microstructural characteristics of cassava fibre-gelatin composites.

1.6 Organisation of the Dissertation

Chapter one introduces the background of the study and highlights the knowledge gaps in some related literature available in the research area. Chapter two is focused on the review of literature on work related to the current study. Chapter three presents the materials and methods used for the research. Various research techniques employed for
the characterization of the material properties of cassava fibres and scaffold developments were described and explained. Chapter four presents the results and discussions of the research findings. Finally, Chapter five summarizes the most relevant outcome of the work.
CHAPTER TWO

LITERATURE REVIEW

2.0 Cassava production and utilization

Cassava, biologically known as *Manihot esculenta* Crantz is a staple root crop that thrives well in poor lands during both favourable and unpredictable rainfall periods. In some countries, it is known as manioc, tapioca or yucca and popularly known in Ghana as “bankye”. Cassava is reported to be one of the most important staple food crops in developing countries (FAO, 2013). The demand for cassava production has increased steadily over the years, especially across tropical countries. According to the United Nations FAO Food Outlook, (2014) report on global food markets, global cassava production in the year 2012 was estimated at 268.4 million tonnes, followed by 278.6 million tonnes (2013) and 291.3 million tonnes in 2014. The report made a vivid observation, indicating this upward trajectory because of sustained growth in Africa and Asia where the demand for value-added food products and industrial application of cassava in the form of ethanol and starch are on the rise respectively (FAO, 2013). Much of this production growth is centred in sub-Saharan Africa, accounting for about 167 million tonnes (57%) in 2014.

Ghana was recognised as the third largest producer of cassava in Africa, contributing an output of 14.755 million tonnes out of the 167 million (FAO Food Outlook, 2014). This makes cassava the most important agricultural commodity in Ghana, as it contributed a substantial 22% to Ghana’s Agricultural Gross Domestic Product (AGDP) (Ennin et al., 2009).
Cassava plant is mainly made up of leaves, the stem and the root tuber. The leaves of cassava plant are spirally arranged on the stem and are usually dark green and reddish with various shades of purple pigmentation occurring in the foliage (IITA 1990). The root consists of adventitious roots which absorb water and nutrients from the soil (IITA, 1990) for the plant. The secondary thickening of the adventitious roots further develops radially around the base of the plant into the fibrous root tubers (IITA, 1990) forming five to eight tubers per plant. The tuber is the main storage region of carbohydrate in the form of starch. The cross-section of a cassava root tuber is made of three major regions (Fig 1).

Figure 1: Cross-sectional view of cassava root tuber (a) (Egbeocha et al., 2016); (b) (Sriherwanto, 2010)

The first region is called the periderm (outer skin or bark) which serves as the outermost layer of the root tuber and comes in different shades of colour from pink to grey (Egbeocha et al., 2016). The second region is known as the cortex which lies beneath the periderm, and is usually about 1.5-2.5 mm thick and white in colour (Egbeocha et al., 2016). The third region is the central portion of the root tuber, which also constitutes
the greater bulk of the tuber serving essentially as storage of starch and is mostly in white. The xylem bundle also called central vascular fibre forms part of the central region and is normally thick and strong. This central vascular fibre is referred to in this study as the ‘thick-core fibre’.

2.1 Traditional uses of cassava in Ghana

Cassava root tuber has a wide range of usage based on its processed products. According to the Ghana Statistics, Research and Information Directorate (2013), about 30% of cassava produced in Ghana is consumed by the farmers themselves, and the rest is sold at the local markets for human consumption and industrial applications. Cassava is estimated to contribute about 30% of the daily intake of calories in Ghana (Odedina and Adebayo, 2012). Cassava is usually sliced into pieces and processed into different traditional products. The methods of processing may involve fermentation, boiling, drying, and milling, and extraction of starch content. Some popular traditional foods made from cassava include “gari” (roasted fermented cassava), “fufu” (pounded cassava), “kokonte” (milled dried chips) (Apea-Bah et al., 2011; Duah et al., 2016).

2.2 Industrial application of cassava and its bioproducts

The industrial application of cassava root tuber and its bioproducts in different fields has increased due to the several reasons such as low cost, availability, recyclability, and biodegradable by-products (Sapuan and Jawaid, 2015). Different bioproducts of cassava such as starch, flour, and bagasse (by-product), have seen frequent industrial use. One viable industrial application of cassava root is the exploitation of the starch, which comprises the main component of the roots (Ceballos et al., 2006). Many tropical countries are into the extraction of starch from cassava including Ghana’s Ayensu
Starch Company Limited (ASCo) at Bawjiase in the Central Region. Cassava starch is usually extracted from the tubers to serve as the raw material for producing glue and adhesives with good affinity to polar substrates like wood and paper materials in the paper and adhesive industry (Nyerhovwo, 2004). Starch is also used in the food industry as a thickening agent and applied in the textile industry for dyeing and sizing of clothes to increase their weight and brightness (Nyerhovwo, 2004). Cassava starch has also been applied in the development of starch-based biodegradable polymer blends (Lu et al., 2009) and the development of tissue scaffolds (Sunthornvarabhas et al., 2011). Cassava flour bioproducts are used in the food and beverage industry for the manufacturing of biscuits, noodles, pastries and alcoholic drinks (Erikson, 2013), and also applied in bio-composites (Kim et al., 2011).

2.3 Cassava bagasse and its constituents

2.3.1 Cassava bagasse is fibrous solid residue which is produced from the industrial extraction of cassava starch and soluble sugars. Cassava bagasse contains no cyanide and its main constituents may vary depending on the crop variety and the varying methods of processing. However, studies have shown that cassava bagasse is made of (5-11 %) moisture, (40-60 %) residual starch and (15-50 %) fibre of the total solid residue (g/100g dry weight basis), and small amounts of lipids and proteins (Pandey et al., 2000). The high moisture content of cassava bagasse makes it challenging to store and transport, therefore, it is usually discharged into the environment to degrade. However, effluents from the bagasse and the degradation by-products pollute the air with a bad odour and contaminate nearby water bodies (Teixeira et al., 2009; Sapuan and Jawaid, 2015; Hermiati et al., 2012).
2.3.2 Cassava cellulose fibre

One of the major components from the cassava bagasse is the natural cellulose fibre, also found in higher plants such as jute, sisal and cotton. It has also been found that some bacteria including Acetobacter xylinum are capable of synthesizing microbial cellulose (Sannino et al., 2009). Cellulose fibre is the most abundant and renewable organic polymer in nature, usually found as the major component in plant cell walls. Plant natural cellulose fibres exist in a composite form because they consist of cellulose microfibrils arranged parallel to each other and cemented in an amorphous matrix of lignin and hemicellulose (Célino et al., 2014). Cellulose, hemicellulose and lignin molecules are bonded together by hydrogen bonds within the fibre bundle. However, chemical bonding is also found to exist between hemicellulose and lignin molecules. Lignin is considered as the adhesive component that holds cellulose and hemicelluloses together (Célino et al., 2014).

2.4 Cellulose fibre: chemical composition and structural organisation

Cellulose is an organic polymer consisting of a linear D-anhydroglucopyranose monomer units joined covalently by β-1,4-glycosidic bonds as shown in Figure 2.2 below. Cellulose is known to be insoluble in dilute acidic/alkaline solutions at normal temperatures due to the concentration of hydroxyls and its hydrophilic nature (Gurunathan et al., 2015). The β-1,4-glycosidic linkages make cellulose more resistive to chemical or enzymatic attack (Aravamudhan et al., 2014). Individual cellulose macromolecules are coupled with hydrogen bonds due to the presence of extensive –OH groups throughout the cellulose chain. These groups consequently give the assembly a unique physical property and the ability to form crystalline structures, forming the basic supramolecular structure of the cellulose microfibrils and the
cellulose fibres (Célino et al., 2014; Aravamudhan et al., 2014). Cellulose fibres have a high tensile strength and are responsible for the mechanical integrity of plants and are therefore considered as the main structural component in plants and natural fibres (Célino et al., 2014).

Figure 2: Chemical structure of cellulose [adopted from Gurunathan et al. (2015)]

The structure and content of cellulose may vary depending on the botanical source. However, cellulose content in plant cell walls usually accounts for 35–50 % of dry weight except for cotton which is made up of pure cellulose (100%) (Célino et al., 2014). Supramolecular structure studies have also shown that natural cellulose fibres exhibit crystalline and amorphous phases intertwined to form the cellulose. The amorphous phase is mainly characterized by the hydroxyl groups on glucose units as detected by X-ray diffraction (Zhang et al., 2008). The crystalline phase also contains large numbers of hydroxyl groups which form many hydrogen bonds owing to a vast network that directly contributes to the compact nature of the cellulose crystal structure.

The chemical compositions of cassava bagasse and peels on a dry basis have been studied by different researchers around the globe. Leite et al. (2017) characterized the chemical properties of cassava bagasse and peels. They found that “the chemical composition of cassava bagasse and peelings, on dry basis, was as follows: 6.7 (±0.6) and 14.8% (±0.8) cellulose, 2.0 and 12.8% total lignin, and 89.9 and 50.3% of
polysaccharides (mainly starch + hemicellulose), respectively”. Aripin et al. (2013) also studied the chemical properties of cassava peels and reported that the cellulose content in cassava peels was found to be 37.9%, hemicellulose 37.0%, holocellulose 66% and lignin 7.5%, respectively. It must be noted that these variations of cellulose content in the cassava peels and bagasse could be due to the crop variety differences and the method of isolation. Plants with high cellulose content and high crystalline regions usually have good mechanical properties because the cellulose microfibrils form the basic structural unit of the plant cell wall by orienting themselves at an angle close to the fibre axis (Célino et al., 2014; Bourmaud et al., 2013).

2.5 Microstructure cassava cellulose fibre

The internal microstructure of elementary cellulose fibre is made of different layers. The outermost layer of the plant, known as the primary cell wall, is made up of a randomly organized network of cellulose microfibrils connected to an amorphous phase of hemicellulose and lignin, which act as a matrix for the cellulose bundles.

![Figure 3: Internal microstructure of an elementary natural fibre](http://ugspace.ug.edu.gh)
Within the primary cell wall are the three inner secondary wall layers (Secondary wall L1, Secondary wall L2 and Secondary wall L3). The middle, secondary wall L2 contains most of the cell materials, and it is found to be the thickest. Crystalline cellulose microfibrils are helically arranged about the long axis of the elementary fibre within the secondary wall L2. The microfibrillar angle between each wall layer is different and it is known to contribute significantly to the mechanical properties of fibre, as well as plants with a higher relative amount of cellulose content and a high degree of crystalline polymerization (Azwa et al., 2013).

2.6 Relevant literature on the industrial application of cassava fibre and the knowledge gap

The production of cellulose fibres from different sources and their application as reinforcement in polymer composite materials have become a target for many engineers due to the desirable properties of the fibres including low cost, availability, low density, high-specific mechanical properties, and image of environmental friendliness (Célino et al., 2014). In fibre reinforced composites, the addition of fibres as reinforcement fillers improves the stiffness and strength of the structure, while the plastic matrix serves as the adhesive to bind the fibres and transfers load to the fibres (Thomas et al., 2011).

Cassava bagasse and its isolated cellulose fibres have also been explored further in different fields, especially in the plastics industry. Teixeira et al. (2009) isolated cassava nanofibrils from cassava bagasse and applied them as reinforcing fillers in a thermoplastic cassava starch matrix, to improve the mechanical properties and the hydrophobic character of starch-based plastic. Aripin et al. (2013) also studied the
possibility of using cassava peels as an alternative fibre in the pulp and paper industry. They aimed to examine the surface morphology of cassava peels and chemically characterize them for the papermaking industry. Wicaksono et al. (2013) incorporated cellulose nanofibres from cassava bagasse as reinforcement fillers in tapioca films to improve the mechanical properties of the plastic film. Work done by Pasquini et al. (2010) showed that the incorporation of extracted cassava cellulose whiskers from cassava bagasse as fillers in nanocomposite rubber films and thermoplastic starch matrices led to a considerable improvement in the storage tensile modulus and also resulted in a decrease of the hydrophilic character for glycerol plasticized films. Farias et al. (2014) characterized a cassava bagasse-polyethylene composite and cassava bagasse. Farias and co-workers reported on the findings of the morphological and physicochemical properties of the cassava bagasse and concluded that the incorporation of cassava bagasse in a low-density polyethylene (LDP) matrix increased the elastic modulus. These previous studies conducted in the past examined the properties of the composite formed with cassava bagasse and cellulose whiskers. Recently, Edhirej et al. (2017) published their findings from their studies on characterization of cassava bagasse reinforced thermoplastic cassava starch. Edhirej and coworkers investigated the influence of the bagasse on the physical, thermal, tensile and structural properties of the composite films. Their findings indicated that composite films with different cassava bagasse size and higher percentage increased the modulus and maximum tensile strength. Although the use of cassava fibre in plastic composite developments resulted in some improvement of the mechanical properties of the composites, the mechanical properties of the single elementary cassava cellulose fibres have not yet been determined. Table 1 below shows the mechanical properties of most of the natural fibres that have found widespread industrial applications, excluding cassava fibre.
Therefore, there is the need to characterize the material properties such as the mechanical properties of the individual elementary cassava cellulose fibre and evaluate its potential application in other industrial fields, such as biomedical engineering.

### Table 1: Mechanical properties of different fibres [Adopted from Célino et al., 2014 and Tan et al., 2015]

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Density (g/cm³)</th>
<th>Young’s modulus (GPa)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flax</td>
<td>1.54</td>
<td>27.5-85</td>
<td>345-2000</td>
<td>1-4</td>
</tr>
<tr>
<td>Ramie</td>
<td>1.5-1.56</td>
<td>27-128</td>
<td>400-1000</td>
<td>1.2-3.8</td>
</tr>
<tr>
<td>Hemp</td>
<td>1.47</td>
<td>17-70</td>
<td>368-800</td>
<td>1.6</td>
</tr>
<tr>
<td>Jute</td>
<td>1.44</td>
<td>10-30</td>
<td>393-773</td>
<td>1.5-1.8</td>
</tr>
<tr>
<td>Sisal</td>
<td>1.45-1.5</td>
<td>9-22</td>
<td>350-700</td>
<td>2-7</td>
</tr>
<tr>
<td>Coconut</td>
<td>1.15</td>
<td>4-6</td>
<td>131-175</td>
<td>15-40</td>
</tr>
<tr>
<td>Cotton</td>
<td>1.5-1.6</td>
<td>5.6-12.6</td>
<td>287-597</td>
<td>7-8</td>
</tr>
<tr>
<td>Kenaf</td>
<td>1.2</td>
<td>14-53</td>
<td>240-930</td>
<td>1.6</td>
</tr>
<tr>
<td>Bamboo</td>
<td>0.6-1.1</td>
<td>11-17</td>
<td>140-230</td>
<td>-</td>
</tr>
</tbody>
</table>

### 2.7 Tissue Engineering

Tissue engineering (TE) is an interdisciplinary field that has evolved rapidly within the health sciences. Tissue engineering is a field that involves a systematic application of the principles and knowledge of engineering and life sciences toward the development of biological substitutes that restore, maintain, or enhance tissue function (Vacanti and Langer, 1993). TE in recent times has been defined as the specialized area of research which adopts the scientific knowledge of chemistry, materials science, engineering, and
medicine with the aim to repair and replace tissues and organs (Cui et al., 2010; Walmsley et al., 2015). In the U. S, tissue engineering has contributed significantly to the development of artificial organ therapy (Khademhosseini et al., 2009). The achievement has been linked to the significant contribution of the knowledge base and understanding in cell and molecular biology (e.g., the isolation and manipulation of cells, genes, and growth factors), development of novel biomaterials and integration of biology to deliver viable cells or grafts in compatible support structures (Butler et al., 2017). Tissue engineering process involves the combination of cells, scaffold, and bioactive cues to regenerate or fabricate a new functional tissue to replace damaged tissue (Flanagan et al., 2006).

2.8 Components in tissue engineering and strategies

Tissue engineering involves four major components; (i) isolated cells (differentiated or undifferentiated), (ii) 3D polymeric scaffolds from natural or synthetic biomaterials which serve as substrate for cells to promote cell activity, tissue production and transplantation (iii) biological signalling molecules such as growth factors and proteins and (iv) a bioreactor, that provides a biological active environment for cell expansion and differentiation (El-Sherbiny and Yacoub, 2013; Asghari et al., 2017). These four components come into play during the tissue engineering process.

Tissue engineering strategy involves two major approaches, namely cell-based and matrix based. During the cell-based approach, isolated cells are expanded in suitable media in vitro and implanted directly into the damaged sites for tissue regeneration. However, with respect to matrix-based approach, the expanded cells are seeded on 3D matrices or porous scaffolds for the cells to proliferate and differentiate into the desired
lineage after which the graft is implanted into the damaged anatomical site for tissue restore or repair. Figure 4 below is a schematic diagram of tissue engineering strategies.

Figure 4: Schematic illustration of tissue engineering strategies [adopted from El-Sherbiny and Yacoub, 2013]

In the matrix-based tissue engineering approach, the design of three-dimensional scaffolds has become one of the fundamental tools to guide tissue formation in vitro and in vivo (Subia et al., 2010). Therefore, the development of novel (polymeric) scaffolds or matrices with the required characteristics is key in this field.

2.9 Scaffold design requirements

Scaffolds for tissue engineering applications have been designed and fabricated from a variety of biomaterials to regenerate different biological tissues in the body. There are key factors that are considered important when designing a scaffold for use in tissue
engineering for the regeneration of almost any kind of tissue type. Some of the factors which profoundly affect the outcome of tissue engineering process and tissue function considered in this study are as follows.

I. **Mechanical property:**

Mechanical characteristic of the polymeric scaffold is one of the major factors considered during tissue engineering applications (Hollister, 2005). Some mechanical properties that are paramount in biomedical applications are Young’s modulus, tensile strength, compressive strength, wear and creep resistance and dynamic stability (Hollister, 2005). Biomaterial scaffolds are required to have mechanical properties that are comparable to the native tissue of which it is intended to regenerate or the anatomical site at which it is being implanted. For instance, in the reconstruction of load-bearing tissues, such as bones, ligaments and cartilage, porous scaffolds with good tensile and compressive strength are required for the scaffold to retain its structure after the implantation of the graft (Yang et al., 2002; Parthasarathy and Sethuraman, 2014). Table 2 shows the mechanical properties of some human tissues. According to Hollister (2005), tissue engineering of hard tissues requires porous scaffolds with compression moduli in the range of 10-1500 MPa and that of soft tissues (0.4-350 MPa) and as such any designed scaffold that cannot provide a mechanical modulus within the above-mentioned range will cause the tissue formed from it to also fail *in vivo* due to excessive deformation. In most tissue engineering applications, proper integration and interfacing between the scaffold and the host tissue are required, and this is possible only if the mechanical integrity of the scaffold match that of the regenerated tissue (Parthasarathy and Sethuraman, 2014).
Table 2: Mechanical Properties of Human Tissues [source: Yang et al., 2002]

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tensile strength (MPa)</th>
<th>Compressive strength (MPa)</th>
<th>Young’s modulus (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical bone</td>
<td>60-160</td>
<td>130-180</td>
<td>3-30</td>
</tr>
<tr>
<td>Cartilage</td>
<td>3.7-10.5</td>
<td>N/A</td>
<td>0.7-15.3 (MPa)</td>
</tr>
<tr>
<td>Ligament</td>
<td>13-46</td>
<td>N/A</td>
<td>0.0065-0.541</td>
</tr>
<tr>
<td>Tendon</td>
<td>24-112</td>
<td>N/A</td>
<td>0.143-2.31</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>N/A</td>
<td>4-12</td>
<td>0.02-0.5</td>
</tr>
</tbody>
</table>

The lack of suitable scaffold or implant–host tissue integration (because of poor mechanical properties of the scaffold) usually leads to implant failure. Tissue scaffolds are also expected to be strong enough to maintain and sustain physiological loads and applied stresses introduced under bioreactor tissue culturing setup and during tissue regeneration in vivo. The surface elastic modulus is known to affect the interactions between cell and biomaterial scaffold (Parthasarathy and Sethuraman, 2014). According to a review by Sanz-Herrera and Reina-Romo (2011), the stiffness of the biomaterial scaffold tends to influence cell differentiation. For instance, Sanz-Herrera and Reina-Romo reported that undifferentiated mesenchymal stem cells differentiated into neuron-like cells when cultured on a less stiff polymeric scaffold, with a stiffness of the order of 1 kPa. However, when these same undifferentiated mesenchymal stem cells were cultured on a stiffer polymeric scaffold (stiffness in the range 10-20 kPa), they differentiated into myoblast-like cells. Therefore, mechanical characterization of biomaterials and polymeric scaffolds is necessary to determine the suitability of the biomaterials for their intended application in tissue engineering.

II. Scaffold architecture (porosity, pore size and pore interconnectivity):

The design of polymeric scaffolds with controlled architectural characteristic including
porosity, pore size and interconnected pore network plays a crucial role in tissue regeneration (Hollister, 2005). Scaffolds are required to be highly porous with an extensive interconnected open-pore network to serve as channels that allow for easy entry of cells within the construct (thereby improving cell seeding efficiency), and guide cell migration for tissue vascularization and formation of new tissue within the scaffolds (Hollister, 2005; Loh and Choong, 2013). Scaffolds with high porous surfaces and pore interconnectivity also provide an avenue for mechanical interlocking between the scaffold and surrounding tissue in vivo to enhance the mechanical integration and stability of the scaffold implant (Loh and Choong, 2013; Karageorgious and Kaplan, 2005). Highly porous scaffolds also increase the release of growth factors and biological cues to cells for cell growth. However, retention of their mechanical stability is often compromised (Loh and Choong, 2013; Hollister, 2005). Scaffolds with high porosity, interconnected pores and sufficient pore size also allow for the easy diffusion of nutrients and oxygen to cells and removal of metabolic waste products and degradation by-products from cells within the scaffold constructs (O’Brien 2011; Loh and Choong, 2013). Most cells usually have a high demand for oxygen and nutrients for their proliferation and tissue growth.

Martin et al. (2004) reported that oxygen demand is one of the major factors that limit cell proliferation, survival and tissue growth due to relatively low solubility, slow diffusion rate and high consumption rate of oxygen. Generally, the diffusional penetration depth of oxygen within native tissues is in the range of 100–200 µm (Muschler et al., 2004). The normal diffusion distances for tissues between capillary lumen and cell membrane ranges between 20–200 µm, therefore, cells within a scaffold construct with a diffusion distance of 200 µm above are unable to survive (Muschler et al., 2004). Also, it has been reported that different tissue architectures require different
scaffold pore sizes (Kim et al., 1998) and therefore, for any scaffold a critical range of pore sizes exist (Murphy et al., 2010). The average pore size within a designed tissue scaffold should be large enough to enable the migration of cells into the constructs for cell attachment. For instance, Table 3 shows some pore size requirements for some native tissues for efficient cellular penetration.

**Table 3: Pore size requirements of some native tissues**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Pore size requirement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>100-400 µm</td>
<td>Hutchmacher, 2001</td>
</tr>
<tr>
<td>Skin</td>
<td>20-125 µm</td>
<td>Yannas et al., 1989</td>
</tr>
<tr>
<td>Peripheral nerves</td>
<td>5-10 µm</td>
<td>Kim et al., 1998</td>
</tr>
<tr>
<td>Liver</td>
<td>45-150 µm</td>
<td>Kim et al., 1998</td>
</tr>
</tbody>
</table>

Cell behaviour has been experimentally determined to be affected by scaffold architecture. Ma et al. (2000) examined the effect of pore size in 3-D polyethylene terephthalate (PET) fibrous matrix on human trophoblast tissue development. Their experimental results indicated that low porosity (LP, porosity of 0.849 with average pore size of 30 µm in diameter) PET scaffolds recorded a higher initial trophoblast cell proliferation rate and metabolic activities than high porosity scaffolds (HP, porosity of 0.896, with average pore size of 39 µm in diameter) scaffolds. In addition, they observed that cells cultured in LP scaffolds spread across adjacent fibres more easily, which led to higher cell proliferation rate. Besides their work, Mandal and Kundu (2009) also studied the effect of 3D silk fibroin scaffolds on cell proliferation and migration of human foreskin fibroblasts. They demonstrated that pore sizes of 200 to 250 µm and porosity of approximately 86% enabled better cell proliferation. Hence it is imperative to characterize any novel biomaterial scaffold to determine the scaffold
surface morphological characteristics and architecture suitability for tissue engineering applications. Apart from the factors mentioned above, scaffolds are also required to be biodegradable with tuneable degradation rate at the same rate as the neo-tissue formation. This is to allow for the host cells to gradually replace the implanted scaffold constructs over time (Ma, 2004; O’Brien, 2011).

2.10 Three-dimensional scaffold fabrication techniques

In nature, cells and tissues in the body are organized into a three-dimensional architecture. Therefore, there are various techniques that have been employed for the fabrication of 3D porous scaffold to facilitate the growth of cells into three-dimensional space in tissue engineering. These fabrication methods are classified as conventional techniques (such as solvent casting and particulate-leaching (Xiang et al., 2006), gas foaming (Ikada., 2006), phase separation (Smith et al., 2006), freeze-drying (Whang et al., 1995; Xing et al., 2010), electrospinning (Ma et al., 2005)), and rapid prototyping methods also known as the solid freeform technique (such as selective laser sintering, 3D printing) (Leong et al., 2009; Loh and Chong 2013). Both conventional and rapid prototyping methods have their advantages and drawbacks, however, conventional techniques have enormous limitations when compared to the solid free form fabrication techniques. Phase separation and freeze-drying techniques have been commonly used in the tissue engineering field for the fabrication of three-dimensional porous scaffolds for different tissue engineering applications (Schoof et al., 2001; Mikos et al., 2004; Sachlos and Czernuszka, 2003; Hua et al., 2002; Smith et al., 2006; Xing et al., 2010). Table 4 shows some processing techniques employed in the fabrication of polymeric scaffolds for tissue engineering applications.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porogen leaching</td>
<td>Control over porosity and pore geometry</td>
<td>Inadequate pore size and pore interconnectivity</td>
<td>Mano et al., 2007</td>
</tr>
<tr>
<td>Solvent casting/particulate leaching</td>
<td>Control over porosity, pore size and crystallinity</td>
<td>Lack of mechanical strength, residual solvents and porogen material</td>
<td>Xiang et al., 2006</td>
</tr>
<tr>
<td>Gas foaming</td>
<td>Free of harsh organic solvents, control over porosity and pore size</td>
<td>Limited mechanical property and inadequate pore interconnectivity</td>
<td>Ikada., 2006</td>
</tr>
<tr>
<td>Melt moulding</td>
<td>Independent control over porosity and pore size</td>
<td>Required high temperature for non-amorphous polymer</td>
<td>Thompson et al., 1995</td>
</tr>
<tr>
<td>Fibre bonding</td>
<td>Easy process</td>
<td>Poor mechanical property, limited applications to other polymers</td>
<td>Mooney et al., 1996</td>
</tr>
<tr>
<td>Electrospinning</td>
<td>Control over porosity, pore size and fibre diameter</td>
<td>Limited mechanical property, decrease thickness with fibre</td>
<td>Liang et al., 2007</td>
</tr>
<tr>
<td>Phase separation</td>
<td>Enables the incorporation of bioactive agents, highly porous structures, and no decrease in the activity of the molecule</td>
<td>Difficult to control scaffold architecture precisely, problems with residual solvent and a limited range of pore sizes</td>
<td>Smith et al., 2006</td>
</tr>
<tr>
<td>Freeze-drying</td>
<td>Produce highly porous structures, high pore interconnectivity and does not require high temperature</td>
<td>Limited to small pore size and long duration time of processing</td>
<td>Mandal &amp; Kundu, 2008</td>
</tr>
</tbody>
</table>
2.10.1 Phase Separation

This technique has been explored by many researchers in the field to produce well interconnected porous structures as tissue engineering scaffolds (Ma, 2008; Olivas-Armendáriz et al., 2010; Martel-Estrada et al., 2010). In general, a phase separation process typically involves four basic stages:

(a) Preparation of polymer solution and placement in a mould

(b) Thermally-induced or non-solvent induced phase separation

(c) Fixing of the separated phase by quenching or gelation

(d) Removal of the solvent or diluent by freeze drying or solvent extraction to produce a microporous structure.

A homogenous polymer solution is first dissolved in a suitable solvent such as molten phenol or naphthalene. The polymer solution is then cast in a mould and induced to be separated into a polymer-rich phase and a polymer-lean phase under two different approaches, either by exposing the homogenous solution to another immiscible solvent or cooling the solution below a binodal solubility curve (Nam and Park; 1999). Lowering the temperature of the solution leads to liquid-liquid phase separation and quenching to form a two-phase solid. The immiscible solvent is then removed by freeze drying or sublimation to produce a porous scaffold (Nam and Park; 1999; Martínez-Pérez., 2011). The thermally-induced phase separation method usually uses thermal energy as a latent solvent to induce the separation of homogenous polymer solution into two or multi-phase system (Nam and Park; 1999; Carlos et al., 2011). Scaffolds produced by this technique are usually limited to those with small pore sizes of 1-20 µm in diameter. The advantage of the phase separation method is that, it can be easily combined with other fabrication technique, such as porogen leaching and rapid prototyping to fabricate scaffolds with controlled pore geometry (Smith et al., 2006)
since the technique gives porous structures with a limited range of pore sizes.

2.10.2 Freeze-drying method

The freeze-drying technique has been adopted in fabricating scaffolds from natural biomaterials because it is useful for biomaterials that can be dissolved in aqueous media (Wu et al., 2010). The freeze-drying technique is founded on the principles of sublimation of solvent, usually water, into the gas phase (Whang et al., 1995). A polymer is first dissolved in a suitable solvent such as distilled water; the solution is then cast into a mould and frozen at a specific low temperature. As the polymer emulsion gets frozen, ice crystals are formed which act as porogen templates by aggregating within the interstitial spaces of the polymer (Sachlos and Czernuszka, 2003). The ice crystals are further removed by lyophilization (freeze-drying) under high vacuum to produce a highly porous foam or sponge-like scaffold with interconnected pores (Whang et al., 1995; Mandal and Kundu, 2009; Subia et al., 2010). Freeze-drying method has been applied to both synthetic polymers such as (PLGA) and natural polymers (such as gelatin, silk, collagen) to produce highly porous scaffolds (Whang et al 1995; Wu et al., 2010; Whang et al 1995; Vepari & Kaplan, 2007, Loh and Choong 2013). Although scaffolds produced by this method are highly porous, they are limited to small pore sizes and long processing time (Boland et al., 2004). It has been reported that varying the freezing temperature, ratio of water to polymer solution and the viscosity of the emulsion influence the size of the ice crystals which act as porogens, to create varying pore sizes in the scaffold (Loh and Choong, 2013; Angulo and Sobral, 2016). Angulo and Sobral (2016) recently examined the effect of processing parameters and solid concentration on the microarchitecture and pore architecture of gelatin-chitosan scaffolds by a freeze-drying technique. Their results showed that
increasing the chitosan content increased the viscoelastic properties of the gelatin-based scaffold. However, the pore size of the scaffold was decreased when compared to pure gelatin scaffolds.

2.11 Biomaterial scaffolds for tissue engineering application

Tissue engineering scaffolds are three-dimensional (3D) porous polymeric structures that are intended to mimic micro-cellular environments for cellular activities such as proliferation, differentiation and growth of new tissues and organs. Scaffolds can be produced from natural polymers (such as collagen, gelatin, elastin, hydroxyapatite, alginate, cellulose, chitosan, hyaluronic acid, agarose, silk, and mixture of polymers obtained by decellularization of tissues), synthetic polymers (such as Polyglycolic acid (PGA), Polylactic acid (PLA), poly(Lactic-glycolic) acid or polyacrylonitrile-polyvinyl chloride, poly(ethylene glycol), PEG and pure biological molecules and other extracellular matrix (ECM) molecules (Khademhosseini et al., 2009; Asghari et al., 2017). The use of natural or synthetic polymers in the fabrication of 3D scaffolds has their advantages and disadvantages. For instance, natural polymers best mimic features of the native in-vivo extracellular matrix components for cells. Natural polymers contain natural surface adhesive properties and bioactive properties which facilitate cell attachment and interactions, thereby improving their biocompatibility once introduced into the body (Dhandayuthapani et al., 2011). However, natural polymers typically lack stable mechanical properties, have a high rate of biodegradation and possess high structure variation (Podlipec, 2015). Synthetic polymers used in the development of scaffolds have a well-defined chemical structure that allows for the control of their material properties such as mechanical strength, processability, rate of degradation and permeability (Khademhosseini et al., 2009), but often induce inflammation or immune
responses and may produce harmful degradation products (Courtenay et al., 2017).

2.12 Gelatin-based scaffolds for tissue engineering applications

Gelatin is a natural protein biopolymer obtained by acidic or alkaline hydrolysis of collagen, which is the principal matrix component of bone, hide and connective tissue found in bovine, fish and pigs. Gelatin is obtained in a faintly-yellow powdered form without taste or odour. There are two main types of gelatin, based on their treatment process. Acidic hydrolysis of collagen yields a Type A gelatin while alkaline hydrolysis of collagen produces Type B gelatin. Gelatin contains about 8–13% moisture and has a relative density of 1.3-1.4 (Gelatin handbook, 2012). Gelatin is known to be soluble in aqueous solution. Gelatin-based biomaterials have been used in many tissue engineering applications as scaffolds for both soft and hard tissue regeneration, wound dressings and drug delivery vehicles due to their biocompatibility, biodegradability, low antigenicity and availability (Liu et al., 2009; Xing et al., 2010; Dubrule 2007, Asghari et al., 2017). Gelatin is made of amino acid sequences, functional groups of proteins and exhibits random helix conformation with flexible water-soluble protein fragments (Cammarata, 2015; Rose et al., 2014). Gelatin solution at low temperatures undergoes a thermally reversible change, where gelatin solution is transformed from sol to gel state. Dissolving gelatin in aqueous solutions at high temperatures tends to break down gelatin macromolecules, leading to low mechanical stability and rapid biodegradation (Yang, 2012). Therefore, gelatin gels are stabilised by chemical crosslinking with water-soluble carbodiimide, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) in the presence of N-hydroxysuccinimide (NHS) (Cammarata et al., 2015; Rose et al., 2014; Xing et al., 2010). Crosslinking with EDC and NHS has been demonstrated to produce a gelatin matrix with good resistance to enzymatic
degradation and improved mechanical properties (Yang, 2012; Kuijpers et al., 2000). EDC is classified as zero-length agent because it introduces cross-links without incorporation of any foreign structures into the network (Kuijpers et al., 2000). EDC activates carboxylic acid residues to bind free amine groups of lysine, which results in the formation of an amide bond (Kuijpers et al., 2000). The reaction scheme between EDC and gelatin is shown in Figure 2.6 below. NHS is normally used in combination with EDC to activate the carboxylic acid group, which in turn, makes the O- acylisourea group less susceptible to hydrolysis, and can increase the efficiency of the crosslinking reaction.

![Figure 5: Reaction scheme of crosslinking gelatin sol with EDC in the presence of NHS](adopted from Rose et al., 2014)

In recent times, gelatin has been combined with other materials to form composites for biomedical application to improve the material properties such as the mechanical properties of gel foams. Xing et al. (2010) combined cellulose microfibres from Kraft hardwood sheets as reinforcement material in a gelatin polymer matrix for tissue
engineering. Askarzadeh et al. (2004) also fabricated gelatin-hydroxyapatite scaffolds for bone tissue engineering.

2.13 Cellulose-based scaffolds for tissue engineering applications

Cellulose natural polymer has recently gained attention in the tissue engineering field, because of its suitable properties, such as potential biocompatibility, availability of reactive surfaces for protein binding, good mechanical strength and biodegradability (Entcheva et al., 2004). Plant cellulose and bacterial synthesised cellulose are chemically similar. However, their macromolecular structures and physical properties differ (Czaja et al., 2007). Cellulose from these sources has seen some application in the tissue engineering field. Cellulose has been demonstrated to be biocompatible for cellular culture (Klemm et al., 2005). Regenerated cellulose scaffolds were shown to be biocompatible for both granulation tissue and bone formation (Renvall & Sjöblom 1991 and Märton et al., 1998). Cellulose has also been shown to have a tunable tensile strength (Syverud et al., 2015). Xing et al. (2010) developed a 3D porous biocompatible microscaffold using wood cellulose microfibres combined with cross-linked gelatin for the growth of brain cell and human mesenchymal stem cells (hMSCs). Their findings showed that, the incorporation of wood cellulose microfibres in the biocomposite as reinforcement increased the tensile strength and Young’s modulus of the scaffolds, as the gelatin matrix served as a binder for the fibres and transferred load to the fibres. Their scaffold also supported the growth of brain cells in vitro. Svenson et al. (2004) isolated bacterial cellulose (BC) from Gluconacetobacter xylinus and developed it as a potential scaffold matrix for tissue engineering of cartilage. Their results indicated that an unmodified bacterial cellulose substrate could support bovine chondrocyte proliferation and growth at a substantial level, while providing significant
advantages regarding its mechanical properties. According to Ko and Iwata, (2001), the
nature of the densely-packed glucan chain structure in cellulose fibres provides them
with sufficient mechanical strength to support cell aggregate structures. Cellulose
fibres also possess several accessible –OH groups that can be functionalized for a vast
range of chemical modifications (Isogai et al., 2011; Ong et al., 2013). These hydroxyl
groups present on its surface facilitate the immobilization of cell adhesive proteins such
as fibronectin (Noiset ,1999). Although cellulose has poor degradation in vivo due to
its hydrophilic nature (Entcheva et al. 2004), it undergoes biodegradation by cellulose
hydrolysis and produces glucose as its final degradation product which according to Ko
and Iwata, (2001) can be potentially removed from a cell culture construct when no
longer needed. Pooyan et al. (2012) fabricated a 3D cellulose-acetate-cellulose
nanowhiskers bio-based composite scaffold for vascular tissue engineering has been
recently fabricated. Their report showed that use of cellulose nanowhiskers (kind of
short fibres) as reinforcement increased the ultimate tensile strength of the scaffolds
from 3.0 MPa to about 8.8 MPa (Pooyan et al., 2012; Pei et al., 2017). These studies
conducted using cellulose from other sources in the biomedical engineering field,
provide the background of this study to further examine the material properties of
cassava cellulose fibres and demonstrate their potential use as a biomaterial for tissue
engineering scaffolds for cellular cultures.
CHAPTER THREE

MATERIALS AND METHODS

3.0 Cassava tubers

Three cassava root tubers of three different genotypes (one local and two cloned genotypes) were harvested from the farm of the Biotechnology and Nuclear Agricultural Research Institute (BNARI), Ghana Atomic Energy Commission (GAEC) and used for this study. The local cassava genotype known as Afisafi was tagged as ‘AF fibre’ in this study, and the other two cassava genotypes with specific names (IITA-TMS-GAEC–160004 and IITA-TMS-GAEC–160006), tagged in this study as ‘ID4 fibre’ and ‘ID6 fibre’ were clones which had not yet been released to local farmers. ID4 and ID6 cassava seeds were originally collected from International Institute of Tropical Agriculture (IITA) and subjected to mutagenesis at GAEC with the purpose of improving the biochemical properties in situ for nutrition, health and industrial benefit.

3.1 Reagents and supplies

N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide, (E7750), Gelatin Type B powder from bovine skin (G9391), N-hydroxysuccinimide (130672) and Phosphate-buffered saline (P5368) were purchased from Sigma-Aldrich, USA. All chemicals were of chemical grade and used without any further purification. 50 mm diameter polystyrene Petri dishes coated with Teflon were purchased from Welch Flurolab. 35 x 10 Corning brand polystyrene Petri dishes were also purchased from Corning Incorporated, NY, USA. Double distilled water (DDWater) was used for all solution preparations.
3.2 Cassava tuber preparation and fibre isolation

The cassava tubers were harvested from the BNARI research farm and washed with running tap water to remove the soil residue, allowed to dry at room temperature and transported to the laboratory. The cassava samples were peeled manually using a kitchen knife after thorough washing with clean water. Water retting method, a process for removing non-cellulosic material attached to fibres to release individual fibres as described by Tahir et al., (2011) was adopted in this research to isolate single elementary fibres and central vascular fibre or thick-core fibres from the tubers (Fig 6). Peeled cassava samples were soaked in labelled water jars or bowls full of clean water for 11 days to allow for degradation of starch and non-cellulosic materials.

![Figure 6: Water retting set up](image)

After the 11th day, the cassava bagasse was washed several times with clean water until most of the starch was washed off to allow for manual separation of the single elementary fibres and thick-core fibres. The isolated fibres were then dried at room temperature for 12 days on plastic trays and clean aluminium foil films. The dried fibres were then packaged in labelled Ziploc storage bags and stored in desiccators for further use and characterization processes. Additionally, some of the dried isolated cassava fibres were later ground into a powdered form using mortar for x-ray powder diffraction.
and thermal degradation experiments. Later, the cassava fibre isolated from the different genotypes with higher mechanical properties (tensile strength and Young’s modulus) was selected and pulverised into small short fibres using kitchen blender, and sieved with U.S.A Standard Sieves No. 35 (with pore opening of 500 µm) and No. 70 (with opening of 212 µm) to obtain uniform short microfibres for the fabrication of the polymer composite scaffold.

3.3 Three-dimensional cassava microfibre-gelatin scaffold fabrication

Three-dimensional cassava microfibre-gelatin scaffolds and pure gelatin scaffolds were fabricated by freeze-drying technique as described in the literature (Wu et al., 2010; Xing et al., 2010). Cassava microfibre-gelatin scaffolds and pure gelatin scaffolds were prepared by the following procedure. 1 wt. % gelatin solution was prepared by dissolving 0.404 g of gelatin (Type B powder from bovine skin, Sigma-Aldrich G9391) in 40 ml double distilled water (DDwater) and heated at 50 °C with stirring for 1 h. Different amounts of dried pulverized ID4 isolated cassava microfibres (0 %, 3 %, 5 % and 7 % relative to gelatin solution) were added to the gelatin solution and gently stirred for even distribution. The mixed solution was then allowed to equilibrate to room temperature. An amount of 0.0184g of N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide, EDC (Sigma-Aldrich E7750) and 0.015778g N-hydroxysuccinimide, NHS (Sigma-Aldrich 130672) were added to the above fibre/gelatin solution and gently stirred using magnetic stir bar at room temperature for 15 minutes to crosslink the mixture.
Figure 7: Preparation of cassava microfibre-gelatin solution and crosslinking

The final concentration of EDC and NHS was 5mM at a molar ratio of 1:1 as adopted from Xing et al. (2010). The crosslinked mixture was then poured into cylindrical 50 mm diameter polystyrene petri dishes coated with Teflon and placed in a 4 °C refrigerator for 12 h to allow for complete gelation. The resulting gel in moulds was then placed in a -20 °C freezer for 12 h until ice crystals were formed. Pure gelatin scaffolds were also prepared without the addition of cassava microfibres. The frozen samples were then lyophilized in a Labconco Freezone freeze-dryer (Fig 8) for at least 36 h. Samples were removed from the freeze dryer and stored in a desiccator after complete drying for further experimental tests and analyses.

Figure 8: Preparation of frozen cassava microfibre-gelatin scaffolds for freeze drying
3.4 Mechanical properties testing

3.4.1 Isolated cassava fibre tensile testing

Isolated cassava fibre tensile specimens were prepared and tested according to ASTM C1557-14 (Standard Test Method for Tensile Strength and Young’s Modulus of Fibres). Individual cassava single and thick-core fibres were carefully selected randomly from each cassava variety and mounted unto cardboard paper with a small amount of cyanoacrylate glue as shown in Fig 9 and Fig 10 below.

![Diagram of mounting tab](image1)

**Figure: 9** Adopted schematic diagram of the mounting tab for the tensile specimen preparation (ASTM C1557, 2014)

![Prepared specimen](image2)

**Figure 10:** Prepared specimen for tensile testing
The tensile tests were performed using a standard Mechanical Testing System, MTS Alliance RT/50 equipped with a 5 lb or 22.2 N load cell as shown in figure 11 below. The setup was connected to a computer with an inbuilt software for live data acquisition and displacement measurements at room temperature of 25 °C. The MTS mechanical tester was calibrated prior to testing. Tensile tests were conducted on specimens with different gauge lengths (10-50 mm) under a constant cross-head displacement rate of 1 mm/min at room temperature. Not less than (n=50) tensile specimens of each cassava variety were prepared and used for the tensile testing.

Figure 11: Setup of the tensile test according to ASTM C1557-14 using MTS Alliance RT/50

For each variety of cassava fibre, at least three determinations of different gauge lengths (10 mm, 20 mm, 30 mm and 40 mm) were conducted to also study the effect of the gauge length on the fibre tensile behaviour. During the tensile testing, fibre fracture that occurred in the gripping region were counted invalid due to causes such as stress concentrations that may be introduced by gripping. The cross-sectional area of the
fractured region or near the fracture location within the gauge length was originally
determined using optical microscopy method. A 3-D image of the fractured region of
each fibre was taken using Keyence 3D Digital Optical Microscope (VHX-900F). The
obtained image was further analysed to determine the cross-sectional area using VHX-
900F digital microscope with an inbuilt image analysis software shown in Fig 12.

Figure 12: Set up of the Keyence 3D Digital Optical Microscope (VHX-900F)

In determining the cross-sectional area of the fractured region, a contour line was
interactively drawn to delineate the fibre cross-section as shown in Fig 13 below and
the original cross-sectional area of the fractured perimeter was automatically
determined by the image software.

Figure 13: Determination of fibre cross-sectional area
The cross-head displacement, force, strain, tensile stress and Young’s modulus (based on the initial fibre cross-sectional area inputted) were recorded by the MTS software. The MTS software initially calculated the tensile strength from the ratio of the peak load and the initial fibre cross-sectional area inputted and determined the Young’s modulus from the initial linear region of the force versus displacement curve measured by the MTS machine. The real tensile strength and Young’s modulus was further calculated manually after the original fibre cross-sectional area of the fractured region was obtained by the optical microscopy method. The real Tensile Strength and Young’s modulus of the fibres were calculated using the following proportions:

Tensile Strength = \( M\sigma_t \times \left(\frac{\text{Int CSA}}{\text{Org CSA}}\right) \)  \hspace{1cm} (Eqn. 1)

Where: \( M\sigma_t \) = machine recorded tensile strength; Int CSA = Initial cross-sectional area of fibre inputted; Org CSA = Original cross-sectional area of the fractured region of fibre obtained.

Young’s Modulus = \( McE \times \left(\frac{\text{Int CSA}}{\text{Org CSA}}\right) \)  \hspace{1cm} (Eqn. 2)

Where: \( McE \) = machine recorded Young’s Modulus; Int CSA = Initial cross-sectional area of fibre inputted; Org CSA = Original cross-sectional area of the fractured region of fibre obtained.

### 3.4.2 Scaffold compression testing

Cylindrical microscaffolds of 8 mm diameter were obtained using biopsy punch with plunger system for the compression testing.
Figure 14: Cylindrical microscaffolds of 8 mm diameter obtained from the biopsy punch

Unconfined compression mechanical testing of microscaffolds was performed using MTEST Quattro Biotense Bioreactor microtester - a custom made microscope-mounted mechanical testing device (ADMET, Norwood, MA) connected with a 10 lb load cell (Fig 15 below).

Figure 15: MTEST Quattro Biotense Bioreactor micro tester mounted on Olympus X18 microscope (ADMET, Norwood, MA)
The mechanical tester allows for simultaneous compression testing, measurement of force and displacement and live imaging (Fig 15). Microscaffolds were mounted on the stage of the MTEST Quattro Biotense Bioreactor micro tester by positioning the microscaffold specimen between two flat steel platen grips using a small amount of cyanoacrylate glue (Fig 16). The live imaging capability of the microscope allowed for capturing of live images of specimens and measurement of the initial thickness or gauge length of the microscaffold after preloading. The live imaging also helped to ensure that the microscaffold specimens did not slip from the grips during compression testing.

Figure 16: Mounting of microscaffold unto the stage of the MTEST Quattro Micro-tester

A preload of 0.5 N was applied to each type of scaffold with different fibre load (0 %, 3 %, 5 % and 7 %), (n=5) before taking the initial gauge length for the stress-relaxation and compression tests. 8 mm cylindrical microscaffold specimens were subjected to unconfined compression loading to a strain endpoint of 40% (load limit) at a strain rate of 0.00833 mm/sec at room temperature of 23 °C to determine the material behaviour under a load. Load and displacement data were obtained and stress and strain
calculations were performed. A compressive stress-strain curve was plotted and the slope of the initial elastic region was calculated for the compressive modulus, as well as measuring the maximum compressive stress at the 40% strain endpoint.

3.5 Physicochemical and thermal behaviour Analysis

3.5.1 X-ray powder diffraction analysis

X-ray diffraction patterns of different varieties of isolated cassava fibres were obtained using Philips Panalytical PW1830/X’pert X-ray Diffractometer, USA, to examine the crystallinity or the crystal structure of the cassava fibres.

Figure 17: Set up of Philips Panalytical PW1830 X-ray diffractometer

Dried cassava fibres of different varieties were ground in a mortar. Powdered cassava samples were then placed in a sample holder and mounted on the Philips PW 1830 generator with a monochromatic CuKα radiation (λ=1.54056), operated at 40 kV and 30 mA. The intensities of the scattered radiation were detected in the range of 2θ = 5°-40°, with a scan stepwidth of 0.03 at the speed of 2°/min. The background signal of the
sample holder was also obtained by performing an x-ray diffraction experiment with same parameters without any cassava fibre sample. The background signal was subtracted from the obtained cassava fibre spectra before any further analysis was performed. In all cases, x-ray diffraction analysis for each type of cassava fibre was replicated to examine the sensitivity of the diffractometer.

To determine the Crystallinity Index (C.I) of the different varieties of cassava fibres, two different methods were used. The first method used was the X-ray diffraction peak height method (an empirical method proposed by Segal et al., 1959) to determine the C.I of cellulose samples. Here, the C.I was calculated from the height ratio between the intensity of the crystalline peak ($I_{200} - I_{AM}$) and total intensity ($I_{200}$) after the subtraction of the background signal as described by (Terinte et al., 2011). Equation 3 below was used for the calculation of the apparent crystallinity.

$$C = \left( \frac{I_{200} - I_{AM}}{I_{200}} \right) \times 100 \ [%] \quad \text{(Eqn. 3)}$$

Where: $C$ expresses apparent crystallinity (%); $I_{200}$ represents the maximum intensity of the peak corresponding to native cellulose crystallographic plane in a sample with the Miller indices (200) lattice diffraction at an angle of $\theta = 22^\circ - 24^\circ$; $I_{AM}$ gives the intensity of diffraction of the non-crystalline material (amorphous band) which was taken at an angle of about $\theta = 18.30^\circ - 21.50^\circ$ in the valley between the peaks (Fig 18a and Fig 18b).
The second method employed in this research was the X-ray diffraction deconvolution method (curve fitting), adopted from Terinte et al. (2011).
The obtained XRD Spectra were further analysed using Gaussian deconvolution functions via ORIGIN PRO Version 17 software to allow for peak separations (i.e. to separate the amorphous or non-crystalline and crystalline contributions to the diffraction spectrum). The deconvolution process was achieved based on the convolution of three sharp peaks, from the following crystalline reflection planes (1-10) at an angle of $2\theta = 14.5^\circ - 15.3^\circ$; (110) at an angle of $2\theta = 15.7^\circ - 16.3^\circ$; (200) at an angle of $2\theta = 22^\circ - 24^\circ$; and one broad peak representing the amorphous band reflection at an angle of $2\theta = 18.3^\circ - 21.5^\circ$ (as described by Wada et al., 2001). In the deconvolution process, iterations were repeated until the maximum F statistic was obtained. In all cases, the F statistic was $>10,000$, which corresponds to a $R^2$ value of 0.995. The Gaussian peak profiles were applied in this study with free adjustment of intensity, position and breadth of crystalline peaks (Terinte et al., 2011). Then the apparent crystallinity (%) was calculated from the ratio of the sum of the area of all crystalline peaks to the total area including amorphous band fraction, following an
equation proposed by Hermans et al. (1947) below:

\[
C = 100\%\left[ \frac{A_{\text{crys}}}{A_{\text{total}}} \right] \]  

(Eqn. 4)

Where: C is the apparent crystallinity [%]; \( A_{\text{crys}} \) is the sum of all crystalline band areas and \( A_{\text{total}} \) is the total area under the diffractograms.

3.5.2 Thermal degradation analysis

Different varieties of cassava fibre were ground into powder in a mortar and later used for the thermal degradation analysis using Discovery Thermogravimetric Analyser, (TA Instruments, USA) as shown in Fig 20 below.

![Figure 20: Set up of Discovery Thermogravimetric Analyser, (TA Instruments, USA) used in this study](image)

Prior to the thermal analysis, the TGA analyser was calibrated with Pans of known mass. About 10 mg of each variety of cassava fibres in a powdered form were placed in platinum High Temperature pans and the samples were heated from room temperature (25 °C) to 800 °C at a heating rate of 10 °C/min under nitrogen atmosphere
with a gas flow rate of 25 ml/min. An inbuilt TRIOS software was used for further analysis of the data obtained.

3.6 Morphological and microstructural analysis by SEM and optical microscopy

The morphology and microstructural characteristics of the isolated cassava fibres and the fabricated scaffolds were examined using Scanning Electron Microscopy (ZEISS Gemini LEO 982 Microscope, USA) and Optical Microscopy (Keyence 3D Digital Optical Microscope VHX-900F) methods.

Regarding SEM analysis, samples were placed on stubs and coated with gold using Denton Vacuum Desk II Sputter Coater to become more conductive and suitable for SEM analysis. Coated samples were then placed in ZEISS Gemini LEO 982 SEM (Fig 21) for imaging. The SEM was operated using accelerating voltage of 5-10 kV and a working distance of 12-18 mm. SEM images and optical microscopy images obtained were processed and analysed using ImageJ software package (NIH, USA) to examine the surface microstructure, porosity, fibre dimensions, and fibre-polymer adhesion. Additionally, thin cross-sections of microscaffolds were obtained using microtome for further SEM analysis to enhance the measurement of pore size and porosity of the scaffolds.

Keyence 3D Digital Optical Microscope VHX-900F was also used to examine the morphology of both isolated cassava fibres and fabricated scaffolds. Images obtained were further analysed using ImageJ software (NIH, USA) for the measurement of fibre cross-sectional area and scaffold pore size calculations.
3.6.1 Porosity and pore size determination

The porosity of scaffolds was determined using the gravimetric method and image analysis technique. With respect to the image analysis technique, SEM surface images and horizontal cross-section images (parallel to the plane of the surface) of microscaffold specimens were obtained for the determination of pore size and porosity. Surface images of the microscaffolds were also obtained through optical microscopy for porosity measurement comparison. The various images obtained were further
processed and examined using ImageJ software package (NIH, USA). Optical images were converted into 32-bit format and filtered using bandpass filter. Surface pores were detected using variable thresholding and edge detection technique as shown in Figure 23 below.

![Figure 23: Surface porosity determination by image analysis, (a): original image (b): image converted into 32-bit (c) : variable thresholding and edge detection](image)

Porosity by gravimetric method was also determined based on the volume fractions of (cassava fibre, void and gelatin) and the actual density of microscaffold or sample composite. The symbols and formulas for the calculation of volume fraction of fibre, gelatin and void in the scaffold are shown below.

### 3.7 Fibre volume fraction calculations

Table 5 below shows the symbol and units as well as formulas employed in the calculation of the fibre volume fraction.
<table>
<thead>
<tr>
<th></th>
<th>Symbol and units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C = “composite” = Fibre + Gelatin + Void</td>
</tr>
<tr>
<td>2</td>
<td>M (“Matrix”) = (gelatin + void)</td>
</tr>
<tr>
<td>3</td>
<td>S = “Solution” = (fibre + gelatin + water);</td>
</tr>
<tr>
<td>4</td>
<td>BC = “batch composite”, before taking a biopsy punch</td>
</tr>
<tr>
<td>5</td>
<td>SC = microscaffold or sample composite obtained by biopsy punch</td>
</tr>
<tr>
<td>6</td>
<td>TWS = total weight of solution</td>
</tr>
<tr>
<td>7</td>
<td>WF/SC = Weight of fibre in microscaffold or sample composite</td>
</tr>
<tr>
<td>8</td>
<td>WF/S = Weight fraction of fibre in solution</td>
</tr>
<tr>
<td>9</td>
<td>WF/BC = Weight fraction of fibre in batch composite</td>
</tr>
<tr>
<td>10</td>
<td>WBC = Weight of batch composite</td>
</tr>
<tr>
<td>11</td>
<td>Wsc = Weight of microscaffold scaffold or sample composite;</td>
</tr>
<tr>
<td>12</td>
<td>WF = Weight of fibre</td>
</tr>
<tr>
<td>13</td>
<td>WG = Weight of gelatin</td>
</tr>
<tr>
<td>14</td>
<td>WFg/S = Weight fraction of gelatin in solution</td>
</tr>
<tr>
<td>15</td>
<td>$\rho_{SC}$ = Experimentally measured density of sample composite</td>
</tr>
<tr>
<td>16</td>
<td>$\rho_f$ = 0.53 g/ml [density of fibre taken from an unpublished preliminary study]</td>
</tr>
<tr>
<td>17</td>
<td>$\rho_g$ = 1.34 g/ml [theoretical density of gelatin, assumed]</td>
</tr>
<tr>
<td>18</td>
<td>$\rho_{GM}$ = density of gelatin in matrix</td>
</tr>
<tr>
<td>19</td>
<td>$V_{SC}$ = Volume of microscaffold or sample composite (determined experimentally)</td>
</tr>
<tr>
<td>20</td>
<td>$V_F$ = Volume of fibre in microscaffold or sample composite</td>
</tr>
<tr>
<td>21</td>
<td>$V_G$ = Volume of gelatin in microscaffold or sample composite</td>
</tr>
<tr>
<td>22</td>
<td>$V_V$ = Volume of void in microscaffold or sample composite</td>
</tr>
<tr>
<td>23</td>
<td>$V_F/SC$ = Volume fraction of fibre in microscaffold or sample composite</td>
</tr>
<tr>
<td>24</td>
<td>$V_F/SC$ = Volume fraction of fibre in microscaffold or sample composite</td>
</tr>
<tr>
<td>25</td>
<td>$V_F/V_M$ = Volume fraction of void in matrix</td>
</tr>
<tr>
<td>26</td>
<td>$V_F/V_{SC}$ = Volume fraction of void in microscaffold or sample composite</td>
</tr>
<tr>
<td>27</td>
<td>$WF_{G/SC} = Weight fraction of gelatin in microscaffold or sample composite</td>
</tr>
<tr>
<td>28</td>
<td>$WF_{G/BC} = Weight fraction of gelatin in batch composite</td>
</tr>
</tbody>
</table>
3.8 Data analysis

Continuous data were obtained from the tensile and compression testing. The thermogravimetric analysis and X-ray powder diffraction tests yielded both continuous data and data in the form of spectra. The continuous data were organised and analysed using Microsoft Excel 2016, IBM SPSS Statistical Software version 24, JMP Pro vs.12 statistical software, MATLAB R2016. Images obtained from the scanning electron
microscopy and optical microscopy were analysed using ImageJ software package (NIH, USA). Origin Pro software was also employed for deconvolution and peak separation of X-ray spectra. The distribution pattern of the continuous data and their normality were tested using Kolmogorove-Smirnov (K-S test) and Shapiro-Wilk tests. Data with KS statistical test showing $p > 0.05$ were accepted to be normally distributed, hence parametric analysis was conducted for such data. Data that deviated from a normal distribution as indicated by $p < 0.05$ from the KS test were subjected to non-parametric analysis. Both descriptive and inferential statistics were conducted in this study. Independent t-test and Mann Whitney u test were performed to examine the difference between the individual single and thick-core fibres with respect to their average mechanical properties. One-way analysis of variance (ANOVA) was conducted to determine whether there were any statistically significant differences among the different varieties of cassava fibres with respect to their averages mechanical properties recorded. Tukey’s HSD post hoc analysis was further conducted to show the means that are significantly different from each other at $p < 0.05$. In all inferential statistics, the alpha level was set to $p=0.05$, indicating significant differences exist at $p<0.05$. Results were presented in the form of tables, charts, curves and spectra.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Isolated cassava fibre of different genotypes

Figure 24 shows digital camera images of cassava fibres of different genotypes isolated from the water retting process in this study.

Figure 24: Isolated cassava fibres of different genotypes (a) ID4 fibres (b) ID6 fibres (c) AF fibres (d) AF single elementary fibre and thick-core fibre

The diameter of the single elementary fibres ranges from (0.4-3.0) mm and the length can reach about 60 mm.

4.1 Mechanical properties of cassava fibre of different genotypes

The study examined the tensile behaviour of the individual single elementary and thick-core cassava fibres isolated from different genotypes. Similarities were found among the tensile behaviour of all the different types of cassava fibres examined in this study. Figure 25 below shows a sample of the typical force versus displacement curves
recorded for Afisiafi (AF) cassava fibres, where the both fibre types exhibited linear
deformation behaviour followed by brittle failure.

![Typical force versus crosshead (displacement) curve for (a) AF Single Fibre and (b) AF thick fibre](image)

**Figure 25: Typical force versus crosshead (displacement) curve for (a) AF Single Fibre and (b) AF thick fibre**

It was observed that all the different types of cassava fibre examined in this study (such as AF single and thick-core fibres) displayed similar tensile behaviour, as shown in figure 25 above. From both curves, it was found that there was an initial small lag (toe region) during the initial application of the load at very low strains, possibly due to the preconditioning of the fibre cells or slack in the machine/grips. Following this, it was found that the gradual application of sufficiently small tensile load led to a linear behaviour representing the elastic region. The slope of the initial linear region of the curve was therefore determined to represent the Young’s modulus. It was also observed that, as the load increased to a peak point the curve deviated from the linear behaviour, possibly be due to permanent plastic deformation in the fibres such as the fracture of some individual fibre fibrils at high tension. Silva et al. (2008) have proposed that the
non-linear behaviour of natural fibres is caused by some collapse of primary cellulose fibre cell walls and delamination between cellulose fibrils. The peak load at which the fibre fractured was determined and evaluated using the cross-sectional area to determine the ultimate tensile strength (UTS) values for the fibres. It is interesting to note that, all the different genotypes of cassava examined in this study showed a similar tensile behaviour pattern as observed through their force versus displacement (crosshead) curves. The similarities in their tensile behaviour suggest the possibility that, individual single and thick-core fibres are made of similar microstructure and therefore, there will not be any significant differences in their mechanical properties.

The mechanical properties of AF single and thick fibres are presented in Table 6 below.

Table 6: Comparison between AF single and thick cassava fibres (gauge lengths between 10 mm – 50 mm)

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>AF Thick Fibre N= 48</th>
<th>AF Single Fibre N=28</th>
<th>U test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength, (MPa)</td>
<td>6.613±4.11</td>
<td>6.625±4.38</td>
<td>662.00</td>
<td>0.914</td>
</tr>
<tr>
<td>Young’s Modulus (MPa)</td>
<td>252.04±131.36</td>
<td>281.43±130.59</td>
<td>574.00</td>
<td>0.291</td>
</tr>
<tr>
<td>Strain at break (%)</td>
<td>3.98±1.92</td>
<td>3.54±1.93</td>
<td>575.00</td>
<td>0.296</td>
</tr>
</tbody>
</table>

Results from the tensile test and Mann-Whitney U analysis showed that there was no significant difference (p=0.914) between the average tensile strength recorded for AF single elementary fibre (6.625±4.38 MPa) and AF thick-core fibres (6.613±4.11 MPa). It was also found that AF single fibres recorded a higher average Young’s Modulus of (281.43±130.59 MPa) compared to AF thick fibres (252.04±131.36 MPa) but their mean difference was not statistically significant (p=0.291). Similarly, there was no
significant difference \( (p=0.296) \) between the average strain-to-failure recorded for AF single and thick-core fibres. The high standard deviation associated with the average tensile properties could be due to factors such as variable gauge length used, the precision of the instrumentation, type of grips, compliance of the machine as well as fibre geometry as proposed by Silva et al. (2008). The data suggest that both AF single elementary fibres and the thick-core fibre (also known as the xylem bundle or the vascular fibre) could have similar atomic structure owing to the statistically insignificant differences in mechanical properties. The minor differences in the mechanical properties of the fibres types could be attributed to the differences in cell wall cross-sectional area between single elementary fibre and the thick-core fibre as proposed by Wang et al. (2014).

Table 7 presents the mechanical properties of ID4 single and thick fibres examined at random gauge lengths.

**Table 7: Comparison between ID4 single and thick-core cassava fibres (gauge lengths between 10 mm – 50 mm)**

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>ID4 Thick Fibre</th>
<th>ID4 Single</th>
<th>U test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N= 23</td>
<td>Fibre N=27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tensile Strength (MPa)</td>
<td>6.57±4.83</td>
<td>7.72±3.75</td>
<td>221.00</td>
<td>0.081</td>
</tr>
<tr>
<td>Young’s Modulus (MPa)</td>
<td>360.09±284.75</td>
<td>369.98±157.90</td>
<td>228.00</td>
<td>0.108</td>
</tr>
<tr>
<td>Strain at break (%)</td>
<td>4.47±4.14</td>
<td>4.07±2.22</td>
<td>281.00</td>
<td>0.566</td>
</tr>
</tbody>
</table>

The study found similarities between ID4 single and thick-core fibres regarding their mechanical properties. From Table 7 above it was observed that ID4 single fibres
recorded a higher tensile strength of (7.72±3.75 MPa) compared to ID4 thick fibre (6.57±4.83 MPa) however, their mean difference was not statistically significant (p=0.081). Similarly, the average Young’s modulus obtained for ID4 single fibres (369.98±157.90 MPa) was marginally higher than that of ID4 thick fibres (360.09 ± 284.75 MPa) but the mean difference was not statistically significant (p=0.108). Results from the tensile test also indicated that there was no significant difference (p=0.566) between ID4 single and thick fibres with respect to their average strain-to-failure. These findings also suggest that the similarities in mechanical properties between of both single fibre and thick-core fibre could be due to their similarities in atomic structure. However, the variabilities in tensile properties could be due to factors mentioned above as proposed by Silva et al. (2008).

Table 8 presents the mechanical properties of ID6 single and thick fibres examined at random gauge lengths.

**Table 8: Comparison between ID6 single and thick core fibres (gauge lengths between 10 mm – 50 mm)**

<table>
<thead>
<tr>
<th>Mechanical properties</th>
<th>ID6 Thick Fibre N= 42</th>
<th>ID6 Single Fibre N=20</th>
<th>U test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength (MPa)</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>364.00</td>
<td>0.399</td>
</tr>
<tr>
<td></td>
<td>5.61±3.20</td>
<td>6.53±3.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young’s Modulus (MPa)</td>
<td>268.76±143.07</td>
<td>315.42±174.05</td>
<td>343.00</td>
<td>0.246</td>
</tr>
<tr>
<td>Strain at break (%)</td>
<td>4.34±3.11</td>
<td>4.66±3.02</td>
<td>383.500</td>
<td>0.492</td>
</tr>
</tbody>
</table>

The tensile test results showed the mean tensile strength obtained for ID6 single fibres (6.53±3.88) was higher than ID6 thick fibres (5.61±3.20 MPa) however, the mean
difference was found not to be statistically significant (p=0.399). Similarly, the average Young’s modulus recorded for ID6 single fibres (315.42±174.05 MPa) was also higher than that of ID4 thick fibres (268.76±143.07 MPa) but the mean difference was not statistically significant (p=0.246). Additionally, it was found that there was no significant difference (p=0.492) between ID4 single and thick fibres with respect to their average strain-to-failure.

In all, these results showed that the average tensile strength, Young’s modulus and strain at break recorded for both single elementary and thick-core fibres of the three genotypes were not statistically different from each other. The variabilities in the tensile properties could be attributed to factors such as test parameters/condition (gauge length, machine compliance, type of grips) as well as fibre microstructure and cross-sectional area measurement as proposed by Silva et al. (2008). Therefore, in terms of practical application, the single fibres could be used interchangeably with the thick-core fibres for higher mechanical strength requirements such as in the fabrication of fibre reinforced composites for industrial applications.

4.1.1 Effect of gauge length on the mechanical properties of the cassava fibres

The effect of different gauge lengths on the mechanical properties of the cassava fibres from different cassava genotypes was examined during the tensile test. Table 9 summarises the mechanical properties of AF fibres at different gauge lengths.
Table 9: Mechanical properties of AF fibre cassava at different gauge lengths

<table>
<thead>
<tr>
<th>Gauge length (mm)</th>
<th>N</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Strain-to-failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>7.23±3.85</td>
<td>223.63±103.30</td>
<td>5.74±1.82</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>5.95±3.06</td>
<td>231.37±73.75</td>
<td>3.91±2.13</td>
</tr>
<tr>
<td>25</td>
<td>7</td>
<td>5.42±3.65</td>
<td>210.15±63.22</td>
<td>2.91±1.02</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>2.33±1.13</td>
<td>139.28±38.07</td>
<td>2.71±1.35</td>
</tr>
<tr>
<td>40</td>
<td>7</td>
<td>4.84±3.28</td>
<td>234.00±107.06</td>
<td>3.01±1.09</td>
</tr>
<tr>
<td>50</td>
<td>4</td>
<td>2.16±1.32</td>
<td>157.90±38.95</td>
<td>1.90±0.96</td>
</tr>
<tr>
<td>F-statistic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.180</td>
<td>0.054</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

From Table 9, it was observed that the magnitude of the tensile strength, Young’s modulus and strain at break of AF fibres tends to be higher at small gauge length (10 mm) compared to larger gauge length (50 mm). The obtained tensile strength for AF fibres decreased from 7.23±3.85 MPa to 2.16 ±1.32 MPa as the gauge length was increased from 10 mm-50 mm. The Young’s modulus recorded for AF Fibres at different gauge lengths varied from 157.90±38.95 MPa to 234.00±107.06 MPa. Strain at break also varied at different gauge length from 1.90±0.96% to 5.74±1.82%. One-way independent analysis of variance (ANOVA) test results showed that gauge length did not have any significant influence on the tensile strength (p=0.180) and the Young’s modulus (p=0.054) of AF fibres as also observed by Silva et al. (2008). However, gauge length was found to have a significant effect on the strain-to-failure (F(5)=4.703, p=0.003) of the AF fibres. The variabilities in the tensile properties could be due to the
anticipated effect of the fibre microstructure and the irregularities of the fibre geometry as proposed by Silva et al. (2008) and Fidelis et al. (2013).

Table 10 below summarises the tensile test results for ID4 fibres at different gauge lengths.

Table 10: Mechanical properties of ID4 fibre cassava at specific gauge length

<table>
<thead>
<tr>
<th>Gauge length (mm)</th>
<th>N</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>8.57±3.18</td>
<td>263.91±120.58</td>
<td>9.83±3.55</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>7.57±3.84</td>
<td>336.48±130.80</td>
<td>4.30±1.95</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>7.76±5.70</td>
<td>356.36±181.25</td>
<td>3.44±1.03</td>
</tr>
<tr>
<td>40</td>
<td>6</td>
<td>5.25±2.73</td>
<td>324.84±97.13</td>
<td>2.81±1.41</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>5.82±3.45</td>
<td>446.11±254.14</td>
<td>1.71±0.58</td>
</tr>
<tr>
<td>F-statistic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P=0.579</td>
<td>P=0.402</td>
<td>P=0.000*</td>
</tr>
</tbody>
</table>

Results from Table 10 shows that tensile strength recorded for ID4 fibres varied from 8.57±3.18 MPa to 5.25±2.73 as the gauge length was increased. Also, Young’s modulus obtained for ID4 fibres varied for different gauge lengths from 263.91±120.58 MPa to 446.11±254.14 MPa. Similarly, the strain at break varied for different gauge length from 1.71±0.58% to 9.83±3.55%. The analysis of variance (ANOVA) test conducted showed that the variabilities in the results for tensile strength (F (5) =0.728, p= 0.579) and Young’s modulus (F (5) = 1.037, p=0.402) were not influenced by gauge length. However, it was found that gauge length does seem to have a significant effect on the strain at break obtained for ID4 fibres.
Table 11 below also summarises the tensile test results for ID6 fibres at different gauge lengths.

### Table 11: Mechanical properties of ID6 fibre cassava at specific gauge length

<table>
<thead>
<tr>
<th>Gauge length (mm)</th>
<th>N</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Strain at break (%)</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>7.89±2.90</td>
<td>210.45±50.68</td>
<td>7.60±2.64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>3.65±1.85</td>
<td>162.22±37.79</td>
<td>4.70±2.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>5.07±2.62</td>
<td>258.24±91.87</td>
<td>4.16±2.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>4.14±2.24</td>
<td>270.81±84.58</td>
<td>2.67±1.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>4</td>
<td>4.73±1.61</td>
<td>335.30±201.71</td>
<td>2.82±1.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>4.73±2.49</td>
<td>317.55±120.41</td>
<td>4.17±4.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5</td>
<td>4.61±1.25</td>
<td>271.17±92.57</td>
<td>2.27±0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-statistic</td>
<td></td>
<td>P=0.015*</td>
<td>p=0.020*</td>
<td>P=0.007*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From Table 11, it was found that the mechanical properties (tensile strength, Young’s modulus and strain at break) do seem to be a function of gauge length for ID6 fibres, unlike AF and ID4 fibres where their tensile strength and Young’s modulus were not significantly influenced by gauge length. Here it was found that the average tensile strength obtained for ID6 varied significantly (F (6) =3.039, p=0.015) for different gauge length from 3.65±1.85 MPa to 7.89±2.90 MPa. Also, the average Young’s modulus obtained for ID6 fibres varied significantly (F (6) = 2.869, p=0.02) from 210.45±50.68 MPa to 335.30±201.71 MPa for different gauge length. Strain at break values obtained for ID6 fibres also was significantly (F (6) =3.469, p=0.07) influenced by different gauge length.
In all, it was found that for all the varieties of cassava examined in this study, different gauge length does seem to influence some of the mechanical properties of the cassava fibres depending on the plant characteristics such as the cassava genotype. The results obtained for most of the mechanical properties (Tensile strength and strain at break) tend to decrease in magnitude as the gauge length increases. These variations in the mechanical properties at different gauge length were also reported by Fidelis et al. (2013), Tomczak et al. (2007), Silva et al. (2010) and Silva et al. (2008) for other lignocellulosic fibres (such as sisal, coir, curaua and piassava) and are consistent with the anticipated effect of the distribution of flaws in the volume of the fibres and mean sizes.
4.1.2 Overall comparison of the mechanical properties of different genotypes of cassava fibre

Table 12 and 13 below present the overall mechanical properties of the cassava fibres isolated from the three cassava genotypes examined at random gauge lengths and specific gauge length.

Table 12: Comparing the mechanical properties of different cassava genotypes (gauge length between 10 mm- 60 mm)

<table>
<thead>
<tr>
<th>Different cassava genotypes</th>
<th>N</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>AF Fibre</td>
<td>76</td>
<td>6.62 ± 4.27</td>
<td>262.866 ± 130.98</td>
<td>3.82 ± 1.94</td>
</tr>
<tr>
<td>ID4 Fibre</td>
<td>50</td>
<td>7.19 ± 4.26</td>
<td>365.433 ± 222.29</td>
<td>3.21 ± 3.22</td>
</tr>
<tr>
<td>ID6 Fibre</td>
<td>62</td>
<td>5.91 ± 3.43</td>
<td>283.813 ± 153.87</td>
<td>4.45 ± 3.06</td>
</tr>
</tbody>
</table>

Welch statistic: P=0.209  P=0.016  P=0.165

Games-Howell column comparison: mean values across each column with different superscripts are significantly different at p<0.05

Comparing the tensile properties of the three cassava genotypes at random gauge lengths (10 mm – 60 mm), it was found that ID4 fibre recorded the highest average tensile strength of 7.19±4.26 MPa, followed by AF fibres (6.62±4.27 MPa) and ID6 fibres (5.91±3.43 MPa). However, their mean tensile strengths did not differ significantly among the cassava varieties (p=0.209) based on Games-Howell multiple comparison test. Thus, the tensile strength recorded by the different genotypes of cassava fibre was not statistically significant at different gauge length. Similarly, the average strain-to-failure recorded for the three different genotypes of cassava fibre was
not significantly different from each other. However, ID6 fibres obtained the highest elongation at break. The study found however, found that the botanical source (genotype) of cassava fibres tend to have significant influence (p=0.016) on the average Young’s modulus recorded for the fibres. The Games-Howell robust post hoc analysis conducted showed that at random gauge length, ID4 fibres obtained significantly higher Young’s modulus (365.433±222.29 MPa) compared to AF fibres (262.866±130.98) at p = 0.012 but not significantly higher when compared to ID6 fibres (283.813±153.87 MPa) at p=0.076. The variabilities in the tensile properties could be due to factors such as different gauge length, irregularity of the fibre cross-section, distribution of defects within the fibre as well as the compliance of the machine and type of grips which was not accounted for in this study.

Table 13 presents the mechanical properties of the cassava fibres examined at specific gauge length of 20 mm.

**Table 13: Comparing the mechanical properties of three cassava genotypes at 20 mm gauge length**

<table>
<thead>
<tr>
<th>Different cassava genotypes</th>
<th>N</th>
<th>Tensile Strength (MPa)</th>
<th>Young’s Modulus (MPa)</th>
<th>Strain at break (%) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF Fibre</td>
<td>11</td>
<td>5.944±3.065 ab</td>
<td>231.365 a±73.747</td>
<td>3.915 a±1.94</td>
</tr>
<tr>
<td>ID4 Fibre</td>
<td>10</td>
<td>7.567 b±3.844</td>
<td>336.485 b±130.803</td>
<td>4.297 a±1.947</td>
</tr>
<tr>
<td>ID6 Fibre</td>
<td>10</td>
<td>3.651 a±1.853</td>
<td>162.218 a±37.788</td>
<td>4.703 a±2.666</td>
</tr>
</tbody>
</table>

F-statistics: P=0.025*  P=0.001*  P=0.731

Tukey HSD column comparison: mean values across each column with different superscripts are significantly different at p<0.05
Interestingly, at this gauge length, one-way independent analysis of variance (ANOVA) test the study found a significant difference in tensile strength and Young’s modulus among the cassava genotypes \( (F(2)=4.205, \ p=0.025 \) and \( F(2)=9.755, \ p=0.001 \) respectively. From the post hoc analysis, it was found that ID4 fibre recorded the highest average tensile strength of \( 7.567 \pm 3.844 \) MPa when compared to ID6 fibre \( (p = 0.019) \) and the highest average Young’s modulus of \( 336.485 \pm 130.803 \) when compared to the other cassava genotypes \( (p < 0.05) \).

Comparing the average tensile strength and Young’s modulus obtained for the isolated cassava fibres from different genotypes to some natural fibres (coconut fibre which is reported to have Young’s Modulus ranging between 4-6 GPa and tensile strength ranging from 131-175 MPa and Sisal fibre with Young’s Modulus ranging 9-22GPa and tensile strength of 350-700 MPa) (Célino et al., 2014), it can be seen that the cassava fibres have relatively low tensile strength and Young’s modulus. However, recent work published by Edhirej et al. (2017) who used cassava bagasse as fillers in thermoplastic starch, reported that a composite with 6% cassava bagasse yielded the highest tensile stress of \( 10.78 \) MPa, which is comparable to the average tensile strength obtained for ID4 fibres \( (7.19 \) MPa) in this study. Similarly, Versino et al. (2015) used 1.5% cassava bagasse as reinforcement in a starch-based composite and recorded an average tensile strength of 4.35 MPa which is lower compared to the tensile strength of ID4 fibres recorded in this study. Edhirej and co-workers also reported the highest modulus obtained for the composite with 6% cassava bagasse to be 581.68 MPa, which is a little higher compared to the isolated single ID4 fibres modulus of 365.433MPa. This variation could be due to the effect of the starch matrix in their composites.
4.2 Examining the X-ray diffraction pattern and crystallinity index of different varieties of cassava fibres

4.2.1 X-ray powder diffraction analysis of AF, ID4 and ID6 cassava fibres

The study examined the x-ray diffraction pattern and the crystallinity index between the single and thick-core cassava fibres of different genotypes. The objective was to examine the differences or similarities in crystalline microstructure among different varieties of cassava.

The study found some similarities and minimal variations in the x-ray diffraction pattern and signal intensities recorded between the single elementary and thick-core fibres for all the cassava genotype examined in the study. Some of the major results are presented below.

Figure 26 shows the x-ray diffractograms of AF single fibres and thick-core fibres.

![Figure 26: Comparison of the x-ray diffractograms of AF single fibres and thick-core fibres](image-url)
From figure 26, it was found that both AF single and thick fibres showed somewhat similar x-ray diffraction pattern with two distinct crystalline peaks but minimal differences in peak intensities and peak broadening, possibly due to differences in the atomic structure. It was further observed that AF thick fibre had its main pronounced crystalline band with the peak intensity of about 1644 count at position \(2\theta=22.061^\circ\) which falls within the reflection angle \((21.90^\circ-22.20^\circ 2\theta)\) assigned to the \((200)\) crystallographic plane of cellulose (Wada et al., 2001). Wada et al. 2001 labelled the crystallographic planes for algal cellulose according to the native cellulose structure.

The AF thick fibres also showed a crystalline peak around \(2\theta=16.823^\circ\) which is also close to the \((15.7^\circ-16.30^\circ 2\theta)\) reflection assigned to the \((110)\) crystallographic plane. These positions were obtained accurately from the deconvolution analysis performed using Gaussian profiles. AF single fibres showed similar crystalline peaks with similar positions. Similarly, from the diffractogram for the AF single fibre, it was found that the main crystalline band occurred at the peak position \(2\theta=21.74607^\circ\) which is close the reflection angle \((21.90^\circ-22.20^\circ 2\theta)\) assigned to the \((200)\) crystallographic plane of cellulose. It was also found that both AF thick and AF single fibres showed an amorphous band at the position of around \((2\theta =17.53^\circ - 18.739^\circ)\) which is close to the \((2\theta =18.30^\circ-18.40^\circ)\) reflection assigned to the amorphous phase as described by Wada et al. (2001). The presence of both crystalline and amorphous bands in the AF fibres depicts its semi-crystalline nature. Based on the similarities of the x-ray diffraction pattern and minimal variation in peak intensities for both AF thick and single fibres it may be assumed that they are made of similar crystalline structures. This could explain the minimal differences in the mechanical properties recorded for both AF single and thick-core fibres.
With respect to ID4 cassava fibres, both ID4 single and thick fibres showed similar x-ray diffraction pattern with three distinct crystalline peaks but differences in peak intensities as shown in figure 27 below.

Figure 27: Comparison of the x-ray diffractograms of ID4 single fibres and thick-core fibres

The more pronounced differences existed in the broadening of the (200) crystalline peaks. From the deconvolution analysis (Appendix-B), it was found that ID4 single (200) crystalline peak occurred at the position $2\theta = 22.24^\circ$ compared to ID4 thick fibres (200) crystalline peak at $2\theta = 22.424^\circ$, however both peak positions are close to the reflection angle ($21.90^\circ$-$22.20^\circ$ $2\theta$) assigned to the (200) crystallographic plane of cellulose (Wada et al., 2001). Although the presence of the crystalline peaks at similar positions was observed between the spectra of ID4 single and thick core fibres, the minimal differences in the peak intensities may suggest that that the isolated single and thick-core cassava fibres could have different atomic structures, which could also explain some variabilities in the mechanical properties recorded for ID4 single and thick-core fibres. Like AF and ID4 cassava fibres, similar diffraction patterns were also
found for ID6 single and thick fibres showing three distinct crystalline peaks at similar positions (Appendix-B).

4.2.2 Overall comparison of X-ray diffractograms of different varieties of cassava fibre and crystallinity index

Figure 28 below shows the overall comparison of the diffractograms of individual single fibres isolated from different genotypes of cassava.

Figure 28: Comparison of X-ray diffractograms of different varieties of cassava fibre

From figure 28, it was observed that ID6 and ID4 fibres showed all the three distinct crystalline peaks described by Wada et al. (2001) for native cellulose structures excerpt for AF fibres that showed two distinct crystalline peaks. In addition to this, there were some variations regarding the peak broadening of the most pronounced (200) crystalline peaks emerging around $2\theta = 22^\circ-24^\circ$ observed for all cassava varieties. AF and ID4 fibres had a much broader (200) crystalline peak compared to ID6 fibres. The presence of this peak which is assigned to the (200) crystallographic plane in native
cellulose structure may indicate the presence of cellulose type 1. It was also found that the intensity of this (200) crystalline peak varied among the varieties of the cassava fibre. The variation in their peak intensities could suggest that the degree of crystallinity among the cassava genotypes would also differ and consequently influencing difference in mechanical properties of the three genotypes. With regard to the amorphous phase, it was found that there was not much difference in the intensities at which the amorphous phase (position 18.30°-18.40 °2θ) emerged for all the cassava varieties. Overall, based on the x-ray diffraction analysis, it can be seen that different genotypes of cassava fibres have some similarities in their diffraction pattern but differences in the peak intensities and number of crystalline peaks observed. The data suggest that the degree of crystallinity of the three different genotypes of cassava fibre would be similar.

4.2.3 *Crystallinity index determination*

One of the important parameters that describe the crystalline structure of cellulose samples is the degree of cellulose crystallinity (also termed Crystallinity Index) (Poletto et al., 2014). The rigidity and flexibility of natural cellulose fibres increases and decreases respectively, with increasing ratio of crystalline to amorphous regions within the cellulose sample (Poletto et al., 2014). This implies that cellulose fibre with a higher crystallinity index is more rigid compared to fibre with low crystallinity index.
Table 14 below presents the crystallinity index values obtained for the different varieties of cassava fibres examined in this study.

**Table 14: Crystallinity index (Cr.I) obtained from the x-ray diffraction analysis**

<table>
<thead>
<tr>
<th>Variety of Cassava Fibres</th>
<th>Type of fibre</th>
<th>Cr.I.&lt;sup&gt;S&lt;/sup&gt; (%)</th>
<th>Cr.I.&lt;sup&gt;H&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF fibres</td>
<td>Single</td>
<td>31.09</td>
<td>90.63</td>
</tr>
<tr>
<td></td>
<td>Thick</td>
<td>39.99</td>
<td>92.18</td>
</tr>
<tr>
<td>ID4 fibres</td>
<td>Single</td>
<td>30.79</td>
<td>89.06</td>
</tr>
<tr>
<td></td>
<td>Thick</td>
<td>25.48</td>
<td>94.81</td>
</tr>
<tr>
<td>ID6 fibres</td>
<td>Single</td>
<td>20.83</td>
<td>81.70</td>
</tr>
<tr>
<td></td>
<td>Thick</td>
<td>34.70</td>
<td>87.29</td>
</tr>
</tbody>
</table>

*Cr.I.<sup>S</sup>: crystalline index proposed by Segal (peak height method) and Cr.I.<sup>H</sup>: crystalline index proposed by Hermans (Peak deconvolution)*

The study used two main methods in determining the crystallinity index of the cassava fibres, namely the peak height method proposed by Segal et al. in 1959 and the peak deconvolution method proposed by Hermans et al. (2001). According to Park et al. (2010), about 70-85% of published papers that determined the crystallinity index of cellulose samples used the peak height method while only 5-10% used the peak deconvolution method. This could be due to the fact that the peak height method is simple to perform compared to the deconvolution method which requires more technical skill. The crystallinity index calculated using the Segal method in this study showed low values for all the genotypes of cassava compared to the Cr.I determined by the Hemans method. These variations have also been reported by Park et al. (2010). Xu et al. (2013) explained that the Segal’s C.I does not truly represent the percentage of the crystalline parts in cellulose samples, mainly because it only considers the crystalline band or peak assigned to the (200) crystallographic plane while neglecting
the other (110) and (1-10) crystallographic planes. From the Segal Cr.I results, it was found that AF single and thick fibres obtained the highest Cr. I of 31.09% and 39.9% when compared to single and thick fibres of ID4 and ID6 respectively, although the differences in Cr.I. for all the cassava varieties was not large. Similarly, Cr.I determined by the peak deconvolution showed AF single and thick fibres obtaining the highest percentage of crystallinity index of 90.63 % and 92.18 % respectively, this suggests that the AF fibres were more crystalline compared to the other fibres. Leita et al. (2017) recently reported crystallinity index of 46% and 77.2-100 % for raw cassava bagasse and chemically treated cassava bagasse, respectively. Their result for the raw cassava bagasse is comparable to Cr.I. obtained for the untreated cassava fibres examined in this study (30.79-39.9%).
4.3 Examining the thermal degradation profile of cassava fibres of different genotypes

4.3.1 Thermogravimetric curves of AF, ID4 and ID6 cassava fibres

The thermal decomposition profile of AF single and thick fibres analysed by the thermogravimetric analysis (TGA) under nitrogen are shown in Figure 29 below.

![Figure 29: Thermogravimetric curve for (a) AF single fibres and (b) AF thick fibres](image)

From Figure 29(a) and (b), it was found that, the thermal profiles for both AF single and thick fibres revealed some differences in the rate of change in mass per temperature curves. The study measured the initial weight loss for each fibre at 3.0 % to examine the temperature at which this loss occurred. It was found that for AF single fibres, the 3.0 % initial weight loss occurred at 73.28 °C however for AF thick fibres, the 3.0 % initial weight loss occurred at 69.11 °C. This initial weight loss as depicted by the rate of weight change curve occurred between (25-100 °C) probably due to the loss of moisture and other volatile materials in the fibre samples. It was also observed that
there was no weight loss between 100-200 °C for both AF single and thick fibres, signifying the working service (thermal processing) temperature range for the AF cassava fibres. This was followed by a major weight loss of 68.58% between the temperatures of 238°-380°C, with the peak at 319°C for AF single fibres and similarly for AF thick fibres, weight loss of 68.47% occurred between the temperatures of 248°C-384°C, with the peak at 358°C in nitrogen atmosphere. This degradation is thought to be related to the decomposition of cellulose, lignin and hemicellulose (200°C - 360°C) (Tomczak et al., 2007). The only difference observed between the single and thick fibres here was found in the thermal derivative curves, where AF single showed two peaks during the degradation of fibre content while AF thick showed one peak. These results suggest that both AF single and thick fibres have similar thermal degradation behaviour. Figure 30 (a and b) below also shows the thermal degradation profile for ID4 single and thick fibres, respectively.

Figure 30: Thermogravimetric curve for (a) ID4 single fibres and (b) ID4 thick fibres
It was observed that for ID4 single fibres, the 3.0% initial weight loss occurred at 63.57 °C while for ID4 thick fibres, it occurred at 60.62 °C. Like AF fibres, it was also found that there was no weight loss between (100-200 °C) for both ID4 single and thick fibres. It was found that the degradation of the fibre content (71% weight loss) for ID4 thick fibres occurred between 254-378 °C, with two peaks at 319°C and 362°C, suggesting the degradation of the cellulose and lignin material in the fibre. Similar behaviour was found for ID4 single fibres, where 69.35% weight loss of the fibre content occurred between 263-377 °C, with two peaks at 318 °C and 364 °C. Based on these results ID4 single and thick fibres have similar thermal degradation profile with Figure 31 (a and b) below also shows the thermal degradation profile for ID6 single and thick fibres, respectively.

Figure 31: Thermogravimetric curve for (a) ID6 single fibres and (b) ID6 thick fibres
Like the thermal behaviour observed in AF and ID4 fibres, ID6 fibres also showed a comparable thermal behaviour pattern with differences in the mass loss peaks. From figure 31 above, it was found that ID6 single and thick fibres exhibited somewhat similar thermal behaviour where their 3.0% initial weight loss occurred at 61.86 °C and 68.19 °C, and the major weight loss because of the degradation of the fibre content, was found to be 67.88% and 68.67% respectively. Again, these results, suggest that both ID6 single and thick fibres have similar thermal degradation profiles with minimal variation in rate at which mass is lost per temperature.

Table 15 below presents the summary of weight loss and temperature variation of different varieties of cassava fibres.

**Table 15: Summary of weight loss and temperature variation for different genotypes of cassava fibres**

<table>
<thead>
<tr>
<th>Variety of Cassava fibre</th>
<th>Initial 3% weight loss temperature (°C)</th>
<th>Rapid weight loss temperature (°C)</th>
<th>Major weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF Single</td>
<td>73.28</td>
<td>252.07</td>
<td>68.58</td>
</tr>
<tr>
<td>AF thick</td>
<td>69.12</td>
<td>248.35</td>
<td>68.47</td>
</tr>
<tr>
<td>ID6 Single</td>
<td>61.86</td>
<td>261.33</td>
<td>67.89</td>
</tr>
<tr>
<td>ID6 Thick</td>
<td>61.19</td>
<td>255.61</td>
<td>68.67</td>
</tr>
<tr>
<td>ID4 Single</td>
<td>63.57</td>
<td>263.07</td>
<td>71.65</td>
</tr>
<tr>
<td>ID4 Thick</td>
<td>60.62</td>
<td>254.24</td>
<td>69.34</td>
</tr>
</tbody>
</table>

From Table 15 it was found that the initial 3% weight loss temperature, rapid weight loss temperature and major weight loss obtained by the different varieties of cassava fibre did not differ significantly.
4.4 Cassava fibre morphology and aspect ratio

Figure 32 below shows the morphology of single cassava fibres from Optical Microscopy and Scanning Electron Microscopy.

From Figure 32b it was observed that both single and thick fibres have similar morphology with the presence of some residual starch (whitish material) around the fibres. Figure 32d also shows the cross-section of cassava single fibre where the internal lumen within the fibre is labelled. It is interesting to note that the pores in the fibres had an average diameter of 146.5±48.25 µm.

4.4.1 Selection of cassava fibre as reinforcement material for composite scaffold fabrication

From the tensile test analysis, ID4 fibre recorded the highest tensile strength and Young’s modulus when compared to the other cassava genotypes both at specific gauge lengths and different gauge length, therefore it was selected as the reinforcement
material for the gelatin composite scaffold development. The diameter of the pulverised cassava microfibres used in the fabrication of composite scaffolds ranged between 9 to 447 µm with an average of 115±102 µm and the length of the pulverised fibres ranged between 86.67-1259 µm with average of 674±355 µm. The fibre aspect ratio (length / diameter) of the pulverised ID4 cassava fibre employed as reinforcement in the composite was calculated to be 11.91 which falls in the category of short discontinuous fibres (Kalpakjian et al., 2001).

4.5 Three-dimensional cassava cellulose microfibre/gelatin scaffold morphology and microstructure analysis

4.5.1 Morphology of scaffolds

This study employed short discontinuous cassava cellulose microfibres from ID4 single variety as a reinforcement material in gelatin composite scaffolds, as shown in figure 33 below. The composite scaffolds were fabricated with different fibre weight fraction relative to gelatin solution (gelatin + water + fibres) before freeze-drying, namely 0%, 3%, 5% and 7%. The actual fibre weight fraction in scaffold composite (fibre + gelatin + pores) after freeze-drying was calculated to be 0%, 75.4%, 84.03% and 88.2%, respectively. The fibre volume fraction of the scaffolds was then determined to be 0%, 8.4%, 11.4% and 15.7%, respectively.

Figure 33 shows the photographs of samples of 3-D cassava fibre-gelatin composite scaffolds fabricated by freeze-drying.
The cassava fibre/gelatin scaffolds showed a 3-D foam-like structure with short discontinuous cassava fibres interspersed randomly within the gelatin matrix and had sufficient stiffness for easy handling. The scaffolds produced had an average thickness of (depth) of 5.2 mm. The surface morphology of the cassava fibre-gelatin scaffolds fabricated appeared to be much rougher (Fig 34 b,c,d) compared to pure gelatin scaffolds (Fig 34a). It was interesting to note that the incorporation of the cassava fibres in gelatin composite scaffolds led to a significant change in the morphology of the scaffolds as observed by SEM and in optical micrographs shown in figures 34 and 35 below. The effect of the fibres on the scaffold surface roughness could improve cell – matrix adhesion during cell culture. This effect of the fibres on scaffold surface morphology was also reported by Xing et al. (2010), who studied the incorporation of nanocellulose fibres from wood kraft in gelatin scaffolds. The random dispersed orientation of the cassava cellulose microfibres within the gelatin matrix as shown in (Figure 37 b, c) below, could serve as contact guidance for directing and spacing of cells to grow along the fibres during tissue culturing, thereby potentially reducing the cluster growth of cells.
4.5.2 Scaffold porosity and pore size analysis

Tissue engineering scaffolds are required to be highly porous with an interconnected pore network to easily facilitate the diffusion of nutrients and oxygen into the matrix and additionally improve cell seeding during cell culture (Hollister, 2005; Loh and Choong, 2013). The pure gelatin scaffold and the cassava microfibre/gelatin scaffolds produced in this study were highly porous with good interconnected pore networks.

![SEM micrographs of the surface structure of gelatin/cassava microfibre foams with various fibre content: (a) 0 wt.%. (b) 3 wt.% (c) 5 wt.% and (d) 7 wt.%). White arrows showing the presence of fibres](image)

Figure 34: SEM micrographs of the surface structure of gelatin/cassava microfibre foams with various fibre content: (a) 0 wt.%. (b) 3 wt.% (c) 5 wt.% and (d) 7 wt.%). White arrows showing the presence of fibres

Figures 34 above and 35 below, show both SEM and the Optical micrographs of the surface structure of pure gelatin and gelatin/cassava microfibre scaffolds with various fibre content: (a) 0 wt.%. (b) 3 wt.% (c) 5 wt.% and (d) 7 wt.%), respectively.
Both figures show the porous structures of the different types of scaffolds fabricated and their interconnected matrices.

Figure 35: Optical micrographs of the surface structure of gelatin/cassava microfibre foams with various fibre content: (a) 0 wt.%. (b) 3 wt.% (c) 5 wt.% and (d) 7 wt.%). White arrows showing the presence of fibres.

The porosity measured by gravimetric method and porosity measured through image analysis for different scaffold types showed similar results. It was found that there were no major differences in the average porosity obtained for scaffolds using different the methods. Pure gelatin scaffolds (0% fibre) obtained a high porosity of 98.9% by density measurements and 92.2% by surface image analysis technique. Scaffolds with 7% fibre load reported an average porosity of 83.4% and 84.1% by gravimetric and image analysis methods, respectively.
Figure 36: Porosity of scaffolds by different techniques

It was found that the thin cross-section porosity for all the scaffolds was lower compared to the surface porosity. These variations were observed in Figure 37 and 38 below through SEM and an Optical microscopy.

Figure 37: SEM micrographs of the cross-section structure of gelatin/cassava microfibre scaffolds with various fibre content:(a) 0 wt.% (b) 5 wt.% and (c) 7 wt.%)
Figure 38: Optical micrographs of cross-section structure of gelatin/cassava microfibre foams with various fibre content: (a) 0 wt.%. (b) 3 wt.% (c) 5 wt.% and (d) 7 wt.%

The pure gelatin scaffold was found to be highly porous compared to the composite scaffolds because in the freeze-drying process, the water in the emulsion forms ice crystals during freezing and the ice crystals then sublime under vacuum drying to leave pores in the scaffold (Zhao and Chang, 2004). The volume of ice crystals is increased when a lot of water molecules accumulate within the matrix. The presence of cassava cellulose microfibres in the gelatin matrix could lead to the absorption of water molecules due to their hydrophilic nature making the water unavailable for pore formation. Also, the presence of cassava fibres in the gelatin matrix could decrease the energy to crystal nucleation (Frydrych et al., 2011) which may lead to a decrease in porosity in the cassava cellulose fibre/gelatin composite scaffolds.

The distribution pattern of the detected surface pores observed in the composite scaffolds is shown in Figure 39 below. The results indicated that about 91% of the
detected surface pores in the cassava fibre/gelatin composite scaffolds had pore diameter between 5-100 µm with mean pore diameter of 36.29 ±12.23 µm.

![Figure 39: Distribution pattern of surface pores detected for fibre-gelatin scaffolds](image)

The pore diameter determined for the composite scaffolds is large enough to allow for the diffusion of oxygen and nutrients to cells within the matrix for growth. Although the freeze-drying technique is known to produce highly porous scaffolds (Mandal and Kundu, 2009; Subia et al., 2010), it is usually limited to small pore size (Mandal & Kundu, 2008).

### 4.6 Mechanical properties of 3-D cassava microfibre-gelatin scaffolds

#### 4.6.1 Scaffold compression test analysis

The study examined the effect of cassava fibres as reinforcement material on the mechanical properties of the gelatin composite scaffolds. Figure 40 below presents samples of the stress-strain curves for the scaffolds fabricated in this study. The scaffolds were compressed at a fixed strain rate of 0.00833 mm/sec to 40% strain endpoint at room temperature.
Figure 40 Stress-strain curves for cassava microfibre/gelatin scaffolds with different weight fraction: (a) 0% fibre scaffold ($\rho=0.0152 \text{g/cm}^3$), (b) 3% fibre scaffold ($\rho=0.0591 \text{g/cm}^3$), (c) 5% fibre scaffold ($\rho=0.072 \text{g/cm}^3$) and (d) 7% fibre load ($\rho=0.095 \text{g/cm}^3$)

The addition of different weight fraction of cassava fibre relative to the gelatin solution (0%, 3%, 5% and 7% respectively) changed the compression behaviour of the scaffolds as shown in the stress-strain curves (figure 40). Cellular solid or polymeric foam is made up of interconnected network of solid struts or plates or walls which form the edges and faces of cells (Gibson and Ashby 1997). Cellular solids or polymeric foams can have open-cell structure, closed-cell structure or open-closed cell structure (as illustrated below in figure 41 by Gibson and Ashby, 1997) depending on the fabrication techniques.
Figure 41: Examples of cellular solids adopted from Gibson and Ashby (1997): (a) 2-D honeycomb, (b) 3-D foam with open cells and (c) 3-D foam with closed cells

The physical mechanism governing the deformation of three-dimensional cellular solids or foams was studied by Gibson and Ashby (1982) through their studies of 3-D open-cell foams and closed-cell foams as shown in Figure 42 below. From the scaffold compression test results shown in figure 40 above, it was found that the 0% fibre scaffold stress-strain curve compared with the stress-strain curve for open-cell foam examined by Gibson and Ashby (Figure 42a).

Figure 42: Sample compression behaviour of foams adapted from Gibson and Ashby (1982): (a) open-cell flexible polyurethane foam, (b) closed –cell flexible polyethylene foam and (c) closed –cell rigid polyurethane foam
The presence of the fibres totally changed the shape of the stress-strain curve obtained for pure gelatin scaffold (Figure 40 b, c, and d respectively), where it began to behave like open-cell and closed-cell foams illustrated by Gibson and Ashby (Figure 42).

According to Gisbon and Ashby, (1982) When polymeric foams are compressed, the cell walls of the foam initially bend leading to the initial near-linear region of the stress-strain curve, this region occurred around strain of about 5%, as shown in this study (Figure 40) and which was consistent with the findings of Gibson and Ashby (Figure 42). The slope of initial linear region of the stress-strain curve was determined as the Young’s modulus of the scaffolds. When a critical stress is reached the cell walls buckle and the foam collapses, owing to the rapid rise in constant stress which is characterised by a non-linear elastic deformation (plateau). The solid cell walls then touch each other at high strains, terminating the plateau to cause the stress-strain curve to rise steeply (densification) usually at high strains of about 60%. From Figure 40 above, it was found that the plateau regime of the stress-strain curves for the composite scaffolds either diminished quickly or the strain at which the densification regime started reduced. (Note that the scaffolds in this study were only compressed to a maximum of 40% strain). According to Gibson and Ashby (1997) this behaviour could be due to an increase in the relative density of the foam which increases the relative thickness of the cell walls. An increase in cell wall thickness would increase the resistance to the cell wall bending and cell collapsing. This would cause a rise in elastic modulus and plateau stress, and the cell walls touching quicker to reduce the strain at which densification begins (Gibson and Ashby, 1997).

Figure 43 shows the average maximum compressive strength at 40% strain endpoint
for scaffolds with different weight fractions of cassava fibres examined in this study.

Groups with different superscripts are significantly different at $p<0.05$

**Figure 43: Effect of cassava fibre load on maximum compressive strength of scaffolds**

From figure 43, it was found that the compressive strength of the scaffolds increased steadily with increasing fibre weight fraction. It was found that the inclusion of the cassava fibres in the gelatin composite scaffolds had significant effect ($p=0.001$) on the compressive strength of the gelatin composite scaffolds. Tukey post hoc multiple analysis showed the average maximum compressive strength for the 0% fibre (pure gelatin) scaffold ($0.036 \pm 0.01$ MPa) was significantly ($p<0.05$) increased with the presence of 3 wt. % fibre, 5 wt.% fibre and 7 wt. % fibre loads, respectively. Although there was no significant difference ($p>0.05$) between compressive strength obtained between 3% fibre and 5% fibre load scaffolds, the 7 wt. % fibre load composite scaffolds recorded a maximum compressive strength of $0.292\pm0.02$ MPa, about eight (8) times higher than the pure gelatin scaffolds. These results indicate the possibility for the efficient stress transfer from the gelatin matrix to the cassava...
fibres.

The results of the Young’s modulus of the scaffolds obtained from the experiment are shown in Figure 44 below.

![Figure 44: Effect of cassava fibre load on the Young’s modulus of gelatin scaffolds](image)

*Groups with different superscripts are significantly different at p< 0.05*

From Figure 44, it was found that the addition of 3 wt. % and 5 wt. % fibre loads to the gelatin scaffolds did not significantly influence (p>0.05) the compressive modulus of the gelatin scaffolds, although the average Young’s modulus of the 0% fibre scaffold was increased marginally from (0.308 ±0.03 MPa) to (0.32±0.03MPa) and further, to (0.35±0.03 MPa) under 3 wt. % and 5 wt. % fibre loads, respectively. However, the addition of 7 wt. % fibre significantly (p=0.001) increased the Young’s modulus of pure gelatin scaffolds from (0.308 ±0.03 MPa) to (1.308 ±0.03MPa), about four times higher.

Overall, it was found that the inclusion of the cassava fibres in the gelatin scaffolds improved the compressive strength and Young’s modulus of the scaffolds. This improvement could be linked to the strong interactions between gelatin matrix and
cassava cellulose microfibres, as well as efficient stress transfer from the matrix to the fibres. Therefore, it can be concluded based on these results that, the strength and stiffness of the cassava fibre-gelatin composite scaffolds were improved with increasing cassava cellulose microfibre load, demonstrating the potential use of these foams in biomedical fields.

**4.7 Preliminary theoretical modelling of the mechanical properties of polymeric foams and discontinuous fibre reinforced composite**

To further understand the mechanical behaviour of polymeric foams or cellular solids and the discontinuous fibre reinforced composites, theoretical constitutive models developed by other researchers that could explain the modulus of the pure gelatin foam and the fibre reinforced composite were applied by using the data from this study. Theoretical models considered in this study were the Gibson-Ashby model, the Iso-strain rule of mixtures and the Halpin-Tsai model (Halpin, 1969).

According to Gibson and Ashby (1982 and 1997) the elastic modulus of cellular foams is related to the geometry of the cell wall material, relative density of cellular solids (1-porosity) and internal microstructure. As explained in section 4.6.1, cellular solids or foams under compression, the cell walls bend initially to give a linear-elastic regime, followed by a buckling of cells when a critical stress is reached to show a plateau with some constant stress, leading into a final regime where cell walls touch to yield an abrupt rise in stress (densification). An increase in the relative density of the foam leads to an increase in the cell wall thickness, which increases the resistance to the cell wall bending and cell collapse and gives rise to a higher modulus (Gibson-Ashby, 1997). The theoretical constitutive model for the Young’s modulus of cellular foams in this
study was:

\[ E_F = \left[ C E_G \left( \frac{\rho_F}{\rho_G} \right)^2 \right] \quad \text{Eqn 17} \]

Where \( E_F \) = Predicted Young’s Modulus of the gelatin foam (MPa)

\( C \) = constant which depends on the cell-wall geometry (assumed to be 1 for open-cell foams)

\( E_G \) = Theoretical Modulus of polymer cell wall material (gelatin), assumed to be 1.5 GPa (Frydrych et al., 2011)

\( \rho_F \) = Density of foam measured from the experiment

\( \rho_G \) = theoretical density of the solid cell wall material (theoretical density of gelatin assumed to 1.3 g/cm\(^3\)).

To further elucidate the effect of the different fibre load on the young’s modulus of the composite scaffolds, the iso-strain rule of mixtures theory was applied, where the Gibson-Ashby model equation was represented in the iso-strain rule of mixtures model to account for the elastic modulus of the pure gelatin matrix. In the rule of mixtures theory, the predicted elastic modulus is dependent on the volume fraction of fibre in the composite. Equation 5 below represents the iso-strain rule of mixture model employed in this study.

\[ E_c = (1 - VF_{F/c}) \times \left[ k C E_G \left( \frac{d}{d'} \right)^2 \right] + VF_{F/c} \times E_F \quad \text{Eqn. 18} \]

Where the \[ k C E_G \left( \frac{d}{d'} \right)^2 \] corresponds to the elastic modulus of pure gelatin foam within the rule of mixture model.
K- Fitting parameter introduced by the researcher to fit the predicted elastic modulus of the gelatin foam to be equal to the elastic modulus recorded in this study.

\( E_c = \) Predicted Modulus (MPa) of the composites with different fibre volume fraction.

\( C = \) constant which depends on the cell-wall geometry (assumed to be 1 for open-cell foams)

\( E_G = \) Theoretical elastic modulus of polymer cell wall material (gelatin), assumed to be 1.5 GPa (Frydrych et al., 2011).

\( \rho_M = \) Density of gelatin matrix (gelatin + pores) in the composite scaffolds.

\( \rho_G = \) Theoretical density of the solid cell wall material (theoretical density of gelatin assumed to 1.3 g/cm\(^3\)).

\( V_{F/c} = \) Volume fraction of fibres in the composite scaffold.

\( E_F = \) Modulus of fibre [using experimentally determined elastic modulus of fibres = 365 MPa]

Additionally, the study also applied the Halpin- Tsai model which is mostly used by researchers (Halpin, 1969; Kordani et al., 2014; Qian et al., 2000; Yeh et al., 2006) when studying random discontinuous fibre composites. This is because the model considers the aspect ratio of the fibres, volume fraction of fibres and the strengths of the polymer matrix and the fibre. The model was expressed as:

\[
E_c = \left( \frac{1 + CnV_{F/c}}{1 - nV_{F/c}} \right) \times E_{\text{matrix}} \tag{Eqn. 19}
\]

\[
n = \left( \frac{E_f}{E_{\text{matrix}}} \right)^{-1} - 1 \left( \frac{E_f}{E_{\text{matrix}}} \right) + C \tag{Eqn. 20}
\]

\[
c = \left( \frac{1}{\alpha} \right) \times 2 \tag{Eqn. 21}
\]
Where, $E_c = $ predicted modulus of scaffolds, $E_{\text{matrix}} = $ Young’s Modulus of pure gelatin scaffold or matrix, $l = $ fibre length, $d = $ fibre diameter, $F_{F/c} = $ Fibre volume fraction in scaffold,

$E_f = $ Modulus of fibre [using experimentally determined elastic modulus of fibres = 365 MPa]

Figure 45, 46 and 47 below shows the results from the theoretical modelling of the elastic modulus for composite scaffolds with different fibre volume fraction using different models.

![Bar chart showing comparison of elastic modulus](chart.png)

**Figure 45: Comparison of the elastic modulus from the compression test and that predicted by the Gibson-Ashby model**

It was found that the Gibson model could well predict the elastic modulus of the 0% fibre load scaffold (pure gelatin scaffold), however the elastic modulus predicted for the 3% fibre load relative to the gelatin solution, was higher compared to the modulus
recorded by the compression test as shown in Figure 45 above. The Gibson-Ashby model mainly depends on the relative density of the scaffold. Although the density of the gelatin matrix in the fibre reinforced scaffolds did not change significantly, the density of the actual scaffold increased with increasing fibre volume fraction (as reported in Figure 40 above). This could account for the differences between the predicted elastic modulus by the Gibson-Ashby model and elastic modulus recorded from the compression test. Additionally, the Gibson-Ashby model does not consider the aspect ratio of the fibres and the randomly dispersed orientation of the fibres in the scaffolds.

The elastic modulus predicted by the iso-strain rule of mixtures and the Halpin-Tsai model are presented in Figures 46 and 47 below, respectively.

![Figure 46: Comparison of the elastic modulus from the compression test and that predicted by the iso-strain rule of mixtures model](image-url)
Figure 47: Comparison of the elastic modulus from the compression test and that predicted by the Halpin-Tsai Model

It was also found that the elastic modulus of scaffolds with different fibre volume fractions predicted by the iso-strain rule of mixture model was higher compared to the modulus recorded by the compression test. This variation could also be due to the fact that the iso-strain rule of mixtures only considers the longitudinal direction of the fibres, while this study fabricated scaffolds with randomly oriented discontinuous fibres.

However, it was interesting to note that the elastic modulus predicted by the Halpin-Tsai model (shown in Figure 47 above) had a good agreement with the elastic modulus recorded from the compression test with some minimal variations. This is because the Halpin-Tsai model accounts for the aspect ratio of the fibres and the random orientation of the discontinuous fibres in the composite scaffolds. Therefore, it can be concluded based on these results that, the Halpin-Tsai model was better suited to explaining the variabilities in the elastic modulus of the cassava fibre-gelatin composite scaffolds.
CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.0 Summary

This study was conducted mainly to investigate the material properties (mechanical properties, thermal degradation behavior, physicochemical and morphological characteristics) of single elementary and thick-core (vascular bundle) cassava fibres isolated from different cassava genotypes and examined the effect of incorporating cassava microfibres as reinforcement on the mechanical properties, and morphological characteristics of gelatin scaffolds. Mechanical properties, thermal degradation behavior, physicochemical and morphological characteristics of individual cassava single and thick-core fibres were also compared. Three-dimensional cassava cellulose microfibre/gelatin scaffolds with different fibre weight fractions were then fabricated using phase separation and freeze-drying methods. Cassava microfibre-gelatin microscaffolds were obtained and subjected to uniaxial compression testing to examine the effect of different fibre weight fraction on the compressive strength, and compressive modulus of gelatin scaffolds. Optical Microscopy and Scanning Electron Microscopy were used to examine the morphology, microstructure and architecture of different cassava microfibre-gelatin microscaffolds. Preliminary theoretical modeling of the elastic modulus of the cassava fibre/gelatin scaffolds was further conducted using Gibson-Ashby model, Iso-strain rule of mixtures and Halpin-Tsai model.

5.1 Conclusion

The following conclusions can be drawn from this study:
1. Different genotypes of cassava fibre showed significant differences in tensile strength and Young’s modulus, with ID4 fibre recording the highest average tensile strength of 7.567 ± 3.844 MPa and highest elastic modulus of 336.485 ±130.803 MPa. The strain at break recorded did not differ significantly among the cassava genotypes. Among these cassava genotypes examined, there was no significant difference in tensile strength, strain at break and elastic modulus recorded by the single elementary fibre and the thick-core fibre, suggesting similar crystalline structure. The results showed that cassava fibre has reasonable mechanical strength and stiffness and can be used as reinforcement filler to improve the mechanical integrity of tissue engineering scaffolds.

2. The tensile strength, Young’s modulus and strain at break of cassava fibres were found to decrease in magnitude as the gauge length increased. Gauge length was found to have significant effects on the tensile strength, elastic modulus and strain at break recorded by ID6 cassava fibre. However, gauge length had significant effect on only strain at break values recorded by AF and ID4 cassava fibres.

3. Different genotypes of cassava fibre showed similar diffraction pattern but minimal variation in peak intensities, with the presence of three crystalline peaks occurring at positions that are assigned to the the (200) crystallographic plane, (1-10) crystallographic plane and (110) crystallographic plane in native cellulose structures and an amorphous band occurring at the peak position close to the $\theta = 18.30^\circ-18.40^\circ$ reflection assigned to the amorphous phase. The crystallinity index determined for the different varieties of cassava did not differ significantly among the cassava genotypes.
4. The single fibres of the three cassava genotypes showed similar thermal degradation behaviour with minimal variation in rate of mass loss. The initial 3% weight loss temperature occurred between 60.62 °C and 73.28 °C due to moisture content and the rapid weight loss occurring at temperatures between 248.35 and 263.07 °C due to degradation of cellulose, hemicellulose and lignin.

5. The addition of cassava fibre had a significant effect on the morphological, microstructure and mechanical properties of the gelatin scaffolds. The cassava cellulose microfibre/gelatin scaffolds fabricated were highly porous with surface porosity ranging between 84 and 90% and had interconnected pores of average size 36.29 ±12.23 µm. The composite scaffolds showed surface architecture suitable for cell seeding and growth for tissue culture.

6. The incorporation of cassava fibres had a significant effect on the compressive strength and elastic modulus of the gelatin composite scaffolds. The 7% fibre load composite scaffold recorded a maximum compressive strength of 0.292±0.02 MPa, about eight (8) times higher than that for the pure gelatin scaffolds. The 7% fibre load scaffold significantly increased the Young’s modulus of pure gelatin scaffolds from (0.308 ±0.03 MPa) to (1.308 ±0.03MPa), about four times higher.

7. Elastic modulus predicted by the Gibson and Ashby model and Iso-strain rule of mixture model had a good agreement with the compression modulus determined for only pure gelatin scaffolds, however the Halpin-Tsai model was able to accurately predict the elastic modulus of the cassava fibre –gelatin composite scaffolds.
5.2 Contribution to knowledge

The study therefore, achieved the project objectives by providing detailed knowledge regarding the material properties (mechanical properties, thermal degradation behavior, physicochemical and morphological characteristics) of single elementary and vascular bundle (thick-core) cassava fibres and demonstrated the use of cassava microfibre as reinforcement in the development of tissue engineering scaffolds. These findings could inform biomaterials engineers and materials researchers about the bulk material properties of cassava fibre during evaluation of potential biomaterials for industrial application at the research and development levels.

5.3 Recommendations

It is recommended that further studies on biocompatibility testing (in vitro cytotoxicity tests) is conducted to assess the response of cells (fibroblasts) in direct contact with the cassava microfibre-gelatin scaffolds for cell viability and growth. Based on the mechanical properties recorded by the cassava microfibre-gelatin scaffolds, chondrocytes and human mesenchymal cells (hMSCs) could also be cultured on these scaffolds to further evaluate biocompatibility, ECM secretion and multilineage differentiation potentials. Scaffolds are also required to be biodegradable with nontoxic degradation by-products to gradually allow host cells to replace the implanted scaffold constructs over time, therefore it is recommended that a biodegradation study is conducted on the cassava microfibre-gelatin scaffolds. Additionally, the mechanical properties of the gelatin composite scaffolds could be optimized for example, the concentration of the gelatin solution could be increased to about 3 wt. % to improve the binding effect of gelatin on the cassava microfibres. Also, fibre aspect ratio could be
systematically varied in future work. Future directions can be also focused on chemically treating the cassava fibres to obtain pure cellulose fibres for comparison of mechanical properties with the non-treated isolated cassava fibres employed in this study.
REFERENCES


Podlipec, R. (2015). *The analysis of cell-biomaterial interaction by advanced experimental techniques as the basis for biocompatibility studies of polymers. UNIVERZA V MARIBORU (SLOVENIA).*


APPENDICES

APPENDIX-A

Analysis of variance showing the influence of gauge length on ID4 Fibre mechanical properties

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Analysis of variance showing the influence of gauge length on AF Fibre mechanical properties

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Analysis of variance showing the influence of gauge length on ID6 Fibre mechanical properties

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Kolmogorov Smirnov and Shapiro-Wilk normality test for AF thick and single fibres

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Variable is normally distributed at p>0.05

Kolmogorov Smirnov and Shapiro-Wilk normality test for ID4 thick and single fibres

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Variable is normally distributed at p>0.05
Table Kolmogorov Smirnov and Shapiro-Wilk normality test for ID6 thick and single fibres

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Variable is normally distributed at p>0.05

Figure 4.4: X-ray diffractograms of (a) AF single fibres and (b) AF thick-core fibres
Deconvolution and curve fitting of x-ray spectra for (a) AF single fibres and (b) AF thick fibres

K-ray diffractograms of (a) ID6 single fibres and (b) ID6 thick-core fibres

Deconvolution and curve fitting of x-ray spectra for (a) ID4 single fibres and (b) ID4 thick fibres
X-ray diffractograms of (a) ID4 single fibres and (b) ID4 thick-core fibres