

**EXTRACTION, VARIABILITY, ENZYMATIC BIOSOFTENING AND  
EVALUATION OF PHYSICO-MECHANICAL PROPERTIES OF  
*AGAVE AMERICANA L.* FIBRE**

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## **CERTIFICATION OF APPROVAL**

This is to certify that this dissertation titled: Extraction, Variability, Enzymatic Biosoftening and Evaluation of Physico-mechanical Properties of *Agave americana L.* fibre has been read and approved as having met the requirements of the Faculty of Natural and Agricultural Sciences, University of the Free State for the award of Doctorate of Philosophy in Consumer Science.

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## **DECLARATION**

I, 'Manonyane Albertina M. Mafaesa, declare that this thesis titled: Extraction, Variability, Enzymatic Biosoftening and Evaluation of Physico-mechanical Properties of *Agave americana L.* fibre except where otherwise indicated, that I herewith submit for the Doctor of Philosophy's Degree qualification at the University of the Free State is my genuine and innovative work and its materials have not been yielded before in full or in part, for the award of any academic qualifications at any other institution of higher education. Any work from the other authors that has been used, is acknowledged accordingly.

.....

SIGNED

.....

DATE

## **DEDICATION**

I dedicate this thesis to my beloved husband, Mr. Rabolou Mafaesa, and children, Mthink'hulu and 'Mamajoin Mafaesa for their words of encouragement, technical, financial, moral support as well as for their dazzling knowledge in information technology offered to shape this study. I also dedicate this study to my late parents who lived by example and from whom I learned how to work hard and achieve my aspirations.



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## LIST OF ABBREVIATIONS/ACRONYMS

°C	Degree Celsius
AGOA	African Growth Opportunity Act
ANOVA	Analysis of variance-variability ratio
BIC	Biobased Industries Consortium
BIO	Biotechnology Industry Organization
BIS	Bureau of Indian Standards
BMBF	Federal Ministry of Education and Research
BMCO	Blue multi-copper oxidoreductases
BMEL	Federal Ministry of Food and Agriculture
BOD	Biological Oxygen Demand
C # 6	Carbon number six
CAM	Crassulacean acid metabolism
CFC	Common Fund for Commodities
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
CoA	Coenzyme A
COD	Chemical Oxygen Demand
CrI	Crystallinity level
DO	Denomination of origin
DP	Degree of polymerization
FAD	Flavin Adenine dinucleotide
FAO	Food and Agricultural Organisation
FMN	Flavin mononucleotide

G	Grams
GDP	Gross domestic product
GFAR	Global Forum on Agricultural Research
GHG	Greenhouse gases
H <sub>0</sub>	Null hypothesis
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HFCs	Hydrofluorinecarbonates
IEC	International Commission on Enzymes
IPGRI	International Plant Genetic Resources Institute
IR	Infrared
IUB	International Union of Biochemistry
IUPAC	International Union of Pure and Applied Chemistry
IYNF	International Year of Natural Fibres
L	Litre
Lac	Laccase
LC	Lignocellulose
LCA	Life cycle assessment
LCCs	Lignin–carbohydrate complexes
LCFs	Lignocellulosic fibres
LGCSE	Lesotho general certificate for secondary education
LHW	Liquid Hot Water
LiP	Lignin peroxidase
ML	Middle lamella
MnP	Manganese peroxidase

MoET	Ministry of Education and Training in Lesotho
N <sub>2</sub> O	Nitrous oxide
NAD <sup>+</sup>	Nicotinamide adenine dinucleotide
NADP <sup>+</sup>	NICOTINAMIDE adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NLF	Natural lignocellulosic fibre
NUFS	Neglected and underutilized fibre species
OECD	The Organisation for Economic Co-operation and Development
PCW	Plant cell wall
PE	Pectinesterase
PFCs	Perfluorcarbonates
PG	Polygalacturonase
pH	The measure of acidity or alkalinity
PL	Pectin lyase
RUE	Resource use efficiency
(S1)	Primary cell wall
(S2)	Secondary cell walls
S <sub>1</sub>	First layer of the secondary cell walls
S <sub>2</sub>	Middle layer of the secondary cell walls
S <sub>3</sub>	Innermost layer of the secondary cell walls
SEM	Scanning electron microscopy
SF <sub>6</sub>	Sulphur hexafluoride
SSA	Sub-Saharan Africa
TDS	Total Dissolved Solid

Tris HCL	Tris hydrochloride
UK	United Kingdom
(U/ml)	Units per millilitre
NaOH	Sodium hydroxide
UN	United Nations
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organisation
USA	United States of America
VOCs	Volatile Organic Compounds
WUE	Water-use efficiency

## ABSTRACT

Unconventional natural textile fibre and enzymatic biotechnologies have positive eco-socio-economic impacts. They are increasingly demanded to substitute the conventional natural and petroleum-based synthetic fibres. The *Agave americana L* fibre is considered as a potential alternative to synthetic fibres. It is a natural, local, long, strong, absorbent, organic, renewable, eco-sustainable and prospective lignocellulosic fibre which is negligible, understudied and underutilised in textiles. This is due to its high lignin content that causes high fibre coarseness, stiffness and less cohesiveness; the properties which make it difficult to spin into a textile yarn. The overall aim of this research study was to extract the *Agave americana L* fibre with a triangulation water retting, investigate the inter-intra-plant fibre variability, enzymatic biosoftening and the physico-mechanical textile properties of *Agave americana L* fibre.

*Agave americana L* leaves were hand-harvested, ribbon stripped and water retted in closed drums to conduct triangulation water retting. The retted fibre was then manually separated from the pithy leaf biomass, washed and dried. The enzymatic biosoftening of the fibre was conducted with individual and sequential methods through biodelignification with MnP, bioscouring with pectinase, bio bleaching with xylanase and biopolishing with cellulase. The physico-mechanical properties and variability of the raw and enzyme-biosoftened fibre were appraised through visual-hand and instrumental evaluation methods.

The triangulation water retting was found highly effective, ecologically sustainable and produced good quality fibre at a comparable time for other plant fibres retted with other eco-friendly methods. Microscopic cross-sectional views revealed that the raw *Agave americana L* fibre is an irregular, compact, multi-cellular, thick composite consists of overlapping ultimate fibres which have large, diverse and polygonal central lumens. The composite is embedded in the natural cementing extracellular fibre components; mainly lignin, hemicellulose pectin and waxes. The enzymatic biosoftened fibre appeared as a flat multi-cellular composite with lumens smaller than those of raw fibre. The longitudinal SEM images show that the raw fibre is an elongated dimensionally varied, compact, rigid, rough-surfaced multi-cellular composite entrenched with impurities and middle lamellae residues. The enzymatic biosoftening processes have eliminated surface impurities and most of the noncellulosic fibre components. This elimination caused apparent fibre morphological

surface smoothness with less irregular cell wall features than the raw *Agave americana L.* fibre.

The structural fibre defibrillation was found subsequent to surface cleanliness and it intensified with the type and number of sequential enzymatic biosoftening processes applied. Thus, sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre experienced the highest biosoftening smoothness and structural defibrillation. The fibre enzymatic biosoftening efficacy was designated by weight loss percentage. The weight loss percentage ranged from 6.2 % to 24 %. The fibre tensile maximum load ranged from 4.64-22.61 N, displacement from 20.12-50.40 mm and initial Young's modulus; from 0-598.3 MPa. The stress-strain curve showed typical viscoelastic behaviour of a brittle fibre with apparent intra-and-inter plant fibre variability. The fibre showed the bending length ranges from 5.6 to 7.4 cm, out of 8cm. The most improved *Agave americana L.* fibre physical properties were observed from the sequential biosoftening processes.

The variability and stress-strain behaviour of *Agave americana L.* fibre are analogous to those of other lignocellulosic fibres. The enzymatic biosoftening of the local *Agave americana L.* fibre increases its serviceable efficacy without polluting the environment and endangering bioreserves. The *Agave americana L.* fibre is a sustainable potential textile fibre because it possesses satisfactory textile mechanical properties and the physical properties which have textile fibre draw-backs which can be improved by eco-friendly enzymatic biosoftening processes to upgrade its textile performance quality and appearance. The *Agave americana L.* fibre's potential textile alternative to synthetic fibres could preserve both synthetic and conventional natural fibre resources.

**Key words:** *Agave americana L.* plant and fibre, biodelignification, bioscouring, biobleaching, biopolishing, enzymatic biosoftening, fibre extraction, lignocellulolytic enzymes, lignocellulosic fibre, triangulation water retting, physico-mechanical properties, and fibre variability.

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# CHAPTER 1: INTRODUCTION, BACKGROUND AND PROBLEM STATEMENT

## 1.1 INTRODUCTION

This is the part of the study; that focuses on an introduction to the *Agave americana L.* plants and fibres. It also states the problem statement, and elaborates on the justification, purpose and significance of the study. It further looks into the conceptual framework, delimitations and limitations, assumptions of the research and defines some operational terms used in the research as well as gives an outline of the rest of the other chapters of the study.

### 1.1.1 Natural fibres bounce back in the 21st century

From the eco-socio-economic point of view, natural fibres are promising comfortable and sustainable alternative to non-degradable and non-renewable synthetic fibres, which pollute the atmosphere with harmful greenhouse gases which causes global warming (Elsasser 2010:248; Fillat *et al.*, 2017:1; Manimekalai & Kavitha, 2017:372). Natural fibres were well-established and played a significant role since the early civilization of mankind to produce textile and technical products to fulfil human needs (Anandjiwala, 2006:1; Sahu *et al.*, 2012:347; Tripathi & Tewari, 2015:1357; Vastrad *et al.*, 2015:198; Draman *et al.*, 2016:9591). For the last 100 years, mass-produced petrochemical man-made fibres have surpassed the natural fibres; in the global market (Konwar & Boruah, 2018:504). This was due to technological modernisation, the short-lived commercial gains of synthetics, their greater uniformity, easy handling triggered by their excellent resilience properties, poor absorbency which results in quick to dry property suitable for wash and wears items, dimensional stability and options for adaptations to fit particular purposes (Elsasser, 2010:89–90; Samuel *et al.*, 2012:780).

However, indiscriminate production, processing and use of synthetic fibres cause severe negative impact on the environment as they pollute the environment since the synthetics are non-biodegradable and non-carbon-dioxide neutral (Mylsamy & Rajendran, 2010:2925). Their production has also resulted in fast depletion of petroleum resources because they are non-renewable (Konwar & Boruah, 2018:504). They are also responsible for global warming because more carbon dioxide is being produced during their production (Edward and Mrinal, 2011:223; Ezeonu *et al.*, 2012:7; Ray *et al.*, 2013:296; Anwar *et al.*, 2014:165; Minderhoud, 2015:2; Omole & Dauda, 2016:48). They further pose problems related to their disposal. They release and

discharge heavy metals and other extracts onto the ground soil and underground water. Their affluent pre-treatment is expensive, so it is likely that it is not avoidable to most fibre production plants and also causes pollution (Allwood *et al.*, 2006:1).

Consequently, there is a turn away from synthetic fibres; towards natural fibres (Savastano *et al.*, 2009:56; Samuel *et al.*, 2012:780; Sisti *et al.*, 2018:98). The value and resurgence of natural fibres is due to their diverse uses in textiles, packaging and composites, cost-effectiveness, comfort, recyclability, 100% biodegradability (Mylsamy & Rajendran, 2010:2925, Teli & Jadhav, 2015:3848), renewability, high energy use efficiency, reduced ecological effects, reduced carbon footprint, carbon utilisation capabilities (Surajarusarn *et al.*, 2019:145), essential physico-mechanical and eco-friendly properties; which grant natural fibres first preference over synthetic fibres (Anandjiwala, 2006:1; Bacci *et al.*, 2010:827; Elsasser, 2010:56; Zimmiewska *et al.*, 2011:98; Verma, 2013:415; Naik *et al.*, 2014:1; Thanesh *et al.*, 2015:117; Oudiani *et al.*, 2015:16; Shah D.U. *et al.*, 2016:1481-1882; Draman *et al.*, 2016:9591).

The current turnaround is robust enough to necessitate an escalating consumption demands for natural fibres warrants intensified research and development for unconventional textile fibre resources (Elsasser, 2010:56; Singha & Rana, 2010:1055-1056; Jacob and Prema, 2008:11; Temesgen & Sahu, 2014:64; Bezazi *et al.*, 2014:195; Darshil *et al.*, 2016:1; Kozlowski & Mackiewicz-Talarczyk, 2012:104; Sahu *et al.*, 2012:347). It has become imperative to scout alternative renewable, eco-friendly and degradable natural sources for textile fibres which can replace conventional synthetic fibres (Martha & Carus, 2015:6; Doshi & Karolla, 2016:1, Djizi & Bouzaout, 2019:113). The exploitation of natural alternative fibres diverts underutilised fibres viable, valuable and diversified eco-socio-economic textile products (Bavan & Kumar, 2010:3604 & 3606; Barth & Carus, 2015:3; Singh, 2015:201).

### **1.1.2 World promotion of natural fibres**

Stringent environmental concerns, the non-renewable resources decrease, the fast-growing ecological developments and consumer awareness (Singh R, 2015:19) have compelled the use of sustainable natural fibre resources to move toward a biobased economic development (Ahmad *et al.*, 2019:1). The United Nations (UN) General Assembly in December 2006 avowed 2009 as the International Year of Natural Fibres (IYNF); with the aim to raise awareness and popularise the ecological, social and economic sustainability of natural fibres, contrary to the synthetic fibre (Adekomaya *et al.*, 2016:4). Natural fibres were promoted with the intention to concurrently

increase the sustainability and income generation options for natural fibre manufacturers, processors and dealers (Mchumo, 2009: ix; Vilane *et al.*, 2012:294; Yusof & Adam, 2013:1).

The most important reasons for natural fibres' promotion revolved around responsible, healthy, sustainable, technical and fashionable choices (Khandual & Sahu, 2016:46). According to the UN, natural fibres are the most responsible choice that boosts the eco-economy and livelihoods, food and fibre security of many small-scale farmers, processors and low-salaried workers in developing countries. The increasing international textile sustainability awareness and production fascinate the economic development and promote the venture into textile industries therefore, allowing countries involved to access the international market with no discrimination. This would fight against poverty and hunger in rural areas (FAO & CFC, 2009: iv). Natural fibres are also a healthier option than synthetic fibres because they provide more natural ventilation, wicking and thermal qualities than synthetics; they are hygienic, less allergenic and irritant, antibacterial, healing and have immune-boosting properties (Khandual & Sahu, 2016:46; Singh R., 2015:17).

Natural fibres are once again described as a sustainable option (Konwar & Boruah, 2018:504). As **such**, they reduce environmental contamination and mitigate climate change (Samuel *et al.*, 2012:780). Due to the Kyoto protocol, global textile markets are heading towards a green economy by reducing greenhouse gas and increasing carbon-neutral production (Yusof & Adam, 2013:1; Silva *et al.*, 2014:1068; Singh R., 2015:2017). Thus they emit and utilise at least an equal quantity of CO<sub>2</sub>, to combat global warming (Barth & Carus, 2015:3 & 5). They need less energy than synthetics during production and produce mainly organic by-products that can decompose to improve soil fertility and structure; and /or be used to generate various ecological, social and economic commodities like electricity (Lee *et al.*, 2011:156; Khandual & Sahu, 2016:46).

Natural fibres are high-tech fibres because they possess inherently high mechanical strength, lightweight and cost little. They also have heat and sound insulation properties better than their counterparts. They are effective geotextiles. Geotextiles are a new, promising outlet for natural fibre production because they decompose over time as the earthworks become stable and stabilize (Khandual & Sahu, 2016:46). Natural fibres are further described as a fashionable choice that is the core of a sustainable fashion development that exists at all stages of their existence (Singh R., 2015:19). Natural fibres are therefore potential eco-fashion garments sources. Thus, natural fibres are sometimes termed sustainably-produced, sustainably-processed and sustainably disposable. Natural fibres are given various names such as green, ethical, recycled, ecological, eco-environmental and sustainable (Khandual & Sahu, 2016:46).

### **1.1.3 The lignocellulosic Fibres**

Lignocellulosic fibres (LCFs) are fibres obtained from the plants and manufactured through the process of photosynthesis (Zimmiewska *et al.*, 2011:98). They are naturally abundant on earth infinite, relatively cheap, easy to process, biocompatible, biodegradable, and renewable and need relatively little energy to produce (Singha & Rana, 2010a:156; Shah D.U. *et al.*, 2016:1481). Thus, lignocellulosic fibres are considered as potential alternatives to fossil synthetic fibres to compensate for the increasing trend of the world's demand for renewable and sustainable fibres (Lee *et al.*, 2014:1-2; Silva *et al.*, 2014:1068; Draman *et al.*, 2016: 9591). Their sustainability properties along with their reasonable strength and stiffness globally, fulfil economic textile interest (Pecas *et al.*, 2018:2; Bora & Padmini, 2019:144). This reduces the dependency syndrome on expensive and exhaustible textile resources (Reddy & Yang, 2005b:191; Bin *et al.*, 2011:422; Abdel-Hamid *et al.*, 2013:1; Shahzadi *et al.*; 2014:246; Msahli *et al.*, 2015:1; Saini *et al.*, 2015:1; Amrita & Anjali, 2016:2).

Lignocellulosic fibres have remarkable mechanical and eco-friendly properties which give them competitive chances against strong synthetic fibres, have recently revived the increasing interest in their development (Kallioinen, 2014:1; Sträuber *et al.*, 2015:67). Conventional lignocellulosic fibres have currently gained ecological market popularity as textile fibres. Moreover the neglected and underutilised lignocellulosic fibres such as *Agave americana L.* fibre, sisal, jute, and hemp are emerging market potential supplements and alternatives to both conventional lignocellulosic and synthetic textile fibres, respectively (Mortazavi & Moghadam, 2009:3307; Shah D.U. *et al.*, 2016:1482). The lignocellulosic fibres have a lesser health hazard relative to their synthetic counterparts. The lignocellulosic fibres also have a lower environmental impact because they reduce and fix greenhouse gases (GHG) in the atmosphere. Thus, they prevent global warming and provide sustainable fibre security (Satyanarayana *et al.*, 2011:215; Balan, 2014:1-2). They also provide socio-economic benefits to people in developing countries where they are mostly produced (Monteirol *et al.*, 2011:4881). However, production and processing of lignocellulosic fibres consume more water and may use synthetic agricultural chemicals which emit greenhouse gases (Barth & Carus, 2015:3 & 5). Contrary to this, *Agave americana L.* fibre production does not need any chemicals or much water for irrigation (Stewart, 2015:3-4).

### **1.1.4 *Agave americana L.* plant origin, taxonomy, variegates and effect**

*Agave americana L.* plant is a monocotyledonous (a plant which generates one small leaf called a cotyledon from the sprouting seed during germination; (mono means single, cotyledon, means

seed leaf)), monocarpic succulent plant with thick fleshy marginal and terminal prickly leaves that form a large rosette (Hulle *et al.*, 2015c:65 & 66, Saraswat & Gope 2017:163); illustrated in figures 1 and 2. *Agave americana* L. plant is the type species for the genus *Agave* which also matures comparatively sluggishly (Adams & Adams, 1998:11). *Agave* (ag-AH-vee) is a scientific name described by Linnaeus around 1750s, for a Greek word “agauos” which means of kings and heroes, admirable, illustrious, magnificent hence noble plants (Castetter *et al.*, 1938:4; Mafaesa, 2006:40; Chattopadhyya & Khan, 2012:33), presumably for some of the species that are large stature and have bold structure and flowers. *Americana* (a-mer-i-KAY-na) means ‘of the Americas or from America because the plant originated from the United States of America and South America (Gonzalez-Valdez *et al.*, 2013:87; Kandhasamy & Vasudevan, 2015:848) and have been naturalized over the world (Castetter *et al.*, 1938:7; Audu *et al.*, 2014:55), even in Lesotho. *Agave americana* L. plant is well-known as century plant (Hulle *et al.*, 2015b:1; Misra & Varma, 2017:45), (from erroneous supposition that it blossoms only once in hundred years which is not the case Castetter *et al.*, 1938:5, it takes more or less than thirty years to bloom, there after it dies (Anandjiwala & John, 2010:183; Chattopadhyya & Khan, 2012:33), century-plant, spreading century-plant and wild century plant, American century plant, American-aloe, agave, American agave, American aloe, Aloe American (L.), “Aloe Vera” (Kolte *et al.*, 2012:1; Misra & Varma, 2017:45), flowering aloe, spiked aloe in English; yucca, Maguey or “miracle of nature” in Mexico, garingboom in Afrikaans (Castetter *et al.*, 1938:7; Tewari *et al.*, 2014:238), The Needle and Thread Plant” (Mylsamy & Rajendran, 2011:76; Zwane *et al.*, 2011:84) and in Lesotho; it is known as “Lekhala le leputsoa” meaning blue agave/aloe (figure 1.1). *Agave americana* L. plant is the commonest, most plentiful and diverse agave species (Rahmani *et al.*, 2015:1). Figure 1.1 illustrates the growing blue *Agave americana* L. plants years before blooming.



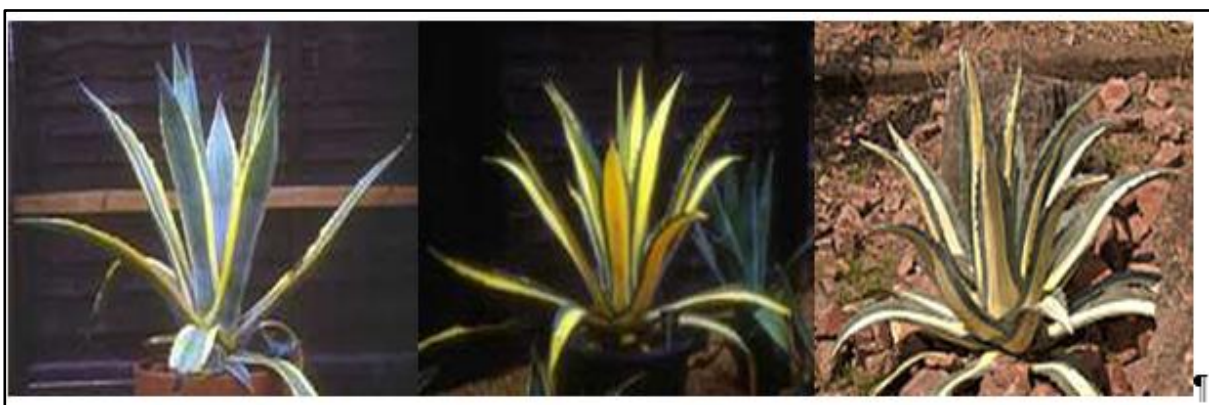
**Figure 1.1: Blue *Agave americana* L. plants (Austin Native Landscaping, 2014)**

Classification of *Agave americana* L. plant is as follows:



- Kingdom: Plantae - plants
- Division: Magnoliophyta–flowering plant
- Class: Liliopsida - monocotyledon
- Order: Asparagales
- Family: Agavaceae (Pronounced – ah-gav-AY-see-ee)
- Genus: Agave L.
- Species: *Agave americana*
- Binomial name: *Agave americana* L. (Mafaesa, 2006:3; Kolte *et al.*, 2012:2; Gonzalez-Valdez *et al.*, 2013:87; Rahmani *et al.*, 2015:1, Krishnadev *et al.*, 2020:2443).

*Agave americana* L. plant in its non-variegated form is commonly powdery-blue-grey in colour (figure 1.1) (Ortega *et al.*, 2019), and variegated varieties; figure (1.2), in which the leaves are variegated with white or yellow borderline or mid or all-over stripes starting from apex to base (Audu *et al.*, 2014:55). The main variegated forms include *Medio-picta* with a wide creamy white stripe that runs down the centre of each leaf and the gradually arched leaves, *marginata* is characterised by marginal creamy-yellow striped leaves, *striata*; have leaves with several yellow to white stripes and *variegata*; have white-edged leaves. A variety of variegated cultivars of *Agave americana* L. tend to be less hardy than the normal more compact and a vivid silver-grey coloured plant that can survive to -12°C if dry (Anandjiwala & John, 2011:183; Chattopadhyay & Khan, 2012:34; Spracklin, 2015:11&12). Figure 1.2 shows the growing different cultivars of variegated blue *Agave americana* L. plants.



**Figure 1.2: A variety of variegated cultivars of *Agave americana* L. plants (Hodgkiss 2016)**

*Agave americana* L. plant leaves are a rich source of strong natural lignocellulosic fibre (Thamae 2008:52), referred to as *Agave americana* L. fibre also called pita fibre (Angela, 2011:36; Vilane

*et al.*, 2014:395; Naimathullah, 2016:2). *Agave americana L.* fibre is a natural and unconventional plant fibre that is scientifically and technologically attractive for its potential characteristics for high-quality fibre production (El Oudiani, 2015a:15). The *Agave americana L.* fibre is a good alternative for synthetic fibres since it is abundantly available and it is also possible to use other components of the *Agave americana L.* plant for value-added by-products production (Hulle *et al.*, 2015b:1).

*Agave americana L.* fibre possesses more prominent prospective eco-sustainable benefits than synthetic fibres. It has lower density and needs simpler processing technologies than synthetic fibres. It is recyclable, eco-responsive, cost-effective, biodegradable, highly disposable and renewable (Kolte *et al.*, 2012:1, Bouaziz, 2014:2, Ortega *et al.*, 2019). However, the *Agave americana L.* fibre has been understudied and underutilised as a textile fibre; although it is worldwide used for technical fibre (paper, rope). The *Agave americana L.* plant is globally used for commercial (mezcal, pectin, pulque, aguamiel and tequila), ornamental and medicinal applications (Bouaziz, 2014:2; Kolte *et al.*, 2012:1). *Agave americana L.* fibre has lately recorded a rapid increase in research for textile potential (El Oudiani *et al.*, 2010:1). The leaves of *Agave americana L.* plant can be harvested from the 3rd year of life, when the base leaves have length more than a meter. Each *Agave americana L.* plant produces 40–50 leaves/year (Hulle *et al.*, 2015c:65). The purpose of the study is to explore alternative social, cost-effective and environmentally friendly methods for extraction, cleaning, bleaching and softening *Agave americana L.* fibre using enzymes in order to improve and add value to its textile applications.

### **1.1.5 Enzymes are Biochemical Products useful for textile bioprocessing**

The term 'Enzyme' was derived from the Greek word '*Enzymos*' denoting 'from the cells or 'in the cell' or Ferments'. Enzymes are the non-living things that are produced by the living animals, plants and microorganisms. They are inanimate like materials. Enzymes are biocatalysts consists of high molecular weight, globular, proteins comprise of elongated lined tangled chains of amino acids that fold a unique and complex 1-3; a three-dimensional structure that speeds up specific biochemical reactions (figure 1.3) which could otherwise be extremely sluggish. Enzymes catalyse biochemical reactions merely by their existence and without being used up in the process (Rehman & Imran, 2014:92).

Primitive man used enzymes way back in the earliest times of civilization in various food and beverage industries as well as in the clothing industry where skins and hide were tanned to

produce soft leather for garments. During the 20th century; the enzymology was intensified and revealed that enzymes are catalytic proteins with specific features that render them ecologically, socially and economically significant. In the 21st century; intensive research and development in existing enzyme-catalysed reactions, impelled the development of new sustainable technologies that address the increasing concern for the green economy (Polaina & MacCabe, 2007: ix-x).

Since then enzymatic wet chemical bioprocessing in the textile has gained interest because the increasing demand for sustainable and cost-effective consumer goods, natural fibre resources depletion, and social as well as environmental safety (Singh *et al.*, 2016b:1), they are harmless and environmental-friendly features that reduce pollution in textile fibre production (Jothi, 2013:2970; Bano *et al.*, 2017:1). Due to its eco-efficiency, safety and non-toxicity enzymatic biotechnology in textiles has increased tremendously (Teli & Adere 2016a:210). Enzymes work under mild conditions that include pH (the measure of acidity); temperature and pressure; they are produced with a smaller amount of energy consumption; they are renewable and biodegradable resources. Enzymes do not need any distinctive corrosion or heat resistant tools to function (Hossain and Uddin, 2011:15; Shrimali & Dedhia, 2016:674; Lima *et al.*, 2016:2). They have reduced environmental impact, contrary to traditional chemical wet processing, which is regarded as costly because effluents need to be specially treated before disposal otherwise; they are environmentally health-hazardous (Hasan *et al.*, 2015:16-17). Increased eco-friendly, sustainable consumer awareness and enzymatic biotechnology developments in the textiles created opportunities to investigate the prospective specific enzyme applications for softening lignocellulosic *Agave americana L.* fibre (Hasan *et al.*, 2015:16-17).

## **1.2 PROBLEM STATEMENT**

Textile sustainability is an increasing global concern due to a shortage of non-renewable textile resources and the production of solid, liquid and gaseous pollution wastes from various sources. There is also scarcity of fibre diversification and security in Lesotho. There are no identified potential plant fibres in Lesotho except *Agave americana L.* fibre. The *Agave americana L.* plant has been acknowledged as a potential local textile fibre source with high fibre yielding capability and can produce fibre with excellent textile fibre properties that include tensile strength, length-to-width ratio and absorbency (Mafaesa, 2006:126). However, it is a neglected, insufficiently investigated and underutilised textile fibre source because, of the absence of cognizance and knowledge of the significance of the plant and fibre (Mafaesa, 2006:92,111-113; Kanimozhi &



Vasug, 2012:220, Hulle *et al.*, 2015b:1). The full potential of the *Agave americana L.* fibre has not been fully explored to add value to the textile fibre security and poverty reduction. The *Agave americana L.* fibre is a by-product in the pharmaceutical industry. These imply that *Agave americana L.* fibre is a natural waste that does not add value to the fibre plant.

Traditionally *Agave americana L.* fibre extraction was done either by boiling the plant leaves and/or hand decortication. Boiling used a lot of energy and hand decortication is tedious, labour intensive and time-consuming. Currently, decortication can be carried out proficiently with mechanical decorticator (Hulle *et al.*, 2015:66), but there are no decorticating machines as yet in Lesotho to extract *Agave americana L.* fibre. Chemical acids and alkalis are successfully used to extract *Agave americana L.* fibre but they also increase fibre surface area and depolymerize the lignocellulosic fibre and reduce its breaking strength. They are also harsh and non-environmental friendly (Hulle *et al.*, 2015:67). The enzymatic retting is a superb, environmentally friendly and non-toxic biotechnology for fibre extraction. But, it is extremely costly and not yet accepted in the textile fibre extraction.

Water retting is the preferred pre-treatment for fibre extraction because it can be affordable, takes a shorter time, yields good quality and quantity fibre that is more uniform than the fibres extracted through dew retting and the aforementioned methods. However, it presents a problem of environmental pollution. Hence why there is a need to investigate an innovative and sustainable strategy that will minimize stated inadequacies of the conventional *Agave americana L.* whole leaf water retting.

The *Agave americana L.* fibre is a lignocellulosic fibre composed of gummy non-cellulosic constituents that include lignin; which leads to inherent and undesirable fibre stiffness, roughness, brittleness, dullness and darkness. These undesirable features contribute to fibre limited applications in textiles. The presence of gum with high lignin content in fibre chemical composition is the principal cause of these undesirable features. The inherent fibre coarseness, stiffness and brittleness limit fibre cohesiveness which is the fibre spinability property for yarn formation (Yang *et al.*, 2011b:377). Thus, the fibre lacks the basic textile properties to function as a textile fibre. It is therefore advisable to remove the gum content of the fibre so as to impart to it the basic softening textile fibre properties, through degumming. Thus, the fibre lacks the basic textile properties to function as a textile fibre. It is therefore advisable to remove the gum content of the fibre so as to impart to it the basic softening textile fibre properties, through degumming. The fibre is used in fibrous state; as a technical fibre that produces cords, ropes, twine and

sackcloth but not as a textile fibre which can be used to produce diversified aesthetic woven textiles. Lignocellulosic fibre softening can efficiently be conducted using chemicals that can damage the cellulose and negatively affect fibres' physical and mechanical properties, because they are harsh, hazardous and non-environmental friendly. These chemical treatments are no longer recommended because of these ecological, social and economic reasons (Pandey *et al.*, 2014b:41, Teli & Adere, 2016b:256). The public, textile researchers, consumers and policymakers are not aware of the possible practical benefits that can be obtained from its effective, efficient and sustainable fibre extraction, softening, upgrading and utilisation. The aim of this research study was to extract the *Agave americana L.* fibre with a sustainable and eco-friendly method and to biosoften the extracted fibre with selected commercial lignocellulolytic enzymes without adversely affecting the environment and its textile fibre properties.

### **1.3 RESEARCH JUSTIFICATION**

Harsh environmental concerns; diminishing non-renewable resources and consumer mindfulness to reduce ecological imbalance are acting synergistically to increase interest in maximizing the exploration, identification and use of alternative, innovative, renewable, sustainable, eco-efficient and organic plants which would lower carbon footprint so as to reduce environmental impacts and increase fibre production and security (Zimniewska *et al.*, 2011:98; Kopania *et al.*, 2012:167; Onyeagoro, 2012:491; Jagannathan & Nielsen, 2013:228-229; Sumi & Unnikrishnan 2015:260, Omole & Dauda, 2016:48).

There are global; ever-increasing demands for textile fibres' alternative sources which are safe and produce no or fewer pollutants including greenhouse gas than non-renewable fibres (Shivankar & Mukhopadhyay, 2019:1). *Agave americana L.* fibre was selected for study because it has a high potential and sustainable prospective textile future. *Agave americana L.* plants are alternative and potential biofibre sources. *Agave americana L.* plants grow wild in Lesotho. The use of wild-grown; organic *Agave americana L.* fibre is sustainable because *Agave americana L.* plants do not need the use of synthetic agronomic chemical inputs; thus it grows wild (Thamae 2008:54), it increases biodiversity and energy renewal and it reduces greenhouse gas emissions and pollution. This exploration study of *Agave americana L.* plants for fibre production can keep an ecological balance in nature because of its cheap means of planting, regional and all year round availability even under adversely extreme weather and soil conditions (Asim *et al.*, 2015:1-2). *Agave americana L.* plants thrive in semiarid and marginal lands and do not compete for

agricultural land; watering or agrochemicals. *Agave americana L.* plants are drought tolerant and use crassulacean acid metabolism, photosynthesis pathway. They, therefore, exhibit a positive impact on the water utilisation efficiency (WUE) from severe drought due to their water-conserving crassulacean acid metabolism (CAM) pathway. *Agave americana L.* plants also, grow well even on adverse soil and weather conditions that are unsuitable for food crops. Thus, they never compete for soil nutrients with food crops (Yang *et al.*, 2011b:380).

*Agave americana L.* plants utilise more carbon dioxide than they produce. The fastest-growing eucalyptus trees yield four times less cellulose than the *Agave americana L.* plants. The *Agave americana L.* plants are among the most promising sustainable 21st century fibre alternative crops that are eco-socio-economically viable future textile biofibre source. New employment prospects can also be generated in the collection and transport of *Agave americana L.* plants for fibre extraction, pre-processing, and the generation of biofibre and by-products. They are the only ones acknowledged as potential textile fibre plants in Lesotho that can function as viable alternate long and strong cellulose biofibres. *Agave americana L.* plant and fibre are also renewable, entirely biodegradable, abundant local textile resources. But *Agave americana L. fibre* is neglected, understudied and underutilised; yet it is obtained from high fibre yielding; multi-purpose plants that are on no account fully exploited, since all their components can be utilised one way or the other (Vogl & Hart, 2003:119; Mafaesa, 2006:7; Ray *et al.*, 2009:731). The exploration of *Agave americana L. fibre* will increase the bio-eco-socio-economic value of new alternative raw textile materials in Lesotho.

Triangulation water retting of *Agave americana L.* is an advanced technique using ribbon and closed tank retting principles which prevent and control environmental pollution as opposed to conventional open water retting. It is a feasible green technology that rets with reduced damage to livelihood and environment. It combines techniques that function hand-in-hand to complement and substantiate one another to increase the effectiveness and efficiency of water retting of *Agave americana L. fibre* through innovative, appropriate and comprehensive strategies which minimise inadequacies of conventional open whole *Agave americana L.* leaf water retting (Honorene, 2017:93). It contributes towards high-quality *Agave americana L. fibre*. Colour and dirt from the retted leaves usually leach out in the first few hours of retting process to produce bright non-stained *Agave americana L. fibre* resulting in light coloured, non-stained fibre. Triangulation water retting process is also faster and more effective than conventional open whole leaf water retting with three to four days. *Agave americana L.* plants are 100% biodegradable. During

triangulation water retting; the by-products generated are mainly organic and their residues can be used as manure to enrich the soil for food production (Hulle *et al.*, 2015b:73; Omenna *et al.*, 2016:275).

Exploring textile features of *Agave americana L.* fibre and sustainable biotechnologies for its upgrading; can prospectively improve and sustain livelihood of Basotho and other developing countries' overall economic growth by alleviating textile insecurity and poverty. *Agave americana L.* fibre can function as an essential alternative to synthetic fibre in textile technology. *Agave americana L.* fibre is dark and dull. It is therefore advisable to soften and brighten it in order to diversify its textile uses and end products. But enzymatic bioprocessing is also a sustainable endeavour. The use of enzymes in this research study is an effort to prevent the depletion of renewable resources and become more environment-oriented and practice renewable resource management for sustainable textile fibres that are to benefit both present and future generations.

It is anticipated that this research study will expand the native knowledge of native *Agave americana L.* fibre and preserve the *Agave americana L.* plants as the source of this fibre. This will further develop the local textile activities and status which will contribute to local eco-socio-economy. Enzyme biotechnology plays a relevant role in the development of sustainable technologies. Enzymes are renewable resources that can be replaced or reproduced easily, at a rate equivalent or quicker than its consumption rate by humans. The enzymatic utilisation fits into the principles of sustainability and green chemistry which demand waste prevention, use of less hazardous and less toxic chemicals and effects, safer products and processes, use of renewable resources, energy efficiency, and use of enzymes instead of harsh chemical reagents. Additionally, it is clear that a sustainable process is not only an environmental option but also a strategic choice (Sumi & Unnikrishnan 2015:261-262).

The upgraded *Agave americana L.* plant and its fibre are expected to play a significant role in Lesotho's economy, by improving earnings and providing employment. This research study is needed to identify the future alternative uses of *Agave americana L.* fibre that contribute to eco-socio-economic activities and increasing developmental opportunities. The exploration of *Agave americana L.* fibre safeguards indigenous knowledge; which entails the consumer awareness and undertakings of local; sustainable fibre resources to empower Basotho. *Agave americana L.* fibre softening can sustainably address livelihood improvement options for Basotho and convey information and develop awareness about indigenous plant fibres. Modern world textile needs demand the improvement of local, indigenous, sustainable textile fibres from renewable

bioresources as economic and ecological reasonable alternatives to limited petroleum-based synthetic fibres (Christy & Kavitha, 2014:26; Dungani *et al.*, 2016:43). It is, therefore, worthwhile that the *Agave americana L.* fibre is further explored and developed for textile utilization as a strategy to combat poverty.

## **1.4 PURPOSE OF RESEARCH**

Currently, in this resource sustainability-era, the natural fibres have gained attention in the textile world as they are decomposable and most crucially, renewable products (Shivankar & Mukhopadhyay, 2019:1). It is a fact that every research work is one based on originality that produces new knowledge instead of summarizing what is already known in a new form. The purpose of this study was not only to explore how the extraction of biofibre from the plant biomass has gained impetus due to the feasibility of an alternative process available to sustainably extract and upgrade the complex plant biomass fibres into textile resource biomaterials but also to study on the ecological, social and cost-effective methods of textile production and processing of *Agave americana L.* to remove the gummy non-cellulose material present on the surface of fibre to maintain its flexibility without causing severe damage to the structure of fibre would be very attractive. In this present investigation, the softening of fibre and quality aspects of *Agave americana L.* fibre will be analysed.

### **1.4.1 The general aims of the study**

The general aim of this research study was to extract the *Agave americana L.* fibre with a sustainable, affordable and eco-friendly method, explore enzymatic biosoftening processes, investigate the inter-intra plant fibre variability and the physico-mechanical textile characteristics of *Agave americana L.* fibre to meet the snowballing consumer eco-sustainability demands for natural fibres and diversify its functionality in the textile industry.

### **1.4.2 Specific objectives**

- To hand harvest *Agave americana L.* leaves for fibre extraction.
- To extract *Agave americana L.* fibre by using triangulation water retting biotechnology.
- To biodelignify *Agave americana L.* fibre using commercial manganese peroxidase (MnP) enzyme to impart the improved textile fibre properties with minimal negative effects on fibre strength.

- To bioscour *Agave americana L.* fibre using commercial pectinase enzyme to remove natural non-cellulosic components from the surface of the fibre, thus to improve its performance properties with minimal fibre damage.
- To biobleach *Agave americana L.* fibres using commercial xylanase enzyme to remove natural colour with pigments and improve fibre whiteness.
- To biopolish *Agave americana L.* fibre using commercial cellulase enzyme to improve the physical properties of fibre in order to impart the required functional textile properties.
- To use the commercial MnP, pectinase, xylanase and cellulase in sequence for biosoftening of the *Agave americana L.* fibre to improve the textile performance properties.
- To determine the weight loss percentage of boiled and enzyme-biosoftened *Agave americana L.* fibre.
- To evaluate the physical properties of raw, boiled and enzyme-biosoftened *Agave americana L.* fibre.
- To evaluate the maximum load, displacement at maximum load and initial Young's modulus as mechanical properties of raw and enzyme-biosoftened *Agave americana L.* fibre.
- To explore the intra-plant (when fibres are extracted taken from different parts of the same plant) fibre variability in *Agave americana L.* fibre physical and mechanical properties.
- To explore the inter-plant fibres extracted from different plants, different parts of the same plant at different levels and of the same leaf variability in *Agave americana L.* fibre physical and mechanical properties.
- Determine the stress-strain curves of both raw and enzyme-treated *Agave americana L.* fibre.
- Determine the morphological structure of *Agave americana L.* fibre in order to predict its textile performance properties.
- To measure the bending length of *Agave americana L.* fibre in order to predict its functionality as a textile fibre.

### 1.4.3 Hypotheses

The overall aims of this research study were to extract *Agave americana L.* fibre with a sustainable and eco-friendly method of fibre extraction, explore enzymatic biosoftening processes, investigate the inter-intra plant fibre variability and the physico-mechanical textile characteristics

of *Agave americana L.* fibre in order to satisfy the growing demand for natural fibres, sustain petroleum fibre resources and diversify its functionality in the textile industry.

Null Hypotheses were used to designate the research questions and expected outcomes.

H0<sub>1</sub>: Hand harvesting of *Agave americana L.* plant is impossible due to its sharp leaf marginal and tip spines.

H0<sub>2</sub>: Triangulation water retting is an ineffective and inefficient fibre extraction technique to disintegrate the non-cellulosic *Agave americana L.* leaf biomass to release fibre bundles.

H0<sub>3</sub>: The triangulation water retting is an environmental-hazardous *Agave americana L.* fibre extraction method.

H0<sub>4</sub>: The age of *Agave americana L.* leaves negatively affect the retting and fibre extraction processes.

H0<sub>5</sub>: The depth of retting water negatively affects the *Agave americana L.* leaves retting and fibre extraction processes.

H0<sub>6</sub>: *Agave americana L.* fibre is not a sustainable alternative textile fibre to the synthetic fibres.

H0<sub>7</sub>: *Agave americana L.* fibre is not a potential textile fibre.

H0<sub>8</sub> *Agave americana L.* fibre is an inconsistent and heterogeneous multi-cellular fibre composite.

H0<sub>9</sub>: There is insignificant intra-plant variability of *Agave americana L* fibre physico-mechanical properties

H0<sub>10</sub>: There is insignificant inter-plant variability of *Agave americana L* fibre physico-mechanical properties.

H0<sub>12</sub>: Enzymatic biodelignification is an inefficient wet processing textile technology to improve the texture of *Agave americana L.* fibre to qualify to be a textile fibre.

H0<sub>13</sub>: The enzymatic bioscouring of *Agave americana L.* fibre is an infeasible alternative to conventional scouring procedure for lignocellulosic fibre softening.



HO<sub>14</sub>: The enzymatic biobleaching of *Agave americana L.* fibre is not expected to remove natural colour with pigments and to improve the fibre whiteness.

HO<sub>15</sub>: Bio-polishing of *Agave americana L.* fibre with cellulase is an ineffective biosoftening biotechnology.

HO<sub>16</sub>: Sequential enzymatic biosoftening is ineffective when compared to an individual enzymatic biosoftening of *Agave americana L.* fibre.

HO<sub>18</sub>: The physical properties of *Agave americana L.* fibre are negatively affected by controlled enzymatic biosoftening processes.

HO<sub>19</sub>: The tensile properties of *Agave americana L.* fibre are negatively affected by enzymatic biosoftening processes.

HO<sub>20</sub>: Enzymatic biosoftening of *Agave americana L.* fibre is not an ecological alternative biotechnology to chemical auxiliaries in textile processing.

HO<sub>21</sub>: The effectiveness and efficiency of the enzymatic biosoftening of *Agave americana L.* fibre is inversely proportional to the fibre tensile strength.

HO<sub>22</sub>: The commercial enzymes are incapable of degrading the non-cellulosic polymers substrates of *Agave americana L.* fibre as a means for bio-softening and up-grading it.

HO<sub>23</sub>: The fibre bending length of *Agave americana L.* fibre is naturally low and is not improved by the enzymatic biosoftening processes.

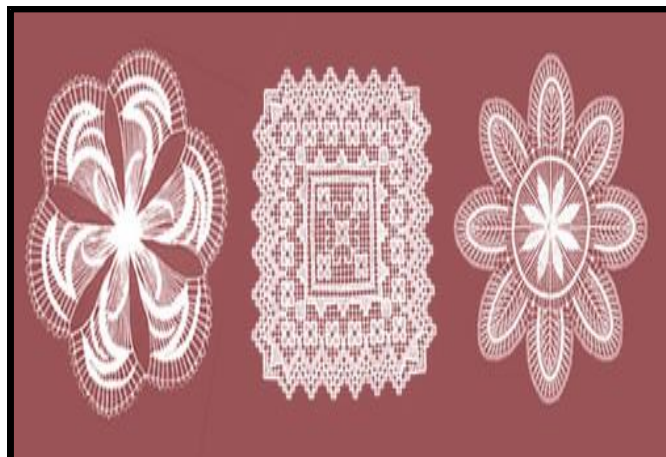
## **1.5 SIGNIFICANCE OF THE STUDY**

Underprivileged Basotho communities, mostly women will be empowered to harvest, extract and use *Agave americana L.* fibre for textile purposes and use its by-products to increase their incomes. This research provides scientifically proven information needed for maximum utilization of *Agave americana L.* plants and fibre to textile scientists and consumers. The socio-economic status of Basotho will be improved by increasing the local textile activities and income generating opportunities in the future. Exploration and communication of *Agave americana L.* fibre as a new fibre in Lesotho will increase the possibilities of eco-socio-economic benefits to domestic users. The research study is of great importance to Lesotho General Certificate of Secondary Education



(LGCSE) Fashion and Textiles curriculum; because it furnishes the curriculum developers, inspectors, implementers, examiners, learners and industrial users with relevant information about this local fibre. Thus; it overcomes the lack of knowledge and bridges gap between imported and local textile fibre demands. It is expected that market value and knowledge of native and local *Agave americana L.* fibre will be broadened.

This research would pave the way to empower craftsmen who will be inspired to use the sustainable local fibre to create the novel traditional souvenirs and modern artefacts like dainty lacework of the Azores (figure1.3 & 1.4), to be sold to tourists and in foreign market, through the African Growth Opportunity Act (AGOA). The AGOA gratifies the recipient countries to produce and promote their varieties of plant fibres to use for textile production and processing in order to reduce dependency on foreign textile inputs importation (Mbugua 2009:3). Figure 1.3 shows a picture of doily lace motives that can be constructed with a textile *Agave americana L.* fibre



**Figure 1.3: The Agave lace (Mara 2013:s.p)**

Figure 1.4 shows a picture of an *Agave* thread and Tenerife lace motives that can be constructed with a textile *Agave americana L.* fibre.



*Figure 1.4: Agave thread and Tenerife lace respectively (Mara 2013:s.p)*

*Agave americana L.* fibre exploration has future potential to improve the livelihood of Basotho through generation of employment in the fibre producing and processing activities and its inclusive value adding benefits. Thus; improve the socio-economic status of Basotho by supporting income generation opportunities that can transpire at various stages. The cottage income generation and employment activities are the first stage. People can prominently shift their main livelihood activities from conventional agricultural fibre production and processing to non-conventional; sustainable *Agave americana L.* fibre processing commercial activities owing to the high-risk rates associated with conventional fibre production and processing. Intensification of *Agave americana L.* fibre researches can add fibre value and upgrade the livelihoods of individual Basotho families through the divergence of income generation activities.

The development of local businesses producing native products specific to the region is likely to evolve when indigenous people are aware and have been inspired by the research information about *Agave americana L.* fibre. There is a potential market niche for the upgraded *Agave americana L.* fibre inherited under the scarcity of fossil natural resources, the potential future global climate change effects and the escalating world's population; or specific traditional culture of the regions locally and in the abroad markets. Unlike conventional fibres, non-conventional fibres that include *Agave americana L.* fibre, are increasingly gaining significance and respond to increasing demand for safe fibre due to their local availability, organic, biodegradable and eco-friendly characteristics that benefit the underprivileged living in marginal areas the most, by improving productivity and incomes, and promoting regional- environmental sustainability.

The third stage is the promotion of industrial expansion across the country. At this stage, the economic status of the Basotho can be promoted through diversification of domestic *Agave americana L.* fibre industries. However, speedily progress in the diversification of *Agave*

*americana L.* fibre usage is likely to be hindered; by the fact that *Agave americana L.* plant is a slow-growing fibre crop. The economically successful and sustainable, *Agave americana L.* fibre-based industries require vigilant planning, quality control and stronger links between research, ordinary and commercial developments (Takane *et al.*, 2010:2; FAO 2012: 11).

This research study triggered public and consumer awareness of viable, environmental and socio-economic benefits of organic *Agave americana L.* fibre production, enzymatic bioprocessing and potential utilisation to improve the functional and aesthetic value. It imparted potential market value to *Agave americana L.* fibre as an alternative, profitable plant fibre from Lesotho. This study encouraged valuing the national textile fibre production, as a means of preserving native raw materials. Exploration of *Agave americana L.* plant and fibre generated potential innovative crafts and businessmen for the nation and thus will nurture economic sustainable development.

## **1.6 CONCEPTUAL FRAMEWORK**

### **1.6.1 The theoretical framework**

Textile sustainability is the theoretical framework of this study. Research and development of sustainable biobased fibre and textile technologies that use eco-efficient, bioprocesses and renewable bioresources denote textile sustainability (Sharma 2013:768). Textile sustainability is a balance between environmental aspects such as pollution (eco-friendly) and climate (CO<sub>2</sub> neutral), social aspects such as safe working conditions and human responsibility and economic aspects such as reasonable price and good quality (Muthu, 2017:9). Sustainable textile fibres and processes capitalize on optimistic textile effects and decrease undesirable ecological, societal and cost-effective effects alongside their production and processing. They are energy-saving, renewable natural resources; nontoxic and healthy for workforces, people living nearby and customers (Curteza, 2012:5; Curteza *et al.*, 2017:7). Sustainable textile fibres are obtained from organic or wild and eco-friendly natural sources that grow without any pesticides, herbicides and chemical fertilizers, they are naturally biodegradable over time and often are also considered hypoallergenic, feel better against the skin and naturally antibacterial (Sirohi, 2016:25-26; Venkatachalam *et al.*, 2017:32).

Sustainable textile fibres and development; are positively influence the Earth and its inhabitants during their production, processing, utilisation and disposal. They neither harm the environment nor deplete natural resources. Thus supports ecological balance (OECD, 2001:7; Khan & Islam,

2015:2; Ozek, 2017:506). *Agave americana L.* fibre is a non-conventional fibre obtained from wild, organic and eco-friendly natural, plant (*Agave americana L.*) that does not use chemical fertilisers, herbicides and pesticides. It is an alternative; and potential textile fibre that can maintain and improve people's quality of life. However, it has never been researched seriously to develop it. Natural retting and enzymatic biotechnological softening of *Agave americana L.* fibre also are viably biotechnologies that utilise alternate bioresources and bioprocesses that are eco-efficient and produce products that are lucrative, more social and eco-friendly (OECD, 2001:5), than conventional chemical technologies (BIO, 2008:2). This implies that the study is based on the relationship between environmental conditions, *Agave americana L.* fibre extraction and biosoftening and upgrading fibre properties.

Figure 1.6 is an illustrative conceptual framework of the experimental processes and anticipated impact flow chart for this research study.

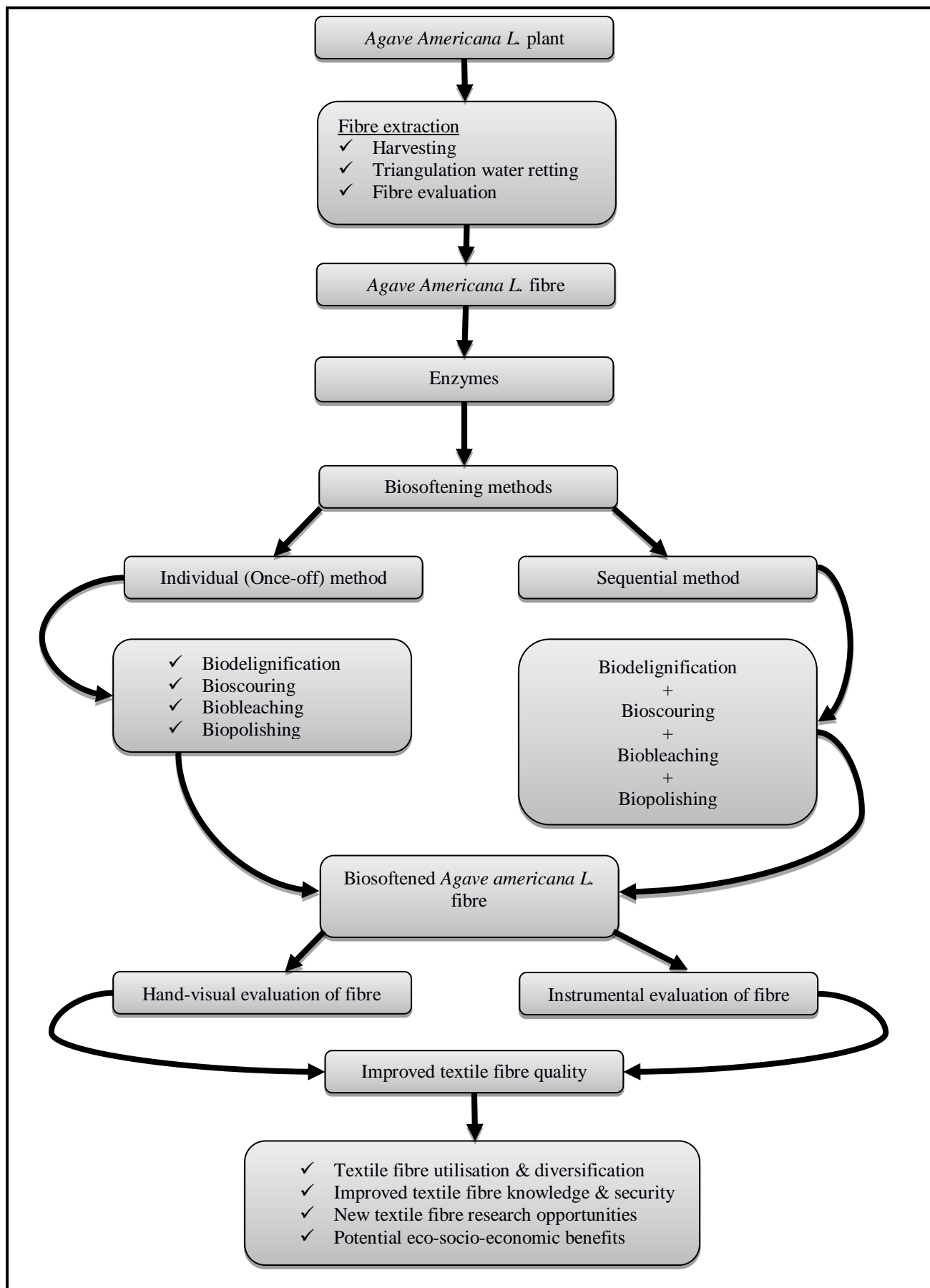


Figure 1.5: Flow chart for experimental procedure and anticipated impact

## 1.7 DELIMITATIONS AND LIMITATION

### 1.7.1 Delimitations (Scope) of the study

- This research study is on extraction, enzymatic biosoftening and the physico-mechanical evaluation of wild *Agave americana L.* fibre, extracted from *Agave americana L.* plants which were harvested from Sehlabeng-sa-Thuoathe in the Berea district in Lesotho.
- The study is further intended to raise awareness about the potential economically, environmentally and socially sustainable benefits that can be created by intensifying the *Agave americana L. fibre* extraction, processing and testing for textile utilisation.
- The experimental work is limited to three plants, each providing six leaves.
- The research is concentrated only on the triangulation water retting method of fibre extraction.
- Intra-and-inter fibre viability concept will be included in the study.
- Fibre quality upgrading biotechnology using the following lignocellulolytic enzymes: pectinase, pectate lyase, xylanase, laccase and cellulose, applied in various ways.
- The main focus of this thesis is on enzymatic biosoftening of *Agave americana L.* fibre as the end product. Yarn and fabric production processes or any value-added textile productions are excluded in the experimental processes in this research, because of too wide scope and limited time factor.
- The study will be more of an exploratory type indicating the possible applications of *Agave americana L.* fibre. Large-scale trials would not be possible due to limited resources. Methods of sustainable *Agave americana L.* plant cultivation, management and production of value added by-products have also been excluded.

### 1.7.2 Limitations of the study

- *Agave americana L.* plant had to be hand-harvested since it is difficult to mechanize its harvesting due to the spiral rosette pattern of development of its leaves.
- *Agave americana L.* plant leaves harvesting, transportation and fibre extraction are challenging because of the spiny margins and tips and the irritating sap that triggers skin dermatitis. The fibre extraction is also labour-intensive and tedious because fibre biomass constituents are closely packed and glued together. Overcoming recalcitrance of *Agave americana L.* fibre extraction and biosoftening is another serious constraint.

- The age of the wild *Agave americana L.* plants; to be used for fibre extraction is unknown. Inadequate knowledge and practical experience in working with enzymes are barriers to the implementation of enzymatic processes in the textile industry.
- The sustainable availability of enzymes in large amounts is a great challenge. The commercial enzymes are extremely costly. The cost is likely to limit the intensity of experimental procedures to be conducted.
- Lack of political support for plant fibre production in Lesotho. The agricultural policies favour the production of wool and mohair (for textile fibres) and the green revolution crops with focus exclusively on food crops, not on non-conventional fibre crops like *Agave americana L.* fibre.

## 1.8 ASSUMPTIONS

- From the eco-socio-economic sustainability, softened *Agave americana L.* fibre is a feasible and promising textile fibre that can be a potential alternative to unsustainable; non-renewable synthetic fibre. It is expected that knowledge generated from this work, will be of value to allow the preservation of inherent *Agave americana L.* plant for development of local textile fibre which would be a possible sustainable prospect for employment and income generation activities that could address the existing societal poverty and environmental problems.
- Bearing in mind the wet processing effects of the lignocellulolytic enzymes on (lingo) cellulosic fibres, it is assumed that *Agave americana L.* fibre will be biosoftened and upgraded from being merely technical fibre to textile fibre after the removal of lignin and other non-cellulose biomass constituents. Thus, *Agave americana L.* fibre bundles will be smoother, softer, finer and brighter than before treatment. Softer and brighter *Agave americana L.* fibre will be a source of diversified fancy textile products.
- Enzymatic biosoftening and upgrading of *Agave americana L.* fibre can become a valuable and eco- friendly alternative of harsh alkaline, chemical and wet-processed fibres, because enzymes catalyse reactions under mild pH and temperature conditions using very little water and are substrate(s) specific.
- The enzymatic removal of non-cellulose biomass constituents in *Agave americana L.* fibre can biosoften and upgrade the fibre without significant negative effects on the fibre performance properties.

- The future of *Agave americana L.* fibre is projected to constantly upsurge with effective support from the textile researchers and policy and decision-makers in the Lesotho government through the development and recognition of suitable policies and regulations complimentary to its wild cultivation, sustainability, diversification and promotion properties and functions.

## 1.9 DEFINITIONS OF OPERATIONAL TERMS

- *Agave americana L.* fibre is a three-dimensional natural lignocellulosic fibre predominantly comprises of cellulose, hemicelluloses and lignin as well as other constituents that occur in minute quantities (Harmsen *et al.*, 2010:13; Novozymes, 2013:69), obtained from the succulent leaves of the monocotyledonous and monocarpic; *Agave americana L.* plant (Bouaziz *et al.*, 2014:1).
- *Enzymes* are compound protein molecules produced by living cells, which act as biocatalysts in chemical reactions that include textile fibre softening (Quarshie & Carruthers, 2014:60).
- *Fibre Biosoftening* is an organic process of using microbes and/or their products to selectively remove the non-cellulosic constituents of the lignocellulosic fibre with the intention to upgrade its textile quality properties (Shroff *et al.*, 2016:524, Suganya 2018:128).
- *Fibre extraction* is a process of separation of non-fibrous plant tissues and other cementing material from the fibre bundles (Vellaichamy & Gaonkar, 2017:1268-1269).
- *Lignocellulolytic enzymes* are enzymes that biosoften and upgrade the plant fibres also termed the lignocellulosic fibres (Morrison *et al.*, 2016:2).
- *Lignocellulosic fibres*: are fibres usually obtained from certain plant parts that include the seeds, fruits, leaves, bast and stalks and roots which are composed mainly of Cellulose, hemicelluloses and lignin chemical polymers respectively; and are intermingled in a hetero-matrix to each other and minute quantities of extractives and ash (Kumar *et al.*, 2016: 150).
- *Mechanical properties* refer to, characteristics of the textile fibre that define its behaviour at maximum load (Kikutani 2009:159).
- The *pH* stands for potential of hydrogen; which is the negative logarithmic measure of the molar saturation of hydrogen ions that indicates the acidity or alkalinity of a substance (Boyd *et al.*, 2011:403)



- *Pre-treatment*: The disturbance of the naturally resistant structure of lignocellulosic fibre biomass to ease enzymatic bioprocessing of the biological processes (Yang *et al.*, 2011a:422).
- *Sustainable textile* fibres are those that refer to textiles produced through the processes that conserve, balance, increase optimistic and reduce adverse social, ecological and economic effects during its production, processing, utilisation and disposal and avoid depletion of natural, safe, non-toxic, efficient renewable and degradable resources, and hearten improvements that satisfy both the needs of current and future generations without jeopardise (Thidell, 2010:2; Rusu, 2011:2; Sharma 2013:768; Smole *et al.*, 2013:1; Hayles, 2015:101).
- *Technical fibres* are fibres produced and utilised for their physical and mechanical properties with aesthetic and comfort properties given little or no attention (Canavan, 2015:536).
- *Textile biotechnology* is also referred to as bioprocessing of textiles is defined as technologies that apply the biological systems, living creatures and their products to conduct and treat industrial textile processes and products respectively (Ezeonu *et al.*, 2012:5; Sharma, 2012:3; Ramachandran & Karthik, 2004:32; Mojsov, 2014:135; Mahabub *et al.*, 2015:18).
- *Textile fibre* is a flexible, fine and smallest inseparable unit with length-to-width ratio of at least 1:100 times; capable of being woven into textiles and/ or fabrics (Morton & Hearle, 2008:3).
- *Underutilized and neglected fibre species (NUFS)* referring to alternative fibres which have a considerably sustainable potential use but mainly unexplored, deserted and underutilised for textile economic benefits (Thies, 2000:1).
- *Water retting* is a chemical and organic process by which the bundles of *Agave americana* L. leaves are immersed in water to rot by combined actions of water and microbes from non-fibrous biomass consisting of pectins and other gummy substances (Nabilah *et al.*, 2012:14; Dungani *et al.*, 2016:46; Omenna *et al.* 2016:275).

## 1.10 OUTLINE OF THE RESEARCH

Subsequent to providing the contextual background, problem statement, justification, purpose, impact and theoretical perspective; of the research study, the organization of this thesis is as follows:

## **Chapter 1: General Introduction**

It consisted of the background, problem statement, justification of research, purpose with the general aims and specific objectives and null hypotheses, methodology, significance, conceptual framework, delimitations and limitation of the study, assumptions, and definitions of operational terms.

## **Chapter 2: Literature Review**

This part of the chapter did interrogate the content of the structure and composition of natural lignocellulosic fibres, the impact of conventional textile fibre production and processing, the textile sustainability: ideas, philosophies and indications and *Agave americana L.* fibre as a potential alternative sustainable textile fibre which covers characteristics of *Agave americana L.* plant as a source of fibre, fibre extraction techniques, structure, properties, composition, the potential sustainability impacts of production and enzymatic biosoftening of *Agave americana L.* fibre.

## **Chapter 3: Research Methodology**

This chapter explains the research design and methodology including the specific data collecting and analysing procedures. The chapter further describes considerations of data credibility that leads to the legitimate research.

## **Chapter 4: Discussions, analysis and interpretation of findings**

In this chapter, findings that emerged from the analyses were discussed by the researcher and reference was drawn from the interrogated literature in chapter 2. A further reference of the theoretical frame work was discussed as well.

## **Chapter 5: Conclusions and Recommendations**

This is the concluding chapter where the researcher dealt with significant points that emerged throughout the study. The chapter further provides implications for this study and suggests the supplementary research alternatives.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 INTRODUCTION

A fibre is defined as a natural or synthetic minute, long, linear and pliable polymer consists of a fibrous structure that resembles a hair that is thousand times longer than its diameter (Sorieul *et al.*, 2016:3).

#### 2.1.1 The basic properties of a typical textile fibre

A fibre is defined as a natural or synthetic minute, long, linear and pliable polymer consists of a fibrous structure that resembles a hair that is very small in diameter in relation to its thousand times higher length. To become a textile fibre, it must have some fundamental properties such as a high length of at least 100 times to its diameter ratio of 10-200 microns (Azanaw *et al.*, 2018:51), elasticity, spinning power, high degree of fineness, outstanding flexibility, reasonable strength and cohesiveness (Srivastava, 2012:1; Sinclair, 2014:3-4). A textile fibre, therefore, is a fundamental unit or the building block from which textiles are manufactured. This implies that textile fibre properties include length that can be classified as filament otherwise staple, size and surface contour. The side-chains size and the presence of crystalline and amorphous areas in the molecule sway the mechanical strength, elasticity and water absorption properties of various fibres. These properties affect the textile serviceability of fibre such as aesthetics, appearance retention comfort, care and durability (Msahli *et al.*, 2015:2).

#### 2.1.2 Textile fibre classification

The fibre functions can be used to classify textiles. The term textile fibres denotes the fibres that qualify to be used in weaving of cloths whereas the technical fibres are the hard wearing rope, making fibre and the fibres used for stuffing upholstering and mattresses are referred to as filling fibres (Sinclair, 2014:3). Table 2.1 represents the textile fibres; classified according to their origin.

**Table 2.1: Schematic representation of textile fibre classification according to origin**

<b>NATURAL FIBRES</b>	<b>MAN-MADE FIBRES</b>
<b>VEGETABLE ORIGIN - CELLULOSE FIBRES</b>	<b>NATURAL POLYMER BASED</b>
<ul style="list-style-type: none"> <li>▪ <b>SEED FIBRES:</b> COTTON, KAPOK</li> <li>▪ <b>BAST FIBRES:</b> JUTE, FLAX, HEMP, RAMIE, KENAF ...</li> <li>▪ <b>LEAF FIBRES:</b> ABACA, SISAL, HENEQUEN ...</li> </ul>	<ul style="list-style-type: none"> <li>▪ <b>REGENERATED CELLULOSE:</b></li> <li>▪ VISCOSE, MODAL, LYOCELL, CUPRO</li> <li>▪ <b>REGENERATED PROTEIN:</b></li> <li>▪ CASEIN, ARACHIN ZEIN</li> <li>▪ <b>CELLULOSE ESTERS:</b> ACETATES</li> <li>▪ <b>RUBBER:</b> ELASTODIENE</li> <li>▪ <b>ALGINATE</b></li> </ul>
<b>ANIMAL ORIGIN – PROTEIN FIBRES</b>	<b>SYNTHETIC POLYMER BASED</b>
<ul style="list-style-type: none"> <li>▪ <b>WOOL</b></li> <li>▪ <b>HAIR FIBRES:</b> ANGORA, MOHAIR, ALPACA ...</li> <li>▪ <b>SILK</b></li> </ul>	ACRYLIC, ARAMID, CHLOROFIBRE, FLUOROFIBRE, MODACRYLIC, POLYAMIDE, POLYESTER, POLYETHYLENE, POLYIMIDE, POLYPROPYLENE, VINYLAL, POLYLACTIDE ...
<b>INORGANIC:</b>	<b>INORGANIC:</b>
ASBESTOS	CARBON, CERAMIC, GLASS, METAL

(Source: Smole *et al.*, 2013:373)

Textile fibres are normally categorised in accordance with their sources or chemical composition. There are two main typical classes: natural fibres and synthetic fibres (Table 2.1) (Mather & Wardman, 2015:2). Synthetic fibres are Man-made fibres because they are developed by people using chemical and mechanical processes. There are two types of man-made fibres: regenerate and truly synthetic fibres (Mussig & Sloomaker, 2010:41-42).

### **2.1.2.1 Natural fibre classification**

Natural fibres are defined as flexible, fine and longer than its crosswise measurement, substances produced by nature in fibre form. The two main classes of natural fibres are organic and inorganics. Inorganic natural fibre class has mineral asbestos; as one well-known example. Organic fibres are subdivided into animal and plant fibres. Their chemical structures include the elements carbon, hydrogen and oxygen with additional nitrogen for animal fibres. (Saxena, 2011:122; Smole *et al.*, 2013:373; Cherif, 2016:14; Pecas *et al.* 2018:4).

### **2.1.2.2 Mineral fibres**

Mineral asbestos fibres are shaped into fibres and are mainly used in the fireproof fabrics. Mineral fibres are fireproof, resistant to acids and are used for industrial purposes (Cherif, 2016:14). Due to its cancerous properties; asbestos is no longer used as a textile fibre.

### **2.1.2.3 Animal fibres**

Animal fibres are largely made up of proteins like silk, hair (wool and mohair) and feathers. The most commonly used type of animal fibre is wool and mohair. Animal fibres are also categorized as:

- Hair or staple fibres such as wool, mohair and speciality hair fibres
- Secretion of filament fibres such as silk i.e. spider silk which is an insect fibre (Elsasser, 2010:35).

### **2.1.2.4 Plant Fibres**

Botanically, plant fibre is described as a single elongated, thick-walled, plant cell of powered sclerenchymatic tissue considered as a very long cell of a specialised secondary cell wall. Vinodhini & Malathy (2009:444), describe a plant fibre as a lengthy, fine, tapering, rigid and thick walled for support; cell that is dead and resonating at maturity; often obtained in the vascular tissue and consists mainly of cellulose, hemicellulose and lignin. In ordinary terms, fibre is a long bundle of unspecialised cells that are cemented to one another by the non-fibrous plant materials (Sorieul *et al.*, 2016:3; Mikshina *et al.*, 2013: 92). Natural plant fibres also referred to as vegetable fibres are cellulose-rich natural fibres derived from plants (Msahli *et al.*, 2015:1; Yu, 2015:30). They are produced as a result of photosynthesis (Zimmiewska *et al.*, 2011:98).

Natural fibres are obtained from two main types of plants depending on the main purpose for which they are produced: basic (main) and secondary fibre plants. Main fibre plants are produced specifically for their fibres while secondary fibre plants are produced for other purposes and fibres extracted from them are waste products e.g. banana fibre, and coir from coconut plant (Nguong *et al.*, 2013:53). The plant fibre can also be classified as soft and hard fibres. The soft fibre is obtained from the phloem tissues of the plant; for instance flax, hemp jute and ramie and the hard fibre are obtained from both the phloem and xylem tissues. The xylem tissue is the hardened wood core of the plant. The *Agave sisalana* (sisal); *Agave americana* L., banana, and palm fibres are the examples of hard fibres (Vinodhini & Malathy, 2009:444).

The most common plant fibre classification system used is the one that correlates with the plant part from which the fibre is extracted (Thomas *et al.*, 2011:11; Smole *et al.*, 2013:370; Teli & Jadhav, 2015:3848, Yu, 2015:30):

- Seed fibres are found around the seeds of a particular plant: kapok, cotton.

- Bast fibres are also called soft fibres. They are clusters of phloem fibres extracted from dicotyledonous stems inner bark. They include nettle flax, kenaf, hemp, ramie, and. Jute. They are considered as the strongest of all plant fibres.
- Leaf fibres are conversely termed hard fibres since they are stiffer with more quantity and quality of lignin than soft fibres. They are extracted from the monocotyledonous leaves' the vascular bundles or veins of xylem and phloem as well as the unsheathing fibres. They include *Agave sisalana* which is commonly called sisal, *Agave americana L.*, henequen, pineapple to mention a few.
- Fruit fibres are obtained from the fruits of the plant: coconut fibre from the coconut palm.
- Stalk and reed fibres are acquired from the stalks of the plants that include corn, rice, barley, oat, straws of wheat and others like grass and bamboo (Mylsamy & Rajendran 2010:2926, Céline *et al.*, 2013:2; Saxena *et al.*, 2011:124; Levetin–McMahon, 2008:299; Zimniewska *et al.*, 2011:100; Chandramohan & Marimuthu, 2011:197; Mather & Wardman, 2015:25; Sajith *et al.*, 2016:1).

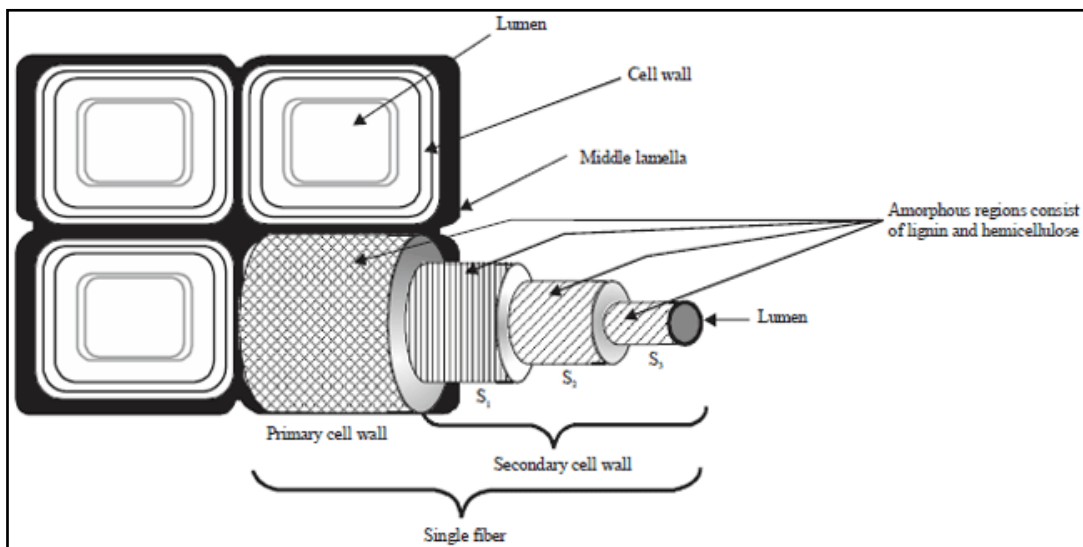
### **2.1.3 Plant fibre cell wall structure**

The plant cell wall is a complicated extracellular medium that surrounds each cell in a plant. Plant-based natural fibres are long and tapering cell, dead and the lumen, which is an empty hole inside the individual cells that transports water within the fibre (Kantharaj *et al.*, 2017:2). They have systematic internal thick cell wall structure and that functions as a support system for the plants to help maintain their shape and structure (Tahezadeh & Karimi, 2008:1623; Burgert & Dunlop, 2011:27; Kallioinen, 2014:1; Woo *et al.*, 2014:60; Pattathil *et al.*, 2015:4280). The plant cell wall (PCW) is a dynamic and complex macromolecular structural frame of plant fibres (figures 3 & 4) that surrounds, protects, supports and strengthens the cell and is a distinctive part of plants necessary for their survival (Caffall & Mohnen, 2009:1879). The quantities and quality of PCW contents change in order for the plant to grow and survive against pathogens (Onofre *et al.*, 2014:276). Natural PCW fibres are multiples of rigid cellulose microfibrils entrenched in the lignin-hemicellulose matrix (figure 2) (Tahezadeh & Karimi, 2008:1623; Burgert & Dunlop, 2011:27; Quiroz-Castañeda & Folch-Mallol, 2013:119; Kallioinen, 2014:1; Woo *et al.*, 2014:60; Pattathil *et al.*, 2015:4280).

#### **2.1.3.1 The Plant fibre cell wall structure**

Plant cell walls are multi-functional structured cells of cellulose micro-fibrils embedded in an unstructured milieu containing various physical and practical constituents (Kantharaj *et al.*,

2017:2). They have a systematic internal cell wall structure. From the inner to the outer part, the natural plant fibre cell consists of primary and secondary cell wall layers and lumen middle lamella layer as illustrated by figure 2.4 (Asim *et al.*, 2015:3; Nishitani & Demura, 2015:177; Sorieul *et al.*, 2016:5). The cell wall cellulose microfibrils are embedded in and cemented by a non-cellulosic components matrix (Kalia *et al.*, 2011:3, Thomas *et al.*, 2011:3, Zimmiewska *et al.*, 2011:104, Dungani *et al.*, 2016:45). The Structural arrangement of natural lignocellulosic fibre cells is presented in Figure 2.1.



**Figure 2.1: Structural constitution of natural lignocellulosic fibre cells (Dungani *et al.*, 2016:44)**

The width of each layer and its components make-up differ in cell types, tissues and plant species. Primary (S1) and secondary cell walls (S2) remarkably vary in the structure of their matrices which designate their diverse functions and highlight the key role of the matrix in strengthening the cell wall fibre (Burgert & Dunlop, 2011:37, Ahmad *et al.*, 2019:2).

### **2.1.3.2 The middle lamella (ML)**

The middle lamella (plural lamellae), is the first, outermost, minor layer, (figure 2.1) that binds adjacent plant cells (Sorieul *et al.*, 2016:5). ML is usually found at the intersection where the walls of neighbouring cells meet. The structure of the ML is different from the other layers because it consists mainly of pectin and different proteins. Pectin is easily hydrolysed into individual cells by acids or enzymes (Ochoa-Villarreal *et al.*, 2012:63; Pu *et al.*, 2013:2; Smole *et al.*, 2013:37; Nishitani & Demura, 2015:177).

### **2.1.3.3 The primary cell wall (S1)**

The S1 is the thin initial outmost layer that propagates at the time of plant growth and is positioned just outside the cell membrane (figure 2.1), and is supple, letting the new cells to grow. The primary wall is comparatively unspecialised and has the same molecular structure in all kinds of cells. The primary cell wall propagates during active plant growth. The S1 comprises of the cellulose, hemicellulose, and pectin polysaccharides but it is dominantly comprised of cellulose molecules, organised into reedy hair-like structures named microfibrils. The microfibrils are mesh worked within lignin hemicelluloses and pectins matrix; which binds them together and reinforces the cell wall (Cziple & Marques, 2008:134; Sträuber *et al.*, 2015:67).

### **2.1.3.4 The secondary Cell wall (S2)**

The (S<sub>2</sub>) is a thick lignified layer inside the primary cell wall with a smaller lumen (figure 2.1). The (S<sub>2</sub>) is formed as cells differentiate after growth. The structure and composition of (S<sub>2</sub>) are extremely individualised, indicating the distinctive structure of the cell (Ördög & Molnár, 2011:61). A large amount of plant photosynthesis occurs in secondary walls. The secondary walls encompass more of the total plant biomass and control the contour, function of cells and their environmental response (Ahmad *et al.*, 2019:2). The secondary cell wall is primarily comprised of cellulose microfibrils and the hydrophobic polyphenol lignin. The hydrophobic polyphenol lignin lowers the water content of the cell wall biomass (Gierlinger, 2014:1).

The S<sub>2</sub> is made up of three distinctive, diverse and successive layers namely S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub> in the order of deposition (figure 2.1), (Mikshina *et al.*, 2013: 93). The S<sub>1</sub> is the outer layer which is nearby the middle lamellae and consists of microfibrils that are oriented crosswise and have a high angle. The S<sub>2</sub> is the middle layer that is the thickest portion of the wall with the least microfibril angle alignment. The S<sub>3</sub> is the inner layer of the cellulose microfibrils, from the ML towards the lumen; the layer also has crosswise-oriented microfibrils. The S3 varies in its make-ups: the proportion amongst cellulose, hemicellulose and lignin in the placement or spiral angle of the cellulose microfibrils (Pereira *et al.*, 2015:10; Thomas *et al.*, 2011:3; Sorieul *et al.*, 2016:5).

The angle at which the cellulose microfibrils are twisted to form spirals of the fibre axis is termed a spiral angle. The spiral angle differs with individual plant fibre depending on the type, development, location in the cell wall and degree of growth. The varying content of both cellulose and lignin, the degree of polymerization, spiral angle and the plant part from which each fibre type is extracted determine the mechanical properties of plant fibres (Dunlop, 2011:37; Thomas *et*

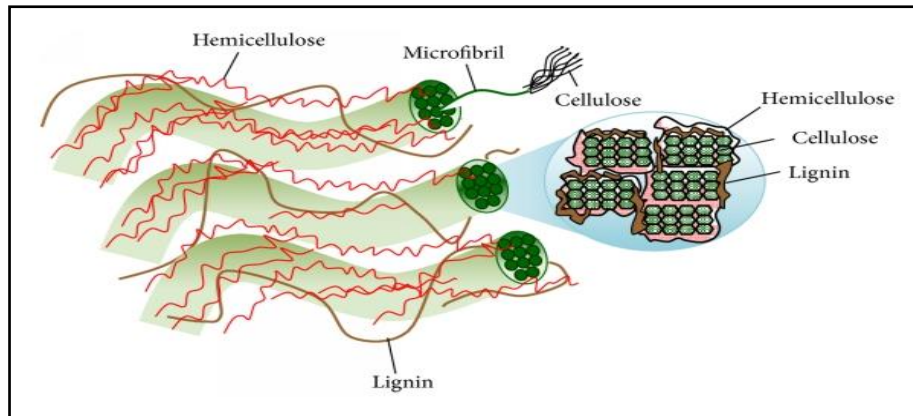


*al.*, 2011:7; Silva *et al.*, 2014:1068; Asim *et al.*, 2015:3; Saini *et al.*, 2015:1; Sorieul *et al.*, 2016:5).

The fibre strength and cellulose content increase proportionally and inversely with spiral angles towards fibre axis. Cellulose fibres have more orderly crystalline regions than amorphous regions distributed throughout the fibre (Thomas *et al.*, 2011:13). The cellulose content grows gradually from S<sub>1</sub> to S<sub>2</sub> whereas the hemicellulose amounts remain unchanged in all layers. Lignin content increases alongside with increase in cellulose content. Hemicellulose molecules are meshed structures interconnected with cellulose fibrils, lignin and pectin that bind them together. The cellulosic fibre strength and rigidity are due to these adhesive properties. There is no pectin but more cellulose in the secondary wall than in the primary wall. As a result, the S<sub>2</sub> is stiffer than the primary wall, and contain no enzymes and glycoprotein (Asim *et al.*, 2015:3).

#### **2.1.4 The structural components of lignocellulosic fibre**

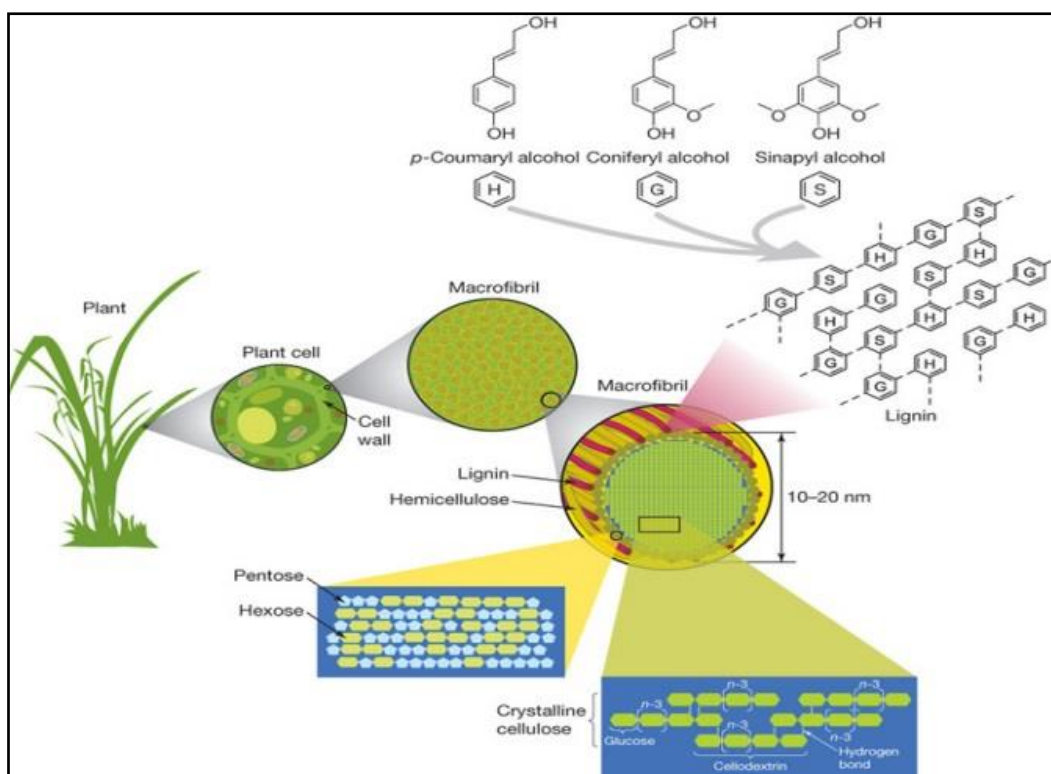
The lignocellulose is the most plentiful, diversified potential and sustainable natural textile materials in the world. It is a viable alternate to non-renewable fossil-based textile resources (Isikgor & Becer, 2015 4499-4500; Lima *et al.*, 2016:1). The lignocellulosic fibres are fibres obtained from a number of plant molecules containing cellulose, with varying amounts of lignin (Kazimierzak *et al.*, 2014:758). Lignocellulose (LC) is a complex carbohydrate polymer; containing predominantly polysaccharides: cellulose (~50%) which is the basis of all plant fibres (Wielgus *et al.*, 2012:291), and hemicellulose (~30%) built from sugar monomers (xylose and glucose) and lignin (~20 %), a highly aromatic material (figure 2.2), (Quiroz-Castañeda and Folch-Mallo, 2013:120; Anwar *et al.*, 2014:164; Yu, 2015:30; Desai *et al.*, 2016:27). In addition to LC polymers, there are other constituents, such as pectin, waxes, inorganic salts, nitrogenous substance and pigments that exist in minute quantities (Linger *et al.*, 2014:12013; Sharma & Malik, 2015:274; Dungani *et al.*, 2016:45). All plant fibres are basically the structural polysaccharides of plants that are chiefly composed of these three polymers (Kakoty *et al.*, 2019:2617) (figure 2.2), hence why they are called lignocellulosic fibres (LCFs). The LCFs are furthermore referred to as photo fibres because they are a result of photosynthesis (Dashtban *et al.*, 2010:36; Naderi *et al.*, 2012:26; Konczewicz *et al.*, 2013:116; Pattathil *et al.*, 2015:4280; Acharya & Shilpka, 2016:297). The natural plant fibres are furthermore termed cellulosic fibres because there is more cellulose than any of their components (Galletti & Claudia, 2011:2; Silva *et al.*, 2014:1068). The characteristic representation of the lignocellulosic fibre structure is illustrated in figure 2.2.



**Figure 2.2: The representation of the physical structure of the lignocellulose fibre consists of the three main constituents (Lee *et al.*, 2014:4)**

The ratio of fibre constituents is determined by the age, source of the fibre, environmental growth, extraction conditions and processing techniques used to obtain the fibres based on the source (Howard *et al.*, 2003:602; Vega *et al.*, 2012:1; Xu *et al.*, 2013:802 & 803; Linger *et al.*, 2014:12013; Saini *et al.*, 2015:1; Sharma Malik, 2015:274; Wahlström & Suurnäkki, 2015:694; Desai *et al.*, 2016:27).

Cellulose molecules are interconnected with hemicellulose and pectin polysaccharides and the non- polysaccharide; lignin in plant cell walls (figures 2.2 & 2.3) resulting in more complex morphologies (Siro & Plackett, 2010:459; Harmsen *et al.*, 2010:7). Cellulose microfibrils are predominantly crystalline while hemicelluloses and lignin are amorphous hetero-matrix (Dashtban *et al.*, 2010:37), that collectively with other minute fibrogenous substances are firmly intermingled along the hierarchical structure of cellulose, from ultimate fibrils to fibre bundles are filling, binding, stabilizing and strengthening the whole fibre structure (figures. 2.2 and 2.3) (Harmsen *et al.*, 2010:7; Siro & Plackett, 2010 459; Quiroz-Castañeda & Folch-Mallol, 2013:119; Thygesen, 2013:378; Ray *et al.*, 2014:37; Lima *et al.*, 2016:1). The LCFs do not refer to individual fibre cells per se but groups of elongated cellulose fibre cells that form microfibrils aggregated into vascular bundles- macrofibrils figures 2.3 & 2.4 (Levetin–McMahon, 2008:299). There are complex lignocellulosic covalent linkages which are impermeable barriers that prevent permeation of solutions and enzymes to cellulosic structures for fibre upgrading (Akin, 2010:18; Yang *et al.*, 2011a:422; Saini *et al.*, 2015:1; Sharma & Malik, 2015:274). Figure 2.3 illustrates the chemical structure of the lignocellulosic fibre consists of cellulose, hemicellulose and lignin.



**Figure 2.3: The representation of the chemical structure of lignocellulosic fibre consists of the three main constituents (Rubin, 2008:843)**

These lignocellulosic components are affiliated to each other at various degrees and composition that depends on the origin, plant species, age differences, quality and environmental conditions (Pu *et al.*, 2013:1-2; Lee *et al.*, 2014:4; Pattathil *et al.*, 2015:4279; Ramamoorthy *et al.*, 2015:110) and determine the textile performance characteristics of the fibre (Thomas *et al.*, 2011:11; Smole *et al.*, 2013:371).

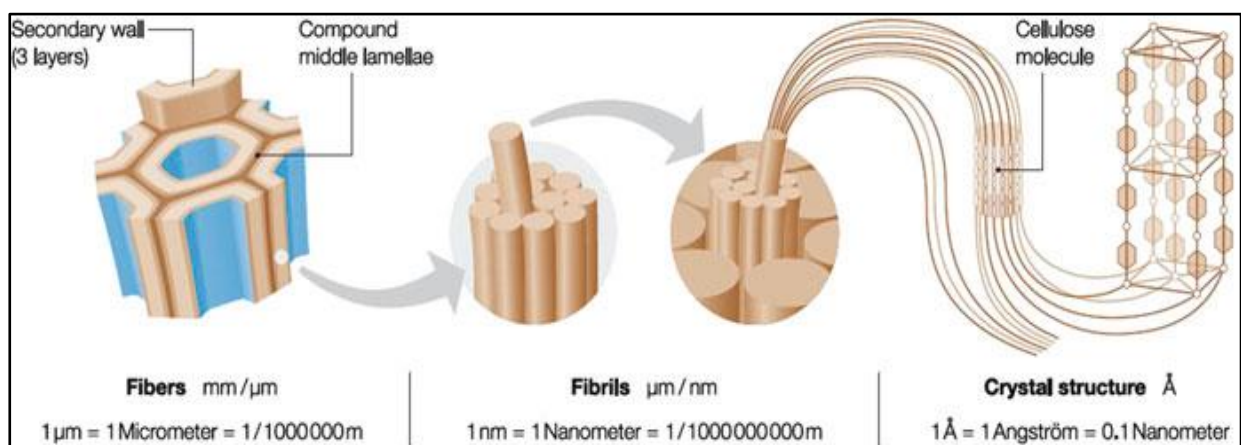
Most of non-cellulosic constituents in lignocelluloses are located on fibre surfaces (cuticle). The cuticles comprise of about 13% proteins, 0.9% pectins, 0.6% wax, 2.0% noncellulosic polysaccharides, ash and other various constituents, all of which safeguard the cells from potential ecological and pathogenic damage during cell growth and development (Michelle and You-Lo, 1998:212; Pereira *et al.*, 2015:10). (Anandjiwala & John, 2011:188; Bin *et al.*, 2011:422; Lee *et al.*, 2011:157; Vega *et al.*, 2012:1, Ramamoorthy *et al.*, 2015:110; Acharya & Shilpka, 2016:297; Lima *et al.*, 2016:1).

#### **2.1.4.1 The physico-chemical structure of cellulose**

(a) The physical arrangement of cellulosic fibre

Anatomically, natural lignocellulosic fibres are insoluble, elongated and tapering cellular filamentous structures called ‘cellulose micro-fibrils (Linger *et al.*, 2014:12013). These cellulose molecular cells have different sizes, shapes and arrangements of cells, depending on the types of fibres in a matrix involving lignin. There is also a central hole called lumen (figure 2.1.) through which each fibre induct water and nutrients during plant lifetime (Satyanarayana *et al.*, 2011:219; Thomas *et al.*, 2011:6; Linger *et al.*, 2014:12013).

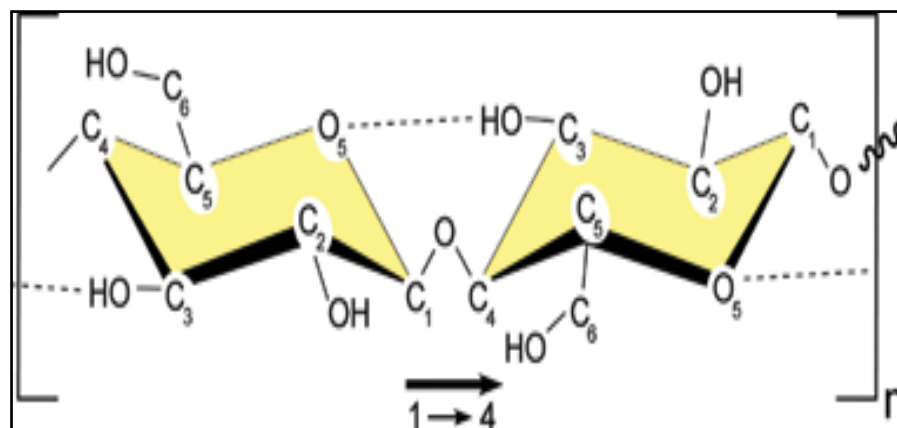
The cellulosic molecules are packed linearly and polygonally along the fibre axis; into ultimate fibrils which are aggregated together to form microfibrils of which in turn are bundled together into macrofibrils (figure 2.4) and the lamellar membrane (Rippon & Evans, 2012:123; Yu, 2015:30). The bundling of ultimate fibrils into microfibrils (Ördög & Molnár, 2011:6:62; Thomas, 2010:11; Yang *et al.*, 2011a:422). In some regions, of the microfibrils cellulose molecules are densely packed into crystalline frameworks termed micelles (figure 2.5) that form crystallite while other regions are amorphous along the length of the fibre (Yu, 2015:30). A typical cellulose microfibril comprises of a diameter of about 10–30 nm and 30–100 cellulose molecules that are in rectilinear sequence; parallel to the longitudinal axis have, a high degree of molecular arrangements that result structurally strong rigid fibre, that has low elongation and pliability (Thomas *et al.*, 2011:7; Wielgus *et al.*, 2012:291; Chen, 2014:31-32; Silva *et al.*, 2014:1068; Shahzadi *et al.*, 2014:247; Sosiati & Harsojo, 2014:33; Saini *et al.*, 2015:1; Mather & Wardman, 2015:2). Figure 2.4 presents the pictorial illustration of the lignocellulosic fibre structural arrangement in the plant cell wall.



**Figure 2.4: Diagrammatic representation of the structural cellulose arrangement of plant fibres in the cell wall (Thomas *et al.*, 2011:7).**

(b) Chemical composition of Cellulose in fibre

Chemically cellulose ( $C_6H_{11}O_5$ ) $_n$ , is a linear condensation biopolymer of anhydroglucopyranose molecules that are revolved to 180 degrees to form a reiterating disaccharide unit termed cellobiose (figures 2.5 & 2.6), connected by beta-linked ( $\beta$ -1, 4-glycosidic bonds) (figures 2.5, 2.6 & 2.7) (Martin & Štefan, 2007:3; Rubin, 2008:842; Harmsen *et al.*, 2010:8; Kalia *et al.*, 2011:2; Lee *et al.*, 2011:157; Yang *et al.*, 2011a:422; Volynets & Dahman, 2011:428; Wielgus *et al.*, 2012:291; Sorek *et al.*, 2014:193) and crystalline forms with inter-and-intra-molecular hydrogen bonds (figure 2.7), creating long straight flat, relatively inflexible, para-crystalline cellulose microfibril consist of both crystalline (a well-ordered structures) as well as non-crystalline (amorphous) structures respectively (Burgert & Dunlop, 2011:36; Galletti & Antonetti, 2011:2; Xu, *et al.*, 2013 803; Mahato *et al.*, 2013:96; Sorek *et al.*, 2014:193; Sharma & Malik, 2015:274) giving a steady, hydrophobic polymer with high tensile strength and stiffness (Ramamoorthy *et al.*, 2015:110; Lee *et al.*, 2011:157). Figure 2.5 shows a repeating cellobiose component that is a basic structural element of a cellulose molecule.



**Figure 2.5: Structure of one cellulose molecule consisting of a repeating cellobiose unit as the basic structural element (Wegner *et al.*, 2013:3)**

The Beta position is the  $-OH$  group on the same side of the ring as the C number 6 makes it hydrophilic in nature. However, the repeated elementary cellulose unit is the dimer cellobiose, (figures 2.5 and 2.6), which is made up of two glucose units linked by the  $\beta$ -1, 4 linkage and intermolecular hydrogen bonds (figure 2.7), through condensation (Kalia *et al.*, 2011:2, Thomas, 2011:11 & 39; Pecas *et al.*, 2018:4). Regardless of its comparative chemical simplicity, the physic-morphological structure of innate cellulose in higher plants is compound and diversified (Harmsen *et al.*, 2010:7; Siro & Plackett, 2010 459; Yang *et al.*, 2011a:422; Pu *et al.*, 2013:2). Figure 2.6 presents an inclusive structural composition and arrangements of cellulose in the plant fibre cell walls.



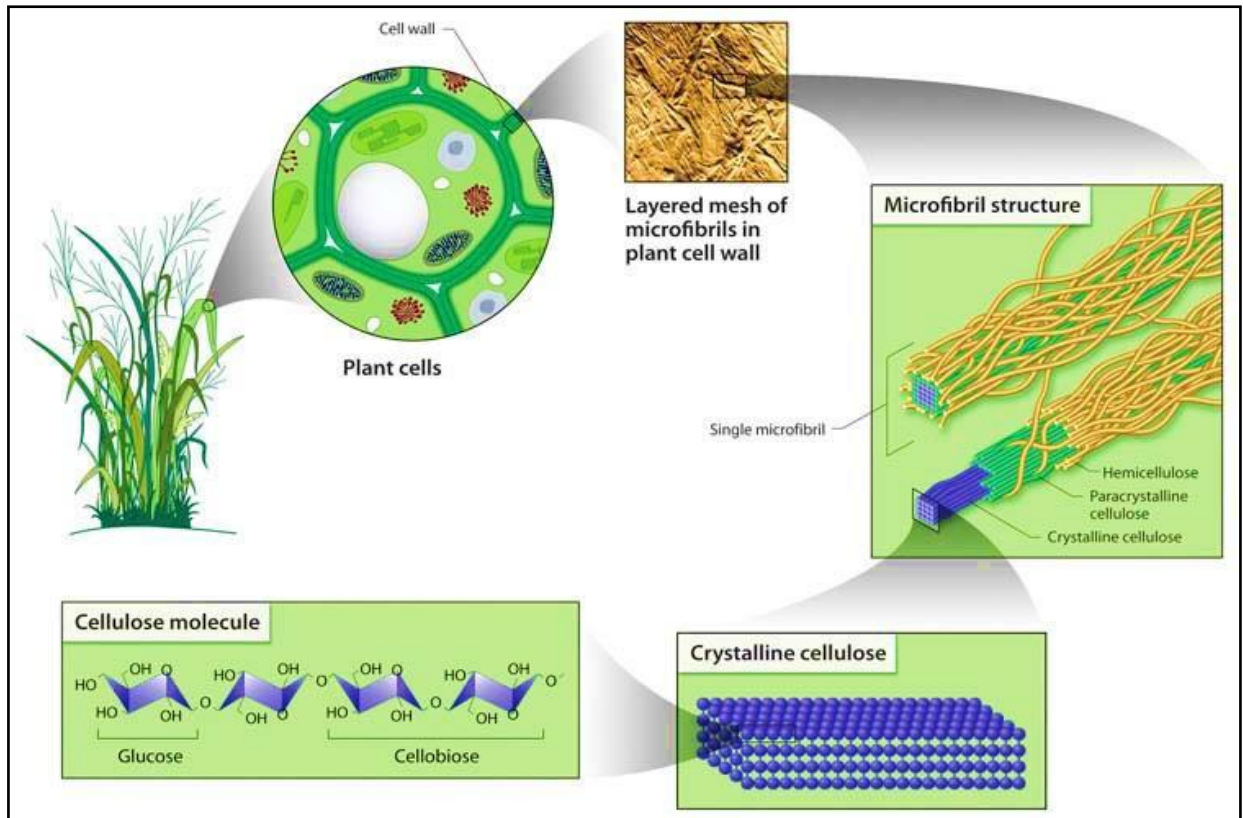


Figure 2.6: A comprehensive structural composition and arrangements of cellulose in the plant fibre cell walls (Quiroz-Castañeda & Folch-Mallol, 2013:121; Kallioinen, 2014:18; Chirayil et al., 2014:21).

Figure 2.7 shows the chemical structure of cellulose chains and its glucosidic Intra- and intermolecular hydrogen bonding.

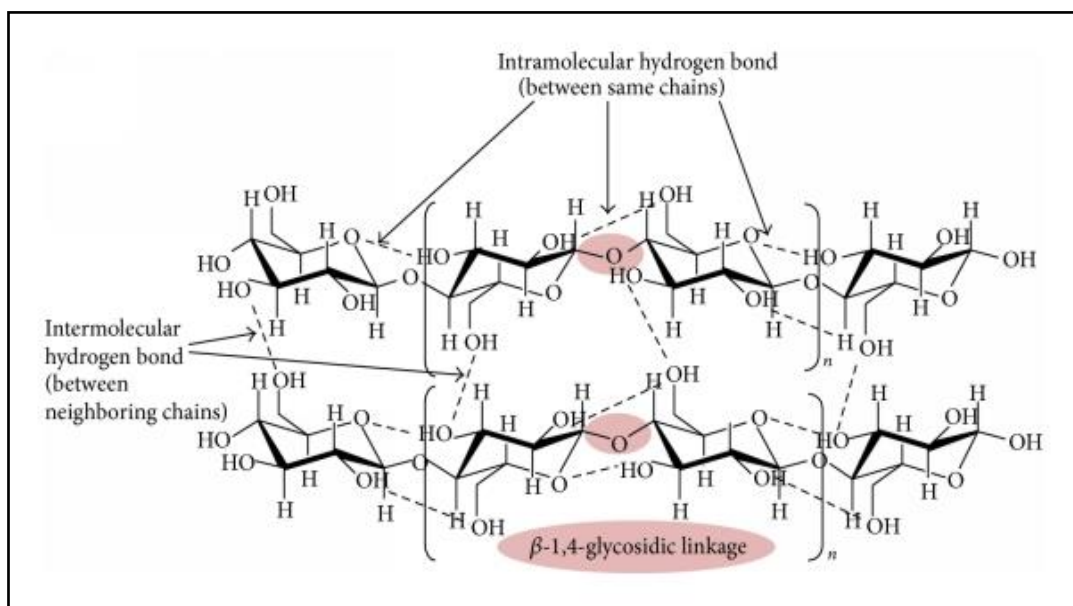


Figure 2.7: Chemical Structure of cellulose chains and its glucosidic linkage: Intra- and intermolecular hydrogen bonding (Lee et al., 2014:6)

Cellulose is the toughest and firmest component of the plant fibre cell walls (Harmsen *et al.*, 2010:8; Lee *et al.*, 2011:157; Wielgus *et al.*, 2012:291). It is formed by the stoking of glucose units obtained through photosynthesis (Wielgus *et al.*, 2012:292) figure 2.7 (Kristensen, 2008:10; Mortazavi & Moghadam, 2009:3307; Konczewicz *et al.*, 2013:116; Ram *et al.*, 2014:12). The nearly pure cellulose and white lignocellulosic fibres are considered to be of good quality when compared to those that are high in lignin, which are usually browner, of lower mechanical strength and thus, of poorer quality (Levetin–McMahon, 2008:299; Takane *et al.*, 2010:1; Smole *et al.*, 2013:371; Rowell, 1992:12).

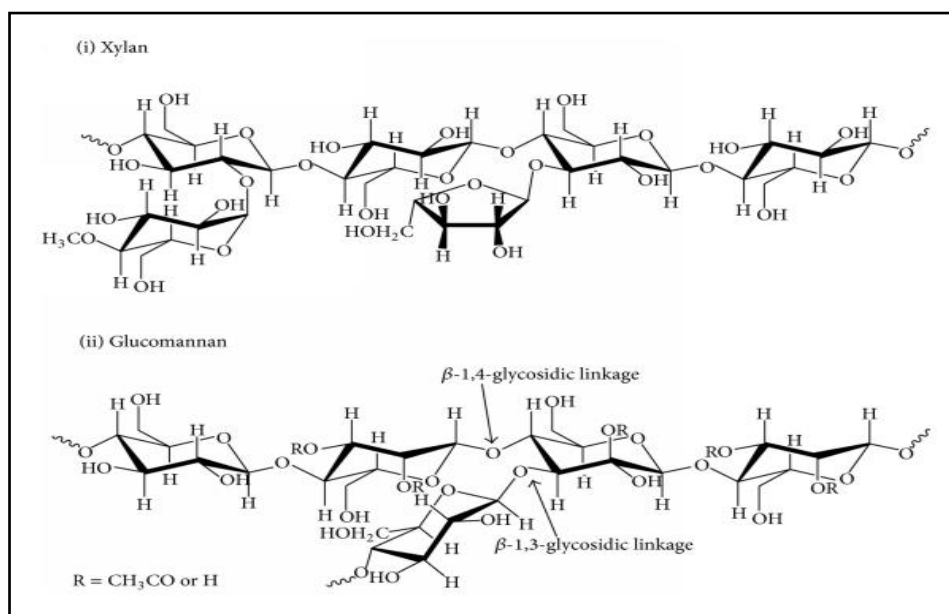
Several cellulosic fibre properties are determined by the degree of polymerization (DP) (Pecas *et al.*, 2018:5). The DP is the sum of glucose units required to compose one polymer molecule. The DP can vary from 500 to 17,000 glucose molecules of  $\beta$  (1, 4)-bound cellulose microfibrils, subject to the type of tissue. Fibres that exhibit eminently high tensile strength and modulus have large amounts of both cellulose content and degree of polymerization and a low microfibrillar angle and vice versa (Bavan & Kumar, 2010:3601; Yu, 2015:30). Progressive removal of the less organised parts leads to the progressive improvement of fibre crystallinity, until almost 100%. The crystallinity is partial due to the presence of hydrogen bonds between the cellulosic chains (figure 2.8). There are however; some hydrogen bonds found in the amorphous phase, even though they are poorly organised (Lee *et al.*, 2011:157; Sahin & Arslan, 2008:79; Rosli *et al.*, 2013:1893; Sorek *et al.*, 2014:194; Pereira *et al.*, 2015:10).

#### ***2.1.4.2 Chemical structure and composition of hemicelluloses***

Hemicellulose (figure 2.8) is the subsequent most plentiful polymer in plant fibre cell wall, after cellulose. It is heterogeneously branched in nature (Kumar *et al.*, 2016:150). Hemicellulose is a collective term for heterogeneous pentoses polymers (D-xylose and L-arabinose), hexoses (mainly D-mannose, less D-glucose and D-galactose and sugar acids that are different in composition (Eichhorn *et al.*, 2001:2109; Saha, 2004 3-4; Rubin, 2008:842; Yang *et al.*, 2011a:422; Pu *et al.*, 2013:2; Ruamsook & Thomchick, 2014:8 & 9). The rate of polymerization of the most hemicelluloses is 70-200 monosaccharide units. Hemicelluloses seal the gap between lignin and cellulose Hemicellulose solubilisation is straight linked to a rise of the biomass porosity (Akin, 2010:15; Eichhorn *et al.*, 2001:2109; Wielgus *et al.*, 2012:293; Volynets & Dahman, 2011:429).

Hemicelluloses are carrying the load and cross-linking cellulosic fibre bundles, lignin, pectin, proteins and non-structural polysaccharides through numerous covalent and noncovalent bonding

substances in the cell wall (Sorek *et al.*, 2014:197; Galletti & Antonetti, 2011:1). As such plant hemicelluloses are different in a number of ways: polydispersity, polydiversity and polymolecularity. Xylan is the most important component of hemicellulose comprising of  $\beta$ -1, 4-linked D-xylopyranosyl remains (figure 2.8). Mannans are the second most common forms of hemicelluloses (figure 2.8) (Shahzadi *et al.*, 2014:247; Aarti *et al.*, 2015:119). Figure 2.8 presents the chemical structures of the most common hemicellulose biopolymers: Xylan and glucomannan.



**Figure 2.8: Chemical structure of hemicellulose compounds (Xylan and glucomannan are the most common biopolymers) (Lee *et al* 2014:5)**

Cellulose and hemicellulose interconnected with non-covalent bonds to the surface of each cellulose microfibril (figure 2.2). Hemicelluloses are made up of 1, 4-linked **b-d**-hexosyl materials. The hemicellulose is different from cellulose in at least three key features. First, it has different sugar units, while cellulose comprises only 1, 4- $\beta$ -D-glucopyranose units (Aarti *et al.*, 2015:119). Secondly, they are branched, with less orientation than cellulose which is truly a linear polymer. Thirdly, the DP for hemicelluloses ranges between 100 and 200 units while that of innate cellulose is 10–100 times higher than that of hemicelluloses (Yang *et al.*, 2011a:422; Mohanram *et al.*, 2013:3; Acharya & Shilpka, 2016:297).

Mechanically, hemicellulose adds little to the rigidity and strength of fibres. It is easier to hydrolyse hemicellulose into sugars than cellulose. Fibres comprising of a higher amount of hemicellulose would not be desirable for producing textile (Mohanram *et al.*, 2013:3; Rosli *et al.*, 2013:1894).



### 2.1.4.3 Chemical structural, composition and functions of Lignin

Lignin is the main non-carbohydrate heterogeneous three-dimensional alkyl-aromatic polymer consists mostly of three; 4-hydroxycinnamyl alcohol syringyl, S, guaiacyl, G and p-hydroxyphenyl, H; monomeric phenylpropanoid units (Pandey *et al.*, 2014b:41); (figure 2.9) in which the aromatic units with 1, 2 or 3 etherified or free hydroxyl groups (Dashtban *et al.*, 2010:37; Li *et al.*, 2014:1176, Kumar *et al.*, 2016:150). The phenylpropane units are derivatives of carbohydrates, coming from the dehydration and cyclisation of sugars that are interconnected through alkyl aryl ether ( $\alpha$ -O-4,  $\beta$ -O-4), aromatic ether (4-O-5') linkages and carbon-carbon linkages (5-5' or  $\beta$ -5) by condensation process (Rubin, 2008:842; Galletti & Antonetti, 2011:2; Levasseur *et al.*, 2013:2; Linger *et al.*, 2014:12013; Gabrič & Pohleven, 2014:52; Shahzadi *et al.*, 2014:247; Dungani *et al.*, 2016:44-45; Sorieul *et al.*, 2016:7). Figure 2.9 illustrates the chemical structure of lignin and its three alcoholic precursors: P-coumaryl-, coniferyl- and sinapyl.

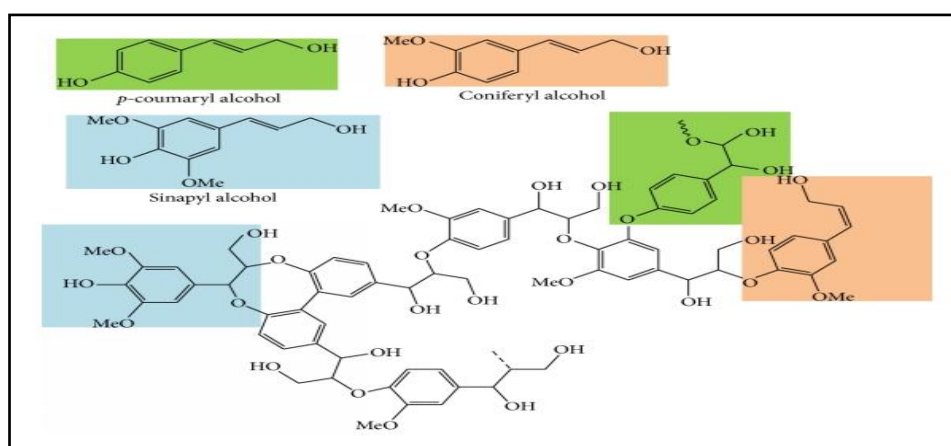


Figure 2.9: Chemical structure of lignin with P-coumaryl-, coniferyl- and sinapyl alcohol precursors (Lee *et al.*, 2014:4)

Lignin in plant biomass is a hard, hydrophobic and insoluble three-dimensional structure (Nagaraja *et al.*, 2014:106). It strengthens plant fibres, affects water movement, nutrients and by-products in the plant cell. Lignin is a much complex, and interconnected molecule with non-crystalline structure glues individual cells and the fibrils creating the cell wall (Eichhorn *et al.*, 2001:2109; Howard *et al.*, 2003:604; Harmsen *et al.*, 2010:11; Volynets & Dahman, 2011:429; Yang Bholay *et al.*, 2012:60; Rosli *et al.*, 2013:1894; Sorek *et al.* 2014:193). The mechanical properties of cellulose are more than those of lignin. Lignin is amorphous in nature (Volynets & Dahman, 2011:429). It is not hydrolysed by acidic medium, but solubilised by hot alkaline

chemicals, readily oxidised and condensed by phenolic chemicals (Yang *et al.*, 2011a:422; Smole *et al.*, 2013:371).

Lignin is the first formed fibre constituent; between the neighbouring cells in a “middle lamella,” connecting other components into tissue, and move into the cell wall passing through hemicelluloses and interlinking the cellulose fibrils. Lignin gives compressive strength and stiffness to the cell wall of the fibre and protects it from physical and chemical damages (Jiang *et al.*, 2018:6480-6481). The lignin content of the fibres affects the fibre organisation, properties, morphology, elasticity and hydrolysis degree (Reddy & Yang, 2005:23; Lee *et al.*, 2011:158; Mahato *et al.*, 2013:96; Pandey *et al.*, 2014b:41, Sorek *et al.*, 2016:197).

#### **2.1.4.4 Structural amorphous components**

##### **(a) Structural pectin**

Pectin is the most common plant cell wall heteropolysaccharide found in the middle lamella, which consists essentially of polygalacturonic acid materials linked by  $\alpha$ -1,4 glycosidic bonds, with limited xylose, arabinose, galactose and l-rhamnose molecules as the side chains (Kalia *et al.*, 2011:12; Ochoa-Villarreal *et al.*, 2012:67; Chiliveri *et al.*, 2016:559). Pectin dissolves in water and seal in the cellulose–hemicellulose biomass in primary cell walls that resides just underneath the cuticle and accrue between cells, in the middle lamella, providing cementing linkage. They add value cell growth and variation and so control the interconnectedness and stiffness of plant tissue. The pectins are most plentiful in the plant's primary cell walls and the middle lamellae (Naderi *et al.*, 2012:26). Pectins that contain a high amount of methylated and acidic sugars; normally galacturonic acid are highly diverged and organisationally very intricate (Caffall & Mohnen, 2009:1879; Thomas, 2010:16; Sorek *et al.*, 2014:197).

Pectin forms a web with other noncellulosic components, such as proteins and waxes (Voragen *et al.*, 2009:263; Zhou *et al.*, 2015:5714). Pectin is the major constituent of plant biomass that cements the fibre fibrils into fibre bundles and controls the shininess and the texture of the fibre (Konczewicz *et al.*, 2013:116). Pectins protect plants against pathogens and wounding. They also promote plant cell growth, enlargement, morphogenesis, cell-cell adhesion, wall structure, motioning, wall permeability, ionic bonding and enzymes inflection, seed hydration, leaf abscission, pollen tube growth and fruit growth (Voragen *et al.*, 2009:263; Ochoa-Villarreal *et al.*, 2012:67). Pectins are anionic in nature. They regulate ion transport and the enzyme penetration

through the walls. They furthermore regulate the water absorbency of the cell wall (Voragen *et al.*, 2009:263).

(b) Structural wax

Waxes are other but minor plant fibre cell wall constituents. They comprise of water and acid-insoluble kind of alcohol (Thomas *et al.*, 2011:12; Volynets & Dahman, 2011:430). They are made up of alkanes, sterols long-chains of fatty acids and alcohols. Natural waxes can be utilised in cosmetics, polishes and coatings, medicines, and insecticides (Volynets & Dahman, 2011:430).

(c) Structural ash

Ash signifies the mineral content of biomass that is dependent upon the soil and ecological environments. In overall, the ash content of woody materials is significantly lower than that of non-woody materials (Reddy & Yang, 2005:24).

### **2.1.5 The basic textile fibre properties**

Fibre properties can be broken down into five arbitrary subgroups that are morphological, physical, mechanical, chemical and topochemical. Morphological properties include; Length, width, length: width ratio, wall thickness and lumen diameter. Physical properties are as follows: Water absorption, drainage, degree of polymerisation, colour, swelling, refractive index, and birefringence. Mechanical properties are the tensile strength, shear strength and stiffness. Chemical properties refer to cellulose, hemicelluloses, pectins, lignin and inorganic constituents. Topochemical properties include the distribution of chemical components and bonds at or near the fibre surface (Morrison, 1999:20). Several fibres are not suitable to become textile fibres. A fibre to qualify as a textile fibre must have the following primary properties: adequate length-to-width ratio of 1:1000, pliability, adequate tensile strength, fineness, lustrous uniform surface and cohesiveness; a property that is induced by fibre softness, in order to form textiles (Gowda, 2007:3; Humphries, 2009:14; Srivastava, 2012:1; Sinclair, 2015:14). The properties of lignocellulosic fibres are mainly influenced by a number of factors that include chemical composition, physical and internal fibre structures, cellulose type, cell sizes microfibrils angle, and faults, which vary with different plants and their parts and varieties (Ahmad *et al.*, 2019:4).

#### **2.1.5.1 Fibre length-to-width ratio**

Morphologically, the length and width of fibre are important but the length: width ratio is a basic factor that determines the potential use of a fibre. The length: width ratio for a typical textile fibre;

should at least be 1:1000 to allow the individual fibres to be spun into a thread (Morrison, 1999:20). The length of the fibre directs its spinability and usefulness. Spinability is the easiness of textile fibres to be twisted into constant, even yarns, with viably satisfactory properties. The fibre length is a property of profitable worth because the cost is largely dependent on the length of fibre. Longer fibres have superior spinning characteristics when compared to shorter ones. However, the length of a fibre is an indeterminate quantity, as the fibres, even in a small haphazard bunch of a fibre, differ immensely in length (Gowda, 2007:27).

The distance across the textile fibre cross-section is normally referred to as its diameter. The diameter of natural fibres varies greatly along its length; because natural fibres are irregular. This compels textile scientists to use an average to measure fibre diameter which, is normally measured in ‘microns’ or ‘micrometres’ ( $\mu\text{m}$ ) which is millionths of a metre (Sinclair, 2015:11). Normally, textile fibre diameter ranges from 10 and 20  $\mu\text{m}$ , although certain textile fibres can reach up to 50  $\mu\text{m}$ . Animal fibre such as silk and wool have a diameter range of silk (10–13  $\mu\text{m}$ ) to up to 40  $\mu\text{m}$  respectively. Synthetic fibres can be produced with a diameter range from 6  $\mu\text{m}$  for microfibres up to over 40  $\mu\text{m}$  for heavy-duty carpet fibres. The fibre with small diameter has superior surface-to-weight ratios and is usually thinner and finer, more pliable, more flexible and softer than thicker fibres. This implies that a fibre has a softer feel and a more improved drape. Thinner fibres are of better quality and the more expensive (Sinclair, 2015:13). Clothes are characteristically manufactured with fibres which have a smaller diameter, than heavy-duty textile fibre which must have a larger diameter for textile applications such as carpets (Sinclair, 2015:12). Textile fibre fineness implies the thinness of fibre; the fibre of smaller diameter (Sinclair 2015:13).

#### **2.1.5.2 Fibre Fineness**

Fibre fineness is one of the principal characteristics needed for textile fibre performance (El Oudiani *et al.*, 2015b:30). The fibre fineness measures the dimensions of the fibre per unit length referred to as linear density. Fibre fineness is described in terms of its diameter as denier which is measured in microns or by the mass per unit length. It, therefore, signifies the cross-sectional size (thinness or thickness) of the fibre (Das *et al.*, 2013:755). Thinner fibres have bigger surface-to-mass percentages and are extra supple; resulting in higher quality fibre with more flexible and softer to touch than thicker fibres. Fibre fineness is an innate property dependent on plant age at harvest. Fibre fineness determines the spinning value of a textile fibre (Sinclair, 2015:16). The increased fibre fineness positively affects the performance properties a fibre and fibre products such as stiffness, torsional rigidity, evenness, and exhaustion of dyes, cohesion, texture tensile

strength, extensibility and lustre (Gowda, 2007:27). It can be detected simply by close examination of the fibre (Kant & Alagh, 2013:2548).

#### ***2.1.5.3 Tensile Strength and elongation***

Fibre performance characteristics of textiles are highly influenced by its tensile strength and extension. Strength or tensile strength is another important fibre property. Fibre tensile strength indicates the maximum pressure the fibre can resist before breaking. It is the breaking tenacity. The rate at which fibre extends before breaking is the elongation at break or breaking elongation. Fibres require different points and forces for elongation at break. It defines the elongation ratio of fibre at the break. Elongation at break is a measure of fibre usefulness within the limits of its breaking load. It is compared to a fraction of the starting length. This is an essential fibre property that makes fibres functional in textiles. Fibres ought to be bent (e.g. at elbow or knee) in order to tolerate high loading throughout processing and utilisation, but they must also be resilient enough to restore their original shapes and sizes. The fibre elongation should preferably be 1-2% or slightly more. Strength and elongation go hand-in-hand (Gowda, 2007:27; Fidelis *et al.*, 2013:150-151; Kant & Alagh, 2013:2549; Sinclair, 2015:16).

#### ***2.1.5.4 Flexibility or Stiffness***

The textile fibre mechanical properties are vital when the fibre is going to be used as a textile. If the textile fibre is used for clothing, it needs to be pliable to allow the body movements while the fibres used for furnishings need to be much harder and more long-lasting (Morrison, 1999:21). Fibre flexibility (stiffness) is another indispensable fibre property that influences fibre quality, fibre processing characteristics and performance properties such as the durability, sensation and drape of textile fibre products. Fibre flexibility is influenced by the organisational structure of the fibre, especially the width and cross-sectional contour of the fibre. The thinner the fibre the more flexible it is alongside its products-yarns and fabrics. The fibre with finely arranged crystalline cellulose molecules is a comparably more rigid fibre with more structurally oriented amorphous cellulose molecules (Sinclair, 2015: 15 & 17).

#### ***2.1.5.5 Defects***

Defects are the factors that cause total or partial harm to the fibre quality. Fibre defects can be divided into two main groups, explicitly a) Main defects and b) Negligible defects (Das *et al.*, 2013:755).

### **2.1.5.6 Density**

Density is the weight to volume ratio of fibre regarding all air spaces in the fibre. It is graded by the feel of weight or weightlessness of two or more fibre bundles, held within a grasp between two hands moved up and down. The sample that feels dense and heavy is rated as 'heavy body' and slack and the lightweight sample is graded 'medium body'. Heavyweight sample is graded high because it spins into good quality yarn (Das *et al.*, 2013:756).

### **2.1.5.7 Colour**

The colour of the fibre is the look the fibre possesses as a result of the way in which fibre reflects light. For example, red, blue, and green are colours. Colour is a quality that makes fibre look attractive and interesting. Plant fibre colour is influenced by retting conditions, water quality and washing conditions (Ali *et al.* 2015:243).

## **2.2 THE IMPACT OF CONVENTIONAL TEXTILE FIBRE PRODUCTION AND PROCESSING**

Worldwide ecological catastrophically elements such as rising temperature, rampant population growth and increasing levels of per capita consumption pressurize the diminishing amounts of agricultural land, oil and mineral reserves and freshwater supplies (Patterson, 2012:29, Kakoty *et al.*, 2019:2617). The growing concerns about the textile ecological, social and economic sustainability and the greenhouse effect; such as high pollution, growing need for decomposable and CO<sub>2</sub> neutral textiles produced with little or no greenhouse gas emissions, environmental guidelines and protocols, scarcities of textile raw materials and increased demand for textiles, deteriorating textile quality and rising textile materials price, inadequate hygienic water; limited textile resources; arable land for fibre production and energy to retain textile industry, have globally intensified the needs and interests in the sustainable and scientific natural lignocellulosic textile research and use (Li & Cai, 2008:81; Hiremath *et al.*, 2012:140; Cohen & Johnson, 2010:13; Connell & Kozar, 2014:41 & 44; Carlos *et al.*, 2014:1; Canavan, 2015:542, Kakoty *et al.*, 2019:2617).

The use of natural lignocellulosic fibre sources that are alternate to non-renewable synthetic fibre sources; is encouraged so as to reduce dependence on fossil fibres, since they are considered sustainable bioresources and have a potential to advance global textile fibre industry (Li *et al.*,

2014:1174). The textile industry is among the industries with the largest production, employment and material wealth impact worldwide (Kakoty *et al.*, 2019:2617). However, the conventional textile industry is among the most environmental polluting which, must be replaced or supplemented with the unconventional environmentally friendly textile industry industries (Thiry, 2011:27; Canavan, 2015:542; Vlad *et al.*, 2015:115; Toprak & Anis, 2017:1)

### 2.2.1 Air pollution

Air-pollution (illustrated in Figure 2.10) is the discharge of exhaust fumes from combustion of textile inputs mainly petroleum resources and airborne fibres (Kjellstrom *et al.*, 2006:818; Cohen & Johnson, 2010:309; Hiremath *et al.*, 2012:141; Toprak & Anis, 2017:12), chemicals, particulates, or biological materials that are harmful to people and/or the living organisms as well as the natural environment into the atmosphere (Tiwari & Babel, 2013:65). Most textile processes produce atmospheric discharges such as air, dust, oil mists, chemical vapours and fragrances. Air-pollution has been recognised as the second most type of pollution concerns subsequent to waste water pollution for the textile industry (Tiwari & Babel, 2013:65). The air emissions, remarkably Volatile Organic Compounds (VOCs) are a serious cause of air pollution (Cohen & Johnson, 2010:13 & 309; Parvathi *et al.*, 2009:3).



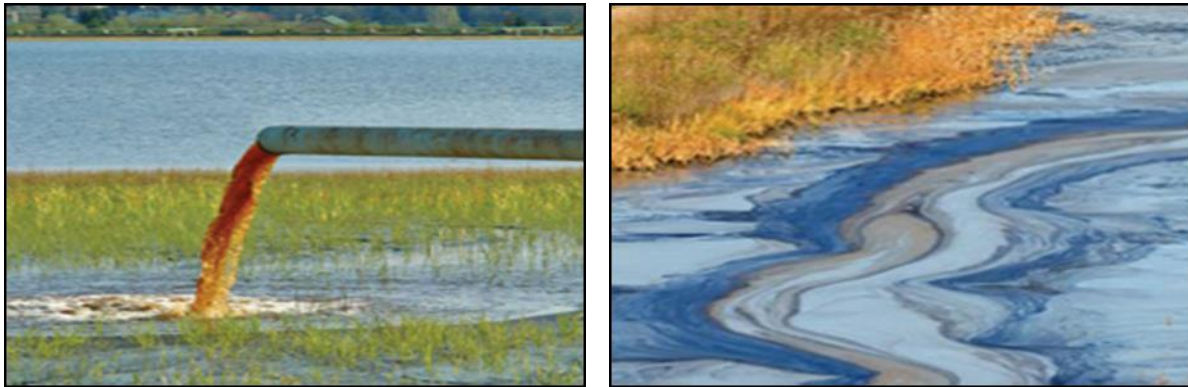
*Figure 2.10: Exhaust fumes as significant sources of air emissions (BMBF) & (BMEL) Germany, 2015:3)*

### 2.2.2 Water pollution

Water pollution (figure 2.11) is the paramount cause of environmental harms allied to the textile industry mainly due to untreated effluents from the dyeing and bleaching activities that have contaminations such as dyes, objects and traces of heavy metals (Hiremath *et al.*, 2012:147). The negative impacts include contaminants in textile emissions that include mineral oils, suspended



solids and other organic composite which are released from the textile production and processing that include scouring, bleaching, mercerizing, desizing, printing, dyeing and other particular substances (Toprak & Anis, 2017:12). large amounts of water is used during textile productivity, and most wastewater produced contains various chemicals, that can cause ecological pollution and harm if discharged into the environment without proper waste management (Kjellstrom *et al.*, 2006:820; Hiremath *et al.*, 2012:147; Cohen & Johnson, 2010:13; Islam & Khan, 2014:3 & 4).



*Figure 2.11: Polluted water effluent from the textile industry (Parvathi et al., 2009:3 & 4)*

### **2.2.3 Solid waste pollution**

Solid waste (figure 2.12) in the textile industry is the product of textile manufacture and usage that fill up the land without rot, being recycled or reused (Islam & Khan, 2014:3 & 4). Biodegradable solid wastes that are eco-produced include natural fabric scraps, yarns and package materials which may not be hazardous. Solid waste from the cutting room may be enormous but can be reduced through efficient and effective recycling and reusing to manufacture other essential textile artefacts (Cohen & Johnson, 2010:14).



*Figure 2.12: Solid waste pollution from the textile industry (<https://www.slideshare.net/bekhter/land-pollution-54848170>)*



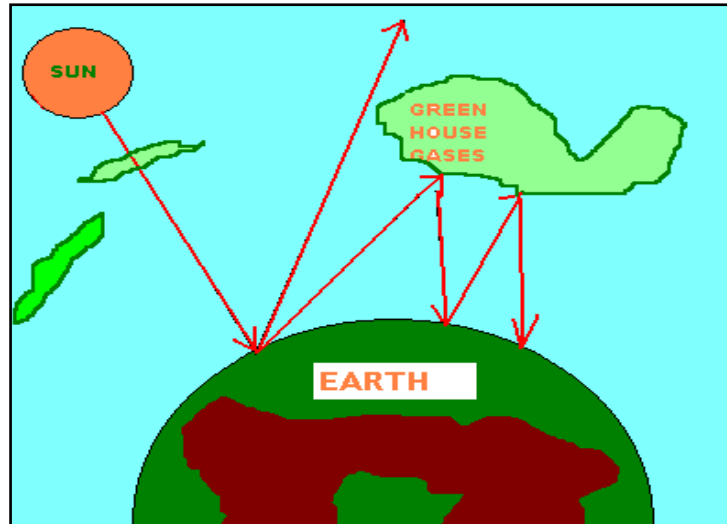
#### **2.2.4 Depletion of resources and other ecological issues**

The other major environmental impacts include energy consumption, substantial exhaustion of resources such as water, fossil fibre, and raw materials. Production of petroleum-based synthetic fibres, transportation, laundering, machines operations and dyeing use a lot of energy and water (Fatma & Jahan, 2016:883). Excessive noise or odour and workspace safety are other environmental issues that must be carefully considered (Parvathi *et al.*, 2009:43; Uygur, 2017:167).

#### **2.2.5 Global warming**

Global warming is an environmental problem caused by greenhouse gases (GHG) especially CO<sub>2</sub> (anthropic emissions) (Toprak & Anis, 2017:4), methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), perfluorcarbonates (PFCs), sulphur hexafluoride (SF<sub>6</sub>) and hydrofluorinecarbonates (HFCs), in the atmosphere forming the gas layer that impacts in the serious environmental problems. This layer acts as a preventive layer that absorbs the infrared (IR) radiations of sunlight from the earth (as illustrated in figure 2.13), which normally would exit into outer space, reflects them back to earth's surface as illustrated in figure 2.13, causing a rise in temperature. The increase in the concentration of GHG in the atmosphere accentuates global warming (Eklahare, 2011:8 & 69; Uygur, 2017:167).

The textile industrial development is one of the causes of global warming because there are detrimental gases released to the atmosphere (Toprak & Anis, 2017:1). The burning of fossil fuels during petroleum-based textile production emits great quantities of greenhouse gases (GHG) in the atmosphere, especially CO<sub>2</sub> (anthropic emissions). Conventional plant fibre production uses some synthetic fertilizers which contribute significantly to global warming. Furthermore, the extreme use of nitrate fertilizers converted into nitrous oxide, is exceedingly detrimental when compared to carbon dioxide (CO<sub>2</sub>) in relation to global warming. Global warming is also worsened by the use of pesticides which release green gases. Organic and wild fibres have a positive impact on global warming because they neither use fertilizers nor pesticides (Chavan, 2014:1; Uygur, 2017:166).



*Figure 2.13: Greenhouse gases absorb and reflect back the IR radiations of sunlight on the Earth (Eklahare, 2011:8)*

### **2.2.5.1 Textiles contribution to Prevention of Global Warming**

Global carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere also has a positive effect on plants, it causes plant stomata to narrow, so water loss is reduced and the efficiency of water usage improves. Plant photosynthesis is also aroused by increased atmospheric carbon dioxide concentrations under sunlight and gives back oxygen (O<sub>2</sub>). This signifies that the production of fibre plants cleans the atmosphere; thus reduces global warming. Increased carbon dioxide (CO<sub>2</sub>) concentration has a fertilizing effect on various fibre crops (BIC 2012:18). But natural animal fibres production increases effect onto global warming in inverse since animals breathe in O<sub>2</sub> and breathe out CO<sub>2</sub> (Parvathi *et al.*, 2009:43; Uygur 2017:167). Intensifying plant fibres can positively contribute to the prevention of global warming, which leads to climate change. Conventional production of natural plant fibres consumes a lot of water and engages the use of toxic chemicals that upon accumulation may be detrimental to human health and the environment (Oecotextiles, 2006:3). The sustainable textile materials and technology that strive to reduce non-renewable resource and energy utilisation and waste of non-toxic and ethically produced are identified substitutes (Parvathi *et al.*, 2009:43). These alternative resources necessitate an eco-friendly care and maintenance. The recycling, reuse, energy recovery or composting by-products and the products are novel strategies to effectively discard the sustainable textile that could provide valuable characteristics to the consumer. The production and manufacturing processes can be improved to various ways such as engaging finest practices in recent systems, executing new, more operative technologies, or substituting current materials and practices for fibre processing

standard that eliminates the use of hazardous chemicals (Fatma & Jahan, 2016:883-884).  
discarding

## **2.3 THE TEXTILE SUSTAINABILITY: IDEAS, PHILOSOPHIES AND INDICATIONS**

Sustainability is the ability to tolerate and continue indefinitely. The word sustainability is imitative from the Latin *sustinere* (tenere, to hold; sus, up) (Ezeonu *et al.*, 2012:2). Sustainability means living a life where an individual is not consuming more earth resources than what is substituted. It is the life stage at which individuals survive along with nature without becoming detrimental, but beneficial to the environment, economy and society. It is a corporation with nature as well as a tool by which individuals act responsibly to maintain or improve the ecosystem (Saxena, 2017:44; Venkatachalam *et al.*, 2017:6 & 23). It implies that the utilisation speed of renewable must be less than the regeneration speed. The utilisation speed of non-renewable resources (high-quality minerals, fossil fibre) must be less than that of renewable resources, used sustainably, can be replaced for them. The pollutant rate of emissions must be less than the rate at which they can be reused, assimilated, or down-graded by the environment (Anupama, 2010:1&15; Hämmerle, 2011:18; Ashby, 2013:321; Ozek, 2017:508).

### **2.3.1 Textile sustainability**

Textile sustainability is aimed at a grand textile production, processing and utilisation to approach, where materials and technologies that make up the textile product and services should be so safe to humans and the environment that they can be continually used through reuse or recycling approach. The environmental and human health impact should be lessened, throughout the entire textile product lifespan from textile raw material up to end-of-life management (Savastano *et al.*, 2010:55; Eisentrau, 2010:9; UNEP, 2011:8-9; Curteza, 2012:5; Patterson, 2012:39-40; Kumari *et al.*, 2013:1381; Vlad *et al.*, 2015:115). Textile Sustainability should balance costs and benefits to nature and people, with consideration for change towards the future in the textile industry. It is a systemic concept that capitalizes on optimism and reduces negative sustainable impacts with textile manufacture and processing (Gardetti & Torres, 2013:4; Khan & Islam, 2015:1-2; FAO, 2012:4). It is also the textile production and processing systems which safeguard that people utilise natural textile resources to minimise textile inputs but maximises quantity and quality of textile products and services as well as human life (Thidell, 2010:2).

It includes production processes utilised to produce in a more ethical manner by preserving an ecological balance and by preventing exhaustion of natural resources and improvement that satisfies the current needs without compromising abilities for future generations to satisfy their textile needs during their time (Curteza, 2012:2-3; Vlad *et al.*, 2015:115). Textile Sustainability, therefore, is the use of textile resources and processes to gratify the contemporary human textile essentials and ambitions without distressing earth's ecological, social and economic balances by preserving biodiversity and natural ecosystems indefinitely while safeguarding transparency, resiliency and adaptability to various textile situations (OECD, 2001:6; Buchholz *et al.*, 2005:1; Kates *et al.*, 2005:10; Thiry, 2011:28; Curteza, 2012:2-3; Dansi *et al.*, 2012:1; FAO, 2012:2).

Textile sustainability empowers people to recognise their potentials and develop their quality of life, safeguard and improve the earth's life sustenance circumstances under which humans can occur in productive harmony (Curteza, 2012:3; Khan & slam, 2015:2). Sustainable textile technology refers to textile production, processing and use which do not utilise harmful resources and /or exhaust resources from the earth without their reciprocal replacement (Venkatachalam *et al.*, 2017:23).

### **2.3.2 The pillars of textile sustainability**

The textile sustainability can be explained through environmental, social and economic textile pillars (Venkatachalam *et al.*, 2017:24). Textile sustainability is based on accomplishing an equilibrium of the ecological, fiscal, and societal pillars of sustainability in textile production, processing utilisation and disposal (Kates *et al.*, 2005:10; Akin, 2010:5; Karte, 2015:2; Murphy, 2012:15; Vlad *et al.*, 2015:115). These three textile sustainability pillars are also known as the textile triple bottom, a line that contributes to the attitude and social science of textile sustainable development (Ozek, 2017:506). Three interrelated dimensions of textile fibre sustainability: textile ecology, textile society, and textile economics (Maia, 2012:185). These three textile sustainability pillars are interrelated and intertwined (figure 2.14) to form an intersection so as to succeed in easing and overcoming the global textile ecological and socio-economic concerns (Basiago, 1999:150, Toprak & Anis, 2017:2).

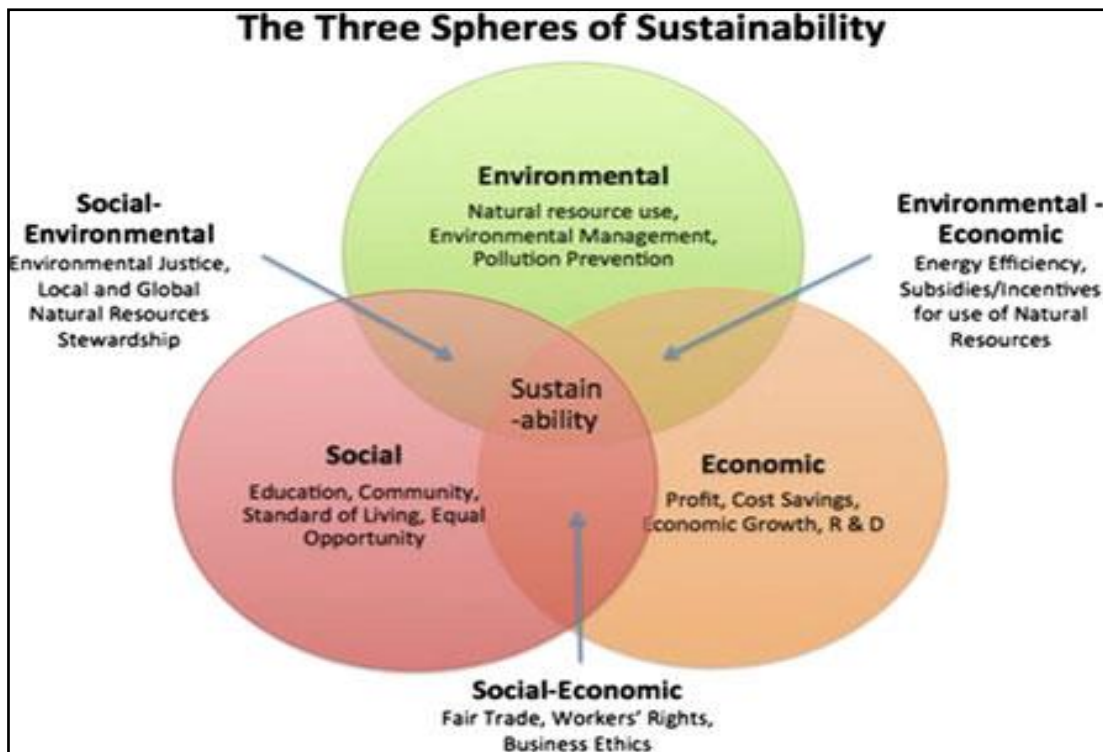


Figure 2.14: Three pillars of textile sustainability (Venkatachalam et al., 2017 24)

The production and processing of *Agave americana L.* fibre, need to be in line with the three essential pillars of textile fibre sustainability: economic efficiency and feasibility (novelty, wealth and efficiency), social equity (dearth, commune, wellbeing and wellness, human rights) and ecological responsibility and respect (biodiversity, land use, climate change) to ensure that this remains so now and in the future (Akin, 2010:1; Maia, 2012:185; Patterson, 2012:40; Theis & Tomkin, 2012:7).

### 2.3.3 Sustainable textile fibres

The sustainable textile fibres include production processes needed to use renewable textile resources and the final textile product that satisfies its purpose and has a fair social impact (Rusu, 2011:2; Canavan, 2015:538). A sustainable textile reflects on the effect of product, process and protocol decisions on how the textile product will be planned and what materials will be used in its construction. Sustainable textile fibres are the textile fibres obtained from renewable recyclable or biodegradable and organic (that cannot be exhausted) inputs and outputs that are ecologically, economically and socially safe, to enhance social well-being. Sustainable textile fibres are environmentally friendly and respective to social and economic quality through pollution prevention (Kumari et al., 2013:1388).

They reduce excessive energy and water use, resource depletion, greenhouse gas (GHG) release, nutrients discharge (resulting into eutrophication), dangerous by-products, effluent associated with the fibre production and processing stages and eco-toxicity from production and processing fertiliser, pesticide and herbicide use. They meet both, quality and eco requirements, and makes the use of resources more efficient (OECD, 2001:6; Vlad *et al.*, 2015:115; Curteza *et al.*, 2017:7; Venkatachalam *et al.*, 2017:31). They are also considered hypoallergenic, comfortable to skin feel and antibacterial by nature (Barth & Carus, 2015:32; Sirohi, 2016:25-26; Curteza *et al.*, 2017:7; Saxena *et al.*, 2017:45; Venkatachalam *et al.*, 2017:32).

#### **2.3.4 The principles of textile sustainability**

- Products and services engaged in textile production, utilisation and disposal are non-toxic, eco-friendly, suitable and intended to be hard-wearing, repairable, recyclable and biodegradable; manufactured and packed with the least amount of material and energy potential.
- Textile manufacturing is envisioned to function in such a way that wastes and eco-incompatible by-products, chemical, ergonomic physical hazard are lessened and eradicated to save resources for most relevant and desired end uses
- Textile workers and their work are respected, designed to safeguard and advance their efficacy and ingenuity; their safety, development and well-being is a priority
- Communities living around textile production, processing, utilisation and disposal areas are valued and their economic, social, cultural and physical wellbeing are enhanced.
- Sustainable economic feasibility is dependent on renewable resources that can be replenished at the same rate as consumption
- The speed with which both renewable and non-renewable textile resources are used should be the equivalent or less than the rate of resource renewals (Curteza, 2012:7).

#### **2.3.5 Textile fibre sustainability and eco-efficacy**

Sustainable development in textile fibres is the development that satisfies both current and future generations' textile needs without any compromise. It is founded economic; eco-friendly and social obligation pillars. The economic pillar supports the textile fibre industry to grow without compromising its integrity; social pillar supports that human rights be respected, with social fairness and social security; eco-friendly pillar supports the textile fibre industry to care for the environment. The textile fibre industry needs to be economically feasible, environmentally

respectful and socially fair to people to be considered a sustainable textile industry (Maia, 2012:185).

Textile fibre industry that is capable of providing reasonable textile fibre goods and services that meet peoples' textile fibre needs and improve their quality of life, while simultaneously decreasing textile ecological impact and resource strain to the Earth's capacity is textile fibre eco-efficient. Textile eco-efficiency, waste reduction, decomposability and end-of-life controllable disposal theories are explained in an easy phrase, creating more with less through. This means textile fibre industry must reduce textile fibre resources intensity; minimise energy intensity in both textile products and services; decrease the amounts and the dispersal of toxic textile-producing substances and decrease the level of toxicity of such substances; encourage recycling and reusing and the use of renewable and durable textile resources and products, thus, increase resources and service intensity (Maia, 2012:185; Patterson, 2012:39-40).

### **2.3.6 Consumer awareness of textile sustainability**

Textile sustainability consumer awareness has motivated the textile industries to look for ways to substitute conventional natural and synthetic polymeric fibres which use unsustainable inputs with the natural fibres that are increasingly being alternatively recognized as a favourable substitute which can encourage the growth of sustainable agriculture, accept environmentally friendly production and processing technologies, foster economic development and strengthen the participation of smallholders in the textile production and processing (Gašparič *et al.*, 2012:202; Stegmaier, 2012:116; Abilash & Sivapragash, 2013:53; Lee *et al.*, 2014:2).

Textile consumers are constantly seeking for decomposable and ecological textiles to safeguard the natural environment (Savastano *et al.*, 2009:56). Currently, there is more stress on eco-friendly fibres, the non-conventional fibres as these fibres have a special advantage of easy availability, are biodegradable, renewable and possess high specific strength when compared to synthetic fibres (Zhu *et al.*, 2013:5172; Christy & Kavitha, 2014:26; Khan & Islam, 2015:1). Exploring the sustainable production of lignocellulosic fibres alternatively opens a wide avenue to utilize these non-conventional fibres to fulfil the growing textile demands and a major socio-economic role which is established by its large influence to the gross domestic product (GDP) and to the employment rate (Savastano *et al.*, 2009:68; Vastrad *et al.*, 2015:198-199; Wegner *et al.*, 2013:4; Dungani *et al.*, 2016:46; Mabhaudhi *et al.*, 2017:1).



Natural cellulosic fibres extracted from the non-conventional resources are considered to have properties comparable to those of conventional cellulose fibres in current use (Li & Zhao, 2015:37). *Agave americana L.* is considered as a promising, economically feasible and suitable alternative source of material for textile fibre but it is underexplored and underutilised (Chattopadhyay & Khan, 2012:33; Sahu *et al.*, 2013:339). The reasons for the under-exploration and underutilisation of *Agave americana L.* fibre crop vary. It may be that its useful qualities have been ignored; feasibly there is on or inadequate processing and marketing or a lack of interest and/or political will on the part of agricultural and textile science research (Thies, 2000:1).

#### **2.4 AGAVE AMERICANA L. FIBRE AS POTENTIAL, SUSTAINABLE TEXTILE FIBRE ALTERNATIVE**

The textile production and processing are identified to be the biggest environmental polluters; resulting in ecological imbalance and depletion of resources. Due to technological advancements, ever-increasing consumption, limitations on the natural textile resources needed to produce fibres and incapacity to escalate the supply corresponding to the demand to make most of the existing fibres either too expensive or unobtainable for commodity presentations, these crises like situations have become a potential threat to humanity. The use of natural, eco-friendly, biodegradable, renewable and recyclable fibres and enzymes for textile processing is being encouraged as a good alternative biotechnology for the local textile industry to produce value-added products for apparel and other household textiles (Curteza, 2012:8-9, Ortega *et al.*, 2019).

The declining supply of raw materials and an increasing global environmental awareness, the naturally growing drought and concerns about climate change, increasing costs, limitations in the availability of land, water, and other resources necessary to grow conventional natural plant fibres are leading to a resurgence of interest to grow unconventional drought and pest resistant natural plant fibres like *Agave americana L.* as potential sources in an effort to improve textile fibre security with the alternative fibres (Karthik & Murugan; 2014:54, Dungani *et al.*, 2016:46).

The sustainable and ecological production and processing of unconventional plant fibres are encouraged since they are less detrimental to the environment and human health (Wegner *et al.*, 2013:4). These still follow the principle of green technology and provide the eco-socio-economic benefits and textile properties that are comparable to those of conventional plant fibres (Li & Zhao, 2015:37). *Agave americana L.* is considered as a promising, economically feasible and



suitable alternative source of material for textile fibre (Djizi & Bouzaouit, 2019:113), but it is underexploited and underutilized (Chattopadhyay & Khan, 2012:33; Sahu *et al.*, 2013:339).

#### **2.4.1 *Agave americana* L. Plant as a source of fibre**

*Agave americana* L. fibre is a pita lignocellulosic fibre also referred to as an istle; extracted from the vascular bundles or veins in *Agave americana* L. plant leaves (Tameem *et al.*, 2016:2). The leaves' vascular bundles consist of both phloem and xylem in addition to the unsheathing fibres Levetin & McMahon, 2008:299. *Agave americana* L. is innate to the USA (Hulle *et al.*, 2015b:1; Davis *et al.*, 2015:2) and Mexico where it is used as a fibre plant. *Agave americana* L. fibre is a hard lignified fibre made up of ultimate fibre cells are bundles of small, short lignified cells unsheathing. *Agave americana* L. plant leaf xylem and phloem (figure 2.16) (Gowda, 2007:2).

Botanically, *Agave americana* L. plant is a slow-growing monocotyledonous (a plant which produces a single small leaf from the seed called a cotyledon when germinating (mono = single, cotyledon = seed leaf)) and monocarpic (growing for a number of many years before flowering that happens once, then dies after that), voluminous, evergreen herbaceous, perennial, *rhizomatous*, plant of a giant botanical genus of the *Agave* (Adams & Adams, 1998:11; Msahli *et al.*, 2007:3951; Mylsamy & Rajendran, 2010:2926; Leal-Díaz *et al.*, 2015:3924; Bouaziz *et al.*, 2014:27).

*Agave americana* L. plant is overwhelmingly the most popular, illustrious and abundant varieties of agave species that belongs to the *Agavaceae* family (Djizi & Bouzaouit, 2019:113, Ortega *et al.*, 2019, Krishnadev *et al.*, 2020:2443), inherent to barren and semi-barren areas of America particularly, Mexico (Hulle *et al.*, 2015b:1); where they were a staple of the Aztec culture, for food, drink, and clothing and really just about anything (Bouaziz *et al.*, 2004:1; Msahli *et al.*, 2004:540; Singha & Rana, 2010a:157; Escamilla-Treviño, 2011:2; Mylsamy & Rajendran, 2011:76; Mara, 2013:1; Chen *et al.*, 2014:320; Bouaziz *et al.*, 2014a:1; Vilane *et al.*, 2014:130; Mielenz *et al.*, 2015:1 Rahmani *et al.*, 2015:1).



**Figure 2.15: *Agave americana L.* Plant; a source of pita fibre**

The *Agave americana L.* plant produces approximately 40-50 leaves per year (Hulle *et al.*, 2015b:1, Krishnadev *et al.*, 2020:2444) and 200-250 leaves throughout its productive period. Each leaf has a weight make up of fibre; 4%, cuticle; 0.75%, other dry material; 8% and moisture; 87.25%. Consequently, a typical *Agave americana L.* leaf weighs about 3% of total weight with each leaf having about 1000 fibres. The diameter of the fibre varies from 100mm to 300mm (Mallikarjun *et al.*, 2017:119). The plant is without stem, but with massive grey-blue (figure 2.15) to blue-green leaves that are alternate and spirally arranged and radiate directly out from the central stalk of the mother plant to form a dense rosette of about 4 m wide (Msahli *et al.*, 2004:540; Boauziz *et al.*, 2014a:1; Hulle *et al.*, 2015b:2). Fibre walls of adult leaves are usually lignified thus, become stiffer and thicker than those of young leaves. *Agave americana L.* is of the plant kingdom, the sources of strong natural hard fibre (Angela, 2011:36; Vilane *et al.*, 2014:395).

The leaves are uphill to flat, widest in the middle, tapered toward the tip and thickened toward the base, the margin straight to undulate; spines (Chambers & Holtum, 2010:10), usually even in size and positioning hardly uneven. The marginal spines at the centre of the leaves range between 3–6 mm whereas those at the slim tips arched from low pyramidal bases are shorter ranging from 1-2 cm in length. The marginal spines are narrowly spaced out: 1-2 cm long. At the broad base area; they are dark brown and packed down. They are light to dark brown in colour. They are fleshy thick, leathery, sword-shaped and covered with a thick cuticle and a coating of wax which make them hairless (Iñiguez-Covarrubias *et al.*, 2001:102; Chandrasekar *et al.*, 2010:2; Mysamy & Rajendran, 2010:2926).

The leaves can grow up to 2.50 m in length and (25 cm) wide and are triangular in cross-section with, brown-red marginal spines that are 5 mm long and 4-11 cm apart, curved like fishhooks and ending in sharp spiny pointed tips (25-40mm long) (figures 2.18 and 2.19) which can pierce through clothes and leather; with ease. These spines are an adaptation of these plants against pestering of plant invaders. The normal *Agave americana L.* plant leaves are of one colour which blue or green except the youngest leaves which have not unfolded which are cream-white, as illustrated in figures 2.16 and 2.17 and acquire colour as they are exposed to sunlight. When the young immature centre leaves unfold, marginal spike impressions are noticeable on the still erect younger leaves as illustrated in figures 2.16 and 2.17. The older leaves often gracefully arch down, figure as illustrated in figure 2.15 (Msahli *et al.*, 2005:540; Bickerton, 2006:219; Chattopadhyay & Khan, 2012:33; Bezazi *et al.*, 2014:2).



**Figure 2.16:** *Agave americana L.* plant with unfolding inner leaves

One leaf of *Agave americana L.* plant creates four times more cellulose fibres than hastiest developing eucalyptus tree. *Agave americana L.* provides a multitude of human and environmental health as well as economic benefits (Tameen *et al.*, 2016:2; Uygur, 2017:165). The leaves serve as the main photosynthetic organs of plants where sunlight energy is trapped and converted into chemical energy reserved for storing carbohydrates (figure 2.18) to be used during flowering (Hulle *et al.*, 2015b:1). In the adult leaves, the fibre walls become thicker and tend to be lignified. The lignin is a strong and watertight body that crosses the cellulosic fibre walls and makes them stiffer. The leaves of *Agave americana L.* are characterized by the abundance of long cellulose leaf fibre bundles representing the vascular system, consist of shorter ultimate fibres, run lengthwise of the leaves and are water-loving in nature, which gives the *Agave americana L.*

leaves their stringency and tenderness. The ultimate fibres are less than 5 mm long and are approximately 25  $\mu\text{m}$  16-32  $\mu\text{m}$  wide (Msahli *et al.*, 2007:3951; Rowell, 2008:18; Manimekalai & Kavitha, 2017:374). The cellulose microfibrils have various types of chemical bonds like covalent, hydrogen and Van Der Waals bonds (Chaabouni & Drean, 2006:367; Bouaziz *et al.*, 2014a:1).



*Figure 2.17: Agave americana L. leaves with explicit marginal spines*



*Figure 2.18: Agave americana L. plant leaves with stored nourishment for flowering*

In reality, outdoor plants typically bloom between the 10<sup>th</sup> and 30<sup>th</sup> years in warm regions and as much as 60 years in colder climates. Indoor plants rarely flower (Anandjiwala & John, 2011:183; Chattopadhyay & Khan, 2012:33). During spring the plant begins bolting and yields its considerable inflorescence held aloft a huge and robust fleshy stalk. Its floral stalk sometimes is termed the trunk which is produced rapidly at maturity. The long narrow flowering stalk that can

grow as high as 3-10 m or so long, bears branched, candelabra-like greenish-yellow flowers (Adams & Adams, 1998:1) up to 9 cm long that are hermaphrodite; (thus possess both male and female organs); bloom in panicles, which contain the plentiful nectar for insects pollinations, but also hummingbirds and bats (Chattopadhyay & Khan, 2012:33; Bouaziz *et al.*, 2014a:1; Bouaziz *et al.*, 2014b:1-2).

*Agave americana L.* plant is clonal, it dies after blooming, but produces suckers/offsets from its roots at the base of each rosette, throughout its life and these remain to continue the lineage, often forming a colony of new plants produced through various meristems (flower, root and shoot) (Bickerton, 2006:219). *Agave americana L.* plant reproduces sexually and asexually using different strategies for reproduction. *Agave americana L.* plant reproduces sexually through self-pollination. The pollinated flowers mature and produce young plants that are attached to the old flowered stalk. At this stage the young plants can be; transplanted into new individual plants (Hamissa, 2008:501).

The pollinated flowers also produce fruits of which after mature release seeds which can also germinate into young individual plants (Adams & Adams, 1998:11-12). *Agave americana L.* plants mainly reproduce asexually, from the rhizomes of the mother plants and bulbs. The vegetative buds on the inflorescence produce plantlets; which can develop roots to form new individual plantlets around the mother plant. This root *reproductive* system, called the *meristem* reproduces plantlets that resemble the mother plants genetically. The *meristem reproduction starts underground and plantlets* shoot springing into the floral stem or root suckers which develop into floral stems. (Bickerton, 2006:219; Zwane *et al.*, 2011:84; Adams & Adams, 1998:12; Sandoval *et al.*, 2012:11).

#### **2.4.2 The drought-tolerant characteristics of *Agave americana L.* plants**

It is forecasted that global climate change is to raise the soil-drying conditions due to the increased heat and drought. This is expected that crops be sensitive to water vapour pressure shortage, ensuing in crop output losses (Davis *et al.*, 2015:4). Increasing competition between agricultural and industrial resource use efficiency (RUE), advocates that crops with greater adverse and extreme growing conditions tolerance and higher water-use efficiency (WUE) vital for sustainable fibre plant production. In countries where water is scarce, the *Agave americana L.* plants have potential to turn into a viable yield and vital tool for promoting textile fibre economic development in those areas and considerably enhancing the quality of life for a number of people



in those communities (Chen *et al.*, 2014:320; Davis *et al.*, 2015:9 Leal-Díaz *et al.*; 2015:3924, Hourí & Machaka-Hourí, 2016:89).

Until recently, productivity, processability and agronomic inputs must be considered seriously when selecting and developing new biofibres for textile production. However, issues such as biodiversity, competition with textile fibre supply, impacts on society, greenhouse-gas emissions, sustainability and water usage, need to be carefully considered. The optimum utilisation of water to produce particular fibre crops is essential due to severe droughts that lead to water scarcity. The *Agave americana L.* plant has high water-use efficiency and can survive under dry growing conditions of arid and semi-arid with minimal competition food crops (Escamilla-Treviño, 2011:1-2; Chen *et al.*, 2014:320; Mabhaudhi *et al.*, 2017:1).

*Agave americana L.* is definitely the most tolerant of all the *Agave* plants. This explicates the reason for its worldwide naturalized spreading (Bezazi *et al.*, 2014:2). It is one of the *Agave* species that have developed diverse biochemical and biophysical mechanisms. The cuticle is the most important protective membrane which is interface characterised. *Agave americana L.* plants are also adaptive for stress protection for adverse weather conditions, even whereby increased water use efficiency is significant (Bernardino-Nicanor *et al.*, 2012:3550). It is extremely resilient to the hostile agro-climatic conditions, especially in peripheral low fertility soils, in beaches, grasslands, riparian zones, rocky slopes, sandy areas, shallow and urban areas, woodlands, very dry hilly terrain and, at a very low growth rate. It tolerates wind, salt, extreme temperatures, drought as well as irregular rainfall. It is well adapted to provide a reliable stage of fibre security to the underprivileged people. The most ideal soil conditions are that which are tolerant of weather conditions and soil that is well-drained (Bouaziz *et al.*, 2004:1; Ravi *et al.*, 2010:111; Davis *et al.*, 2017:314). The *Agave americana L.* leaves are dense succulent plants with large leaves that store a lot of water. During severe and prolonged drought, they lose some viscosity, become floppy and limp to some extent. To avoid transpiration and subsequently loss water, *Agave americana L.* leaves are naturally coated with wax that gives them the characteristic bluish-green colour. In addition to its thickness, *Agave americana L.* leaves are very fibrous. The fibres run parallel to the length of the leaf from base to the apex (Stewart, 2015:3-4).

*Agave americana L.* species is extremely drought tolerate because it takes the water-conserving crassulacean acid metabolism (CAM) photosynthetic pathway *Agave americana L.* plants are strategic plants; that open their stomata during the night when it is cool and during the day when it is hot; they close them to preserve absorbed moisture and this improves their exceptional water

use efficiency (WUE), thus, permits it higher productivity (Nobel, 1990:1; Kant, 2010:1; Bernardino-Nicanor *et al.*, 2012:3550; McNally, 2015:1; Davis *et al.*, 2017:314; Hourri & Machaka-Houri, 2016:89). As such *Agave americana L.* species photosynthesizes during the day, with closed stomata, after it has opened the stomata, to absorb and fix CO<sub>2</sub> at night when temperatures are lesser than during the daytime, thus lessening water loss by transpiration from plant to the atmosphere (Iñiguez-Covarrubias *et al.*, 2001:101; Borland *et al.*, 2009:2879; Chambers & Holtum, 2010:18; Li *et al.*, 2014:1; Stewart, 2015:13). Its WUE in the dry and semi-dry lands is up to six or more times than the WUE of any of the current conventional fibre crops for fibre biomass (Chambers & Holtum, 2010:20; Chen *et al.*, 2014:320; Cushman *et al.*, 2015:4179; Kandhasamy & Vasudevan, 2015:849).

The *Agave americana L.* responds vigorously to its environment in the drought seasons where it will pucker up to decrease the entire leaf surface for evaporation, so as to improve crop yields in arid and semi-arid areas (Leal-Díaz *et al.*, 2015:3924; Mielenz *et al.*, 2015:1; Yang *et al.*, 2015:3). In times of extreme heat or sun, it changes its leaves' angles to either increase or reduce sunshine effects (Bernardino-Nicanor *et al.*, 2012:3550; Bouaziz, *et al.*, 2014b:1-2). *Agave americana L.* has adapted well to marginal, harsh, local growing conditions, of Sub-Saharan Africa (SSA), countries that include Lesotho, thus offers potential sustainable textile production and processing conditions (Chivenge *et al.*, 2015:5689). It has developed efficient mechanisms and structural characteristics, which make them productive regardless of harsh semi-arid growing environments, where soil surface temperatures regularly surpass 55°C and water, as well as nutrients, are severely limited (Iñiguez-Covarrubias *et al.*, 2001:101; Pandey *et al.*, 2014:82). *Agave americana L.* Plant has proven to be the most productive and cold-tolerant *Agave* species tested (New Phytologist, 2014:739).

In addition, the succulent and fibre-rich nature of *Agave americana L.* leaves and their rosette arrangement are an essential tool for this fibre plant for the photosynthetically active radiation, greatest absorption and fixation of CO<sub>2</sub> and channelling of water to their moderately superficial root systems for the successful photosynthesis even in weather extreme periods (McNally, 2015:1; Mielenz *et al.*, 2015:1-2). The roots of *Agave americana L.* plant root systems, also respond accordingly to water scarcity: they contract during the dry season, to minimise water loss, but they also fast to create fine roots in order to rapidly absorb water after a short- existed rain occasion. In addition, dead *Agave americana L.* leaves accrue at the base to buffer the living tissue of the leaf from elevated heat temperatures, which can array from 50 to 55°C (Kolte, *et al.*; 2012:1-2,

Mylsamy 2011:12; Davis *et al.*, 2015:1-2; Stewart, 2015:3). *Agave americana L.* plants flourish in both the urban and rural areas at all seasons of the year even when water and nutrients are scarce (Saraswat & Gope, 2014:1; Kandhasamy & Vasudevan, 2015:849). It thrives in these regions where traditional annual crops cannot, because of its shallow rooting system too (Chattopadhyay & Khan, 2012:33; Bouaziz *et al.*, 2014b:1). But they need easy-to-drain soils and are very resistant to drought and high temperatures, and can tolerate frost, if it is not regular, down to 27 °C below zero (Mylsamy & Rajendran, 2011:76; Rahmani *et al.*, 2015:1).

*Agave americana L.* plants have the prospective future to sustain textile demands associated with global growing populations. Literature indicates that *Agave americana L.* plants are very easy to cultivate since they grow wild and require little care and maintenance, (Bouaziz *et al.*, 2004b:2; Davis *et al.*, 2017:324). They require no watering once well established. Their input costs are less for them to survive, regenerate & maintain on a justifiable basis. It is usually full-fledged in patches and fencing crop in uninhabited and wild environments (as illustrated in figure 2.21), where soils are dry and unsuitable for crop cultivation (Mielenz *et al.*, 2015:1; Stewart, 2015:4). They therefore, have a comparative benefit in peripheral lands because they are naturally grown to endure stressful situations (Baldermanna *et al.*, 2016:107).

### **2.4.3 Processing techniques for obtaining *Agave americana L.* fibre**

There are various ways to extract the natural plant fibres from fibre plants. *Agave americana L.* fibre is an example of such fibres that need such to cleaning before use (Thomas *et al.*, 2011:11; Smole *et al.*, 2013:370).

#### **2.4.3.1 Selection and harvesting of leaves from a plant**

##### **(a) Selection**

To obtain and determine good quality and quantity of the *Agave americana L.* fibre content, the selection of the plant leaves has to be done properly. The *Agave americana L.* plants with sturdy, dense, clean, statuesque, blemish-less and healthy foliage have to be selected for textile fibre extraction. The flowering plants should not be used for textile fibre extraction. The *Agave americana L.* plants are ready for textile fibre harvest from the third year onwards. The mature leaves by then with the length of more than a metre are harvested. The *Agave Americana L* fibre content varies with a varied range of 2.5%–4.5% (Hulle *et al.*, 2015c:65).

##### **(b) Harvesting**



Established *Agave americana* L. plant leaves that are at the lower sides of the plant can be harvested from 3-4-year old plants and from the fifth year onwards regular harvesting of leaves can be done for fibre extraction, when the leaves are equal or more than a metre (Hulle *et al.*, 2015b:1). Mature lower leaves, at an angle of more than 45° to the upright are cut away from the plant with a sharp heavy duty knife. The leaves are transported to fibre extraction place, after harvesting. The leaf marginal and tip spikes are detached, before fibre extraction (Iñiguez-Covarrubias *et al.*, 2001:105; Hulle *et al.*, 2015a:2; Hulle *et al.*, 2015c:66; Kolte *et al.*, 2012:2; Teli & Adere, 2016b:257).

#### **2.4.3.2 Fibre Extraction Processes**

The quantity and quality of lignocellulosic fibres; are determined among others; by the used extraction method. Extraction of *Agave americana* L. fibre is the separation of cellulosic fibres from the buttressing substances which include pectin or lignin, wax, ashes, resin, fats and other carbohydrates (Bora & Padmini, 2019:145). The principle for plant fibre extraction is to degrade and remove the non-cellulosic components of the leaf biomass so as to obtain the cellulosic fibre bundles (Ebisike *et al.*, 2013b:37; Gašparič *et al.*, 2013:202; Van Dam, 2002:7). The *Agave americana* L. fibres lie embedded longitudinally in the leaves. In order to soften the fibres various preliminary chemical and mechanical processes are used to degrade plant leaf biomass, so as to achieve with ease better results for fibre extraction (Dungani *et al.*, 2016:46, Bora & Padmini, 2019:145).

*Agave americana* L. fibre extraction is cost-effective and harnessing its alternative potentials to synthetic fibre properties will contribute enormously to the textile industrial inventive world growth (Angela, 2011:35). Fibre extraction process is named after the method of fibre separation from the leaf biomass (Chandrasekar *et al.*, 2010; Ales & Sumanasiri, 2016). The methods used for the extraction of *Agave americana* L. fibres are comparable to sisal fibres extraction methods (Thomas *et al.*, 2011:9). Generally, separation of non-fibrous constituents from the fibre is attained manually or mechanically and followed by physical, chemical or enzymatic processing of fibres (Konczewicz *et al.*, 2013:118). The proper extraction process usually results in high-quality fibre with essential length, fineness and strength, high cleanliness, optimum effectiveness and uniformity (Van Dam, 2002:7; Kolte *et al.*, 2012:2; Selvam & Arungandhi, 2013:96).

##### **2.4.3.2.1 Mechanical fibre extraction**

Mechanical fibre extraction means that the process is carried out manually through a process known as decortication (Van Dam, 2015:12), where manual decorticators using modern machinery or basic tools like knives, cans, lids of aluminium containers, scrape away biomass pulp, perhaps after pounding the leaves with a mallet. Traditional hand decortication is a very slow and tedious process that requires too much time and manpower. Alternatively, fibre decortication can be conducted effectively using a modern machine-driven decorticator. The leaves are then crushed by a spinning wheel set with blunted teeth that cannot damage fibres (Chandrasekar *et al.*, 2010:3). The mechanical fibre extraction processes such as fibre separation washing and drying must be carried out immediately effect or else the adhesives in the leaves harden, triggering the pulp to stick to the fibres and make it challenging to properly clean the fibres. The removal of non-cellulosic fibre components from the cellulosic fibre components cannot be done efficiently using mechanical extraction methods (Thomas *et al.*, 2011:10; Satyanarayana *et al.*, 2011:219; Angela, 2011:44; Kolte *et al.*, 2012:3; Ray *et al.*, 2014:37; Saraswat & Gope, 2014:1; Hulle *et al.*, 2015b:2; Hulle *et al.*, 2015c:66-67; Ales & Sumanasiri, 2016:2; Teli & Adere, 2016b:257).

#### 2.4.3.2.2 Retting Process

Retting refers to a method of fibre extraction by which intercellular noncellulosic leaf biomass that intimately binds together fibre bundles is degraded chemically in order to separate it from the cellulosic fibres (Ray *et al.*, 2015a:101, Bora & Padmini, 2019:144). Chemical retting can be achieved by using inorganic chemicals (such as acids, bases surfactants and chelators) or organic chemicals such as enzymes. Enzymes can be obtained commercially or produced by using micro-organisms (Das *et al.*, 2014:270-272; Hulle *et al.*, 2015b:2; Sisti *et al.*, 2018:101).

##### (i) Inorganic Chemical Retting

Inorganic chemical retting yields high-quality fibre, although it is viewed as relatively costly, and not eco-friendly because of high energy and chemical utilisation (Thomas *et al.*, 2011:11; Das *et al.*, 2014:270). Lignin and hemicelluloses are easily hydrolysed into smaller molecules through the use of acids in cellulosic fibre extraction. Acid treatment forms reactive groups that assist fibres to fibrillate, resulting in high fibril crystallinity. Contrary to that, alkali **breaks** up the lignocellulosic components between fibres and splits structural bonds between lignin and cellulose, which results in an enlarged surface area and an increased degree of polymerisation which reduces fibre strength (Hulle *et al.*, 2015c:67).

## (ii) Organic Chemical Retting

Retting is a chemical organic fibre extraction process when water and microorganisms or enzymes combined actions ferment and softens the *Agave americana L.* plant leaf biomass to release the lignocellulosic fibre (Omenna *et al.*, 2016:275, Manimekalai & Kavitha 2017:373). Good quality lignocellulosic fibre is usually extracted by the organic retting method. Organic chemical retting does not produce any toxic materials, and the by-products are completely biodegradable. Organic retting of *Agave americana L.* plant can be achieved through water and enzymatic retting (Thomas *et al.*, 2011:9; Hulle *et al.*, 2015b:2; Sisti *et al.*, 2018:101).

## (iii) Water retting

Water retting is an organic process whereby, the *Agave americana L.* plant leaves are submerged in water, to allow aerobic and/or anaerobic bacteria to digest away noncellulosic plant biomass leaving cellulose fibres intact (Gowda, 2007:22; Savastano *et al.*, 2009:56; Konczewicz *et al.*, 2014:119; Thygesen, 2013:373; Teli & Adere, 2016b:257). It is a microbial biodegradation process which allows ecological and easy separation of fibre from pith. After harvesting, the leaves are immersed in water for some time (about 2-3 weeks) during which the non-cellulosic biomass that connects the fibre with other plant constituents is fermented and degraded by retting micro-organisms (Sisti *et al.*, 2018:104).

When adequately fermented, the fibres can be easily separated from the leaf biomass (Hulle *et al.*, 2015b:2) and produces good quality fibre even though fibre quality is mainly dependent upon retting conditions and duration (Parida *et al.*, 2011:5268; Srinivasakumar *et al.*, 2013:3). If the leaf retting is overdone, fibres quality decreases (Ray *et al.*, 2015a:102). Under-retting leads to partial removal of adhesive materials and extraction of fibre becomes a challenge (Savastano *et al.*, 2009:57; Ebisike *et al.*, 2013a:96; Ebisike *et al.*, 2013b:36; Saravana & Kumar, 2016:3600). Hence, the retting progress must be checked prudently at intervals to prevent fibre destruction. The natural water retting is a slow process (Bhikhu & Shah, 2015:69), but it is cost-effective (Teli & Adere, 2016b:257). Overdue

Retting duration is dependent upon temperature, atmospheric retting conditions which include sometimes nutrients, thickness of the leaf and type of bacteria and the existence or lack of oxygen. Organic water retting is a potential option for physical or inorganic chemical lignocellulosic fibre extraction processing. The fibres are separated from the woody leaf biomass using mechanical methods. The water retting method poses no special disposal problems because the material is

biodegradable (Vogl & Hart, 2003:127 & 124; Elsasser, 2010:56; Koztowski, 2012:70). Retting utilizes the microorganisms that require lower energy input, no chemical and mild environmental conditions (Thomas *et al.*, 2011:9; Koztowski, 2012:70; Bezazi *et al.*, 2014:1; Van Dam, 2015:12).

The good quality *Agave americana L.* fibre basically relies on a controlled retting process. The *Agave americana L.* leaves' quality, thickness and quantity, the nature of retting water, weather and ecological situations which can affect temperature during retting, the existence, number and activity rate of microbes, the pre-treatment of the leaves are some of the most important factors controlling retting rate (Ray *et al.*, 2015a:105, Ray *et al.*, 2016:59). Plant fibre retting must be carefully monitored or else the resultant fibre would either be under or over-ret. Under-retted *Agave americana L.* fibre is usually coarser in texture with cuticular debris and over-retted fibre is easily destroyed during extraction and end up shorter, thinner and less strong than normal (Nair *et al.* 2013:23).

#### (iv) Enzymatic Retting

Enzymatic Retting is another class of organic retting. Enzymatic retting uses industrially produced enzymes to degrade the pectin materials binding the fibre bundles. It is quicker than water and dew retting methods and yields softer, more pliable and spinnable fibres (Hulle *et al.*, 2015b:2). It is potentially easier to control and a cheaper method of fibre extraction because the retting enzyme can be reused a number of times before it is disposed. The enzyme concentrations can be higher to hasten the retting process (Hulle *et al.*, 2015c:68, Kolte, *et al.*, 2012:3). The enzymatic retting is potentially an effective fibre extraction that can minimise environmental impact with no significant strength loss of cellulosic fibre (Hernández-Hernández *et al.*, 2014:1). Enzymatic retting leads to an improved fibre quality, predominantly the greater fibre fineness feature (Koztowski, 2012:74).

#### 2.4.3.3 Washing and drying of extracted fibre

After fibre extraction with any of the discussed methods, fibres are washed in water until clean and dry thoroughly. The washing and rinsing of the fibres must be proper to get rid of the fibres binding tissues (Angela, 2011:44; Saraswat & Gope, 2014:1). Fibre should be thoroughly dried because moisture content determines fibre quality. Non-natural drying is highly recommended for best fibre quality but it is not viable for all (Chandrasekar *et al.*, 2010:3). Before packing for use,

dried fibres should be combed and sorted according to different grades (Hulle *et al.*, 2015c:65; Boguslavsky *et al.*, 2007:3).

## **2.4.4 The structure, properties and composition of *Agave americana* L. fibre**

### **2.4.4.1 The structure**

*Agave americana* L. fibres are multiple-celled fibres, extracted from the leaves of monocotyledonous, naturally occurring uncultivated *Agave americana* L. plant. The *Agave americana* L. fibre in a leaf comprises of xylem, phloem and diverse unsheathing cells that are scattered all over in leaf condensed milieu. The cells are lignified and stiff in nature. The entire fibro-vascular bundle is called fibre. *Agave americana* L. fibre is irregular, rotund; narrowing to a point, with one thicker side; that is from the base side of the leaf. The fibre from the underside of the leaf is better-quality than the coarse one obtained from the upper side of the leaf (Mafaesa, 2006:92; Hulle *et al.*, 2015b:3; Hulle *et al.*, 2015c:65).

The *Agave americana* L. fibre is obtained in bundles which have a minimum length of 65.2 cm up to 2.8 m long (Mafaesa, 2006:96) Each *Agave americana* L. fibre is made up of numerous cells called ultimates or ultimate cells. The ultimates are overlapping and bound by waxy and sticky materials such as lignin, hemicelluloses and pectin (Smole *et al.*, 2004:47; Msahli *et al.*, 2007:3952; Ray *et al.*, 2014:37), to form the technical filament fibre that is relatively thick. The *Agave americana* L. ultimate fibre, is thin with a diameter of about 150  $\mu\text{m}$  (Hulle *et al.*, 2015c:69) and it is very difficult to obtain and thin to be spun on its own (Chaabouni *et al.*, 2018:1).

*Agave americana* L. fibre is lengthy with a varying diameter which is determined by a number of ultimates across the section (Hulle *et al.*, 2015b:68). The ultimate fibre cells have the following parameters: the average length of around 10.1  $\mu\text{m}$  (1 to 7.5 mm) (Msahli *et al.*, 2006:10; Msahli *et al.*, 2007: 3951) and the average width of 3.1  $\mu\text{m}$  which is lower than of flax ultimate fibre cells of about 10 to 30  $\mu\text{m}$  and sisal ultimate fibre cells with a diameter of about 24  $\mu\text{m}$  (Iñiguez-Covarrubias *et al.*, 2001:102). *Agave americana* L. fibre bundles are very strong with 39% to 49% strain range due to the spiral nature of ultimate fibre cells. This structure explains the mechanical strength of this technical fibre (Msahli, *et al.*, 2007:3953; EI oudiani *et al.*, 2009:15; Kolte *et al.*, 2012:2; Saraswat & Gope, 2014:1; Hulle *et al.*, 2015c:68 – 69).

#### **2.4.4.2 The properties of *Agave americana L. fibre***

*Agave americana L.* fibre is a natural cellulose fibre that possesses excellent specific properties for textile fibre such as reasonable strength, moisture absorbency, low density and appropriate length (Smole *et al.*, 2004:47). But it is stiff to function as a textile fibre for making diversified and value-added products, without special softening treatment (Msahli *et al.*, 2015:1). *Agave americana L.* fibre is flexible, smooth & lustrous and has burning characteristics comparable to ordinary cellulosic fibres (Chattopadhyay & Khan, 2012:33).

##### **(a) The Physical and Mechanical Properties**

To find the full potential textile benefits of *agave americana L.* fibre there is a need to understand its chemical and physical structures along with properties, defects, strength dimensions, variability and crystallinity; to mention a few examples (Dungani *et al.*, 2014:4), because the textile fibres are complex and diverse. The performance characteristics and end uses of any textile fibre depend on the fibre type and structures, age, production and processing methods. (Singha and Rana, 2010b:1057). Comprehensive knowledge of textile structure will facilitate an intelligent assessment of standards and brand of merchandise and will develop the ability to distinguish quality in textiles and, in turn, to appreciate the proper uses for the different qualities. The knowledge of the physical, mechanical and chemical structures and properties of the individual fibre will facilitate the intelligent exploration procedures and potential end uses (Bavan & Kumar, 2010:3601-3602). The physical structure that is identifiable with the low powered microscopes or the naked eye is the macrostructure. The physical properties include length, diameter, surface appearance, colour, and light reflection. Conversely, the fibre microstructure is detected with the more sophisticated systems textiles (Smith and Block 1982:31)

The micro properties such as tensile strength and elasticity are determined by the type of chemical molecules and their mode of arrangement within the fibre. The microstructure affects the chemical properties such as dye fixation, sunlight resistance and resistance to mould and fungi (Smith and Block 1982:65). The performance of textiles and textile products of durability, maintenance comfort, appearance and appearance retention as well as cost are affected by the properties of the fibre(s) from which they are made (Kalebek & Babaarslan 2016:3). Durability performance properties are affected by the tensile properties that are due to of a fibre. The load performance of any textile fibre is dependent upon the molecular structure and also the spacing of the molecules. The crystalline structural textile is more durable and therefore more serviceable for specific

purposes than the amorphous structural textile. Comfort performance properties are affected by the fibre moisture absorbency, elasticity, hand and feel of the fibre. The textile fibre that is more absorbent is more comfortable to use next to the skin. The lignocellulosic fibres are highly absorbent and strong (Smith and Block 1982:31 &70), thus, provides good maintenance, durability and outstanding comfort performance characteristics to the textile end products. They are therefore good for skin-contact towels and clothing that includes sportswear as well as dippers. However they lack self-extinguishing properties, they can burn rapidly even after removal from the flame. They are therefore not good for children's sleepwear and protective clothing unless they are treated with flame proof or flame resistant finish. Fibre content can also play such a significant role in determining textile performance properties because fibres are the basic units of textiles (Hollen *et al.* 1988:5).

Different fibre separation techniques produce fibres with differences in physical, mechanical and morphological properties, which later influence the properties of the textile end product. The physico-mechanical properties like colour, length diameter and tensile strength influence the processing and end use performance characteristics of textile fibres. For example water retting of plant fibres produces the better quality fibre with greater uniformity mechanical extraction and dew retting. Efficiency in processing activities produced acceptable physical properties, colour and mechanical strength of the fibres (Zakaria *et al.*2020:7208-7212).

The *Agave Americana L.* fibre is a lignocellulose fibre. Thus, it is organic chemical cellulose complex molecules consists of carbon, hydrogen and oxygen atoms bond together at reactive sides to provide it with unique structures that could determine performance characteristics and the end uses in textile. This chemical and physical textile knowledge is needed to analyse, evaluate and anticipate the textile potential performance characteristics, benefits and end uses of *Agave americana L* fibre.

The *Agave americana L.* fibre is a technical multicellular fibre bundle consists of numerous polygonal ultimate fibres glued together by noncellulosic fibre components with imbalanced proportions along the fibre length axis (Rippon & Evans, 2012:123, El Oudiani *et al.*, 2015b:30). The *Agave americana L.* fibre exists as bundles created from numerous ultimate fibres detained together by waxy and sticky constituents (Chattopadhyay & Khan, 2012:34), due to its high tenacity of 2.94 g/d when dry and 2.3 g/d when wet, higher extensibility of 50% of its length before rupture when compared to other lignocellulosic fibres of which most don't exceed 10% and have average linear density of 24 tex. It is a coarse fibre which is superior and more flexible than



other fibres from *Agaveceae* (Chattopadhyay & Khan, 2012:36; Mbugua, 2014:48; El Oudiani, 2015a:15). The *Agave americana L.* fibre tensile strength is proximate to those of other lignocellulosic fibres like sisal or flax. Surprisingly it has extreme lower initial Young modulus and higher rupture work than other sister lignocellulosic fibres (El Oudiani *et al.*, 2010:1; Mylsamy & Rajendran 2011:76; Kolte *et al.*, 2012:1; Phologolo *et al.*, 2012:17).

Literature indicates that *Agave americana L.* fibre strength intensifies with plant leaf age. The leaf's age is determined by the level and position on the plant and the position of the fibre in the leaf. Fibre from the leaves of the lower levels is the strongest of all when compared to those obtained younger middle-aged and top youngest leaves. The youngest leaf fibre is less extensible (Hulle *et al.*, 2015c:69 & 70). The high amount of cellulosic hydrogen bonds binds the fibre chains, stiffens the fibre structure and reduces its ability to be distorted. Contrarily, fibre extracted from the oldest leaves possesses fewer hydrogen bonds because of lignin and other non-cellulosic sticky components. *Agave americana L.* fibre also exhibits low density and high water absorbency (El Oudiani *et al.*, 2009:335; El Oudiani *et al.*, 2010:1; Smole *et al.*, 2013:371; El Oudiani, 2015a:19 & 21; Hulle *et al.*, 2015a:24). Fibres obtained from the middle part of the plant provide the highest tensile strength and stiffness (Djizi & Bouzaouit, 2019:113). Thus, the top and the bottom leaves of the plant have to be avoided for fibre extraction (El Oudiani *et al.*, 2015a:27-28).

*Agave americana L.* fibre bundles have high tensile strain that exceeds 60% because they consist of single fibres which have zigzag relaxation arrangements which then, allow them to be straightened before distortion (Thamae *et al.*, 2009:73 & 97). This is contrary to other lignocellulosic fibres which have only up to 10% length extension before breaking. When forces are applied, these fibres are first straightened out before being pulled apart to a breaking point. This feature is thought to increase the strains of the *Agave americana L.* fibre (Bunsell, 2009:87; Hulle *et al.*, 2015b:3). It is the potential alternative source of biotextile fibre with minimal pollutants emission (Kolte *et al.*, 2012:1; Phologolo *et al.*, 2012:17). *Agave americana L.* fibre is a natural resource that possesses abundant antibiotic and hygienic properties for use to manufacture fabrics that can be used effectively to treat and dress open wounds to prevent bacterial infections (Kandhasamy & Vasudevan, 2015:848).

(b) The texture

*Agave americana L.* fibre is strong and durable (Tameem *et al.*, 2016:2). The dried raw *Agave americana L.* fibre is coarse, harsh and rigid in texture (Hulle *et al.*, 2015a:23-24; Hulle *et al.*,



2015b:4) and hard-surfaced due to the presence of lignin on their surface, which is the distinctive characteristic of all the leaf fibres (Mafaesa, 2006:92; Hulle *et al.*, 2015a:4, Hulle *et al.*, 2015c:70). It has a limited textile value, since it is more rigid and coarser than the bast fibres. But they can be processed to be soft and flexible. The potentially useful *Agave americana L.* fibres remain underutilised and underexplored because of the increased importance of synthetic fibres for cordage use and agricultural and processing limitations (Msahli *et al.*, 2006:9; Msahli *et al.*, 2007:3952, Mylsamy, 2011:4).

(c) The colour

The colour of the *Agave americana L.* fibre can be off-white to yellowish rust, subject to the handling method and time elapse for fibre extraction (Msahli *et al.*, 2006:11; Hulle *et al.*, 2015b:1; Hulle *et al.*, 2015c:70). Retted fibre discolouration is due to retting situations, quality of water and washing (Das *et al.*, 2013:755).

(d) Lustre

The uneven surface and cross-sectional shape of *Agave americana L.* cause it to be semi-dull in appearance. Its uneven cross-section throws light in all directions, with few bright lights. The correctly extracted and a single fibre shine up and look attractive (Hulle *et al.*, 2015b:4).

(e) The burning characteristics

The burning characteristics of *Agave americana L.* fibre are comparable to those of other plant fibres. When approaching the flame, it does not shrink away from the flame; rather it flares up immediately when coming in contact with the flame. After ignition, it continues to glow even when removed from the flame until it finished. It has a burning odour that resembles a burning paper. It produces soft leathery ash (Chattopadhyay & Khan, 2012:3; Hulle *et al.*, 2015c:70).

(f) Chemical Composition

Like most naturally occurring lignocellulosic fibres, *Agave americana L.* fibres bundles are composed of 67-80% cellulose fibrils interleaved in a 15% hemicellulose and 5-18% lignin, 0.26% waxes, and 8% moisture (Paudel & Qin 2015:219, Saraswat & Gope 2017:165)., which are comparable and range along with the percentage contents of other lignocellulosic fibres but the *Agave americana L.* fibre lignin content is lower than that of other plant fibres (Chattopadhyay & Khan, 2012:34). Pectin, water-soluble materials and ash are also existing along with major

contents even though it is in small quantities (Singha & Rana, 2010b:1057; Bouaziz *et al.*, 2014:1; Hulle *et al.*, 2015c:72). The property of each constituent contributes to the overall properties of the fibres. For example, the raw *Agave americana L.* fibre is difficult to spin for use in textiles due to relatively high lignin content which causes the fibre to be stiff. Karolia & Bhoj (2016:18) confirmed that the stiffness of the fibre reduces its cohesiveness and entanglement properties for fibre spinability. Cellulose micro-molecules are bound to one another, with various types of chemical bonds like covalent, hydrogen and Van Der Waals bonds (Chaabouni *et al.*, 2006:367; Chattopadhyay & Khan; 2012:36; Paudel *et al.*; 2015:219).

The fibre disintegrates when exposed to strong chemicals, but it was resistant to weak ones (Msahli *et al.*; 2006:9 & 11; Kolte *et al.*, 2012:2; Mbugua, 2014:48; EI Oudiani *et al.*, 2015:16). Like other natural plant fibres, *Agave americana L.* fibre is hydrophilic and has the moisture content of 9.19 % and moisture regain ranging from 10 -17% which is equivalent to that of other lignocellulosic fibres such as sisal 11%, jute 13.75%, linen 12%, ramie 8.5% (EI Oudiani *et al.*, 2009: 436; Singha & Rana, 2010:156). It is assumed that its hydrophilic behaviour occurs because of free hydroxyl groups on the chemical structures of the fibre, that effect polarity which attracts the already polarized water molecules (Mafaesa, 2006:121; Msahli *et al.*, 2006:11; Mbugua, 2014:48; Izijesu & Thamae, 2015:39). The *Agave americana L.* fibre is an eligible prospective source of textile fibre (Chattopadhyay & Khan, 2012:34, Mbugua, 2014:48). This natural fibre can be upgraded to a soft pliable and valuable textile fibre (Bouaziz *et al.*, 2014:1; Hulle *et al.*, 2015c:72).

(g) The effects chemicals on *Agave americana L.* fibres

*Agave americana L.* fibres are not easily damaged by weak acids and alkalis, thus, the weak acidic and alkaline bleaches, detergents and dyes can successfully be used with no effect on structure and tenacity of *Agave americana L.* fibres. However strong acids and bases can dissolve and distort these fibres. The bonds connecting the ultimate *Agave americana L.* fibres are more unstable to acids than alkalis so this can lead to loss of fibre tensile strength. In contact with concentrated bleaches can bleach fibres but with persistent exposure, the fibre strength is weakened. Chlorine bleach must be used for a short time and then rinsed out thoroughly to prevent further fibre damage to the (Hulle *et al.*, 2015b:4-5; Tameem *et al.*, 2016:2).

#### **2.4.4.3 The uses of *Agave americana* L. plant and fibre**

*Agave americana* L. planting reserves nature in many ways as described under 2.3.5 (Escamilla-Treviño, 2011:2; Manson, 2013:1; Davis *et al.*, 2015:1-2.). It has great potential to be upgraded into new sustainable textile fibre crop that can be used for various domestic, commercial, ornamental and medical applications (Kozłowski *et al.*, 2004:61; Mafaesa, 2006:5 & 42; Chandrasekar *et al.*, 2010:2; Singha & Rana, 2010b:1057; Chattopadhyay & Khan, 2012:33; Manisha *et al.*, 2012:69; Singha & Rana; 2010a:156, Saraswat & Gope 2014:1; Kandhasamy & Vasudevan 2015:849; Rahmani *et al.*, 2015:1). *Agave americana* L. plants were employed in the brewing of alcoholic beverages, as fodder source, a natural sweetener, a food source, a defence against pests and technical resources such as roofing and reinforcing composite materials by ancient Mexicans (Isikgor & Becer, 2015:4498).

The *Agave americana* L. is a resourceful plant with versatile environmental, economic, and social products and by-products such as biofibre, biofuel, biogas and bio-alcohol; are obtained and sustainable jobs are created as such it has the potential of transforming lives of poor communities (Tameem *et al.*, 2016:2, Krishnadev *et al.*, 2020:2443). The *Agave americana* L. plants can be densely grown (as illustrated in figure 2.19), as a natural hedge and biofence to secure humankind properties since they are impermeable to cattle and people due to their long-leaf rosettes with side margins and the vicious end-spikes at the tips. The dried stems of flowering are used as a waterproof roof thatching and building materials and stools in Lesotho (Thamae, 2008:55-56).



**Figure 2.19:** *Agave americana* L. plant forming a dense biohedge

In ancient times, *Agave americana* L. leaves' terminal spines were adapted and used as natural needles or awls which were naturally attached to the leaf threads or fibres as illustrated in figure 2.20 to create embroidered leather called *piteado* and to sew coarse textiles produced for by native

nations mostly Mexicans (Mara, 2013:1; Zwane *et al.*, 2011:84). To prepare the cord, the leaf cuticle and the non-cellulosic biomass were detached from the fibres, but the spine was not removed. the fibres were then twisted into the required twine ready for use with the fibre attached and when the work was complete the remaining thread and /or point was cut off and discarded (Castetter *et al.*, 1938:72), (figure 2.20).



**Figure 2.20: *Agave americana L.* leaf fibre thread and needle (<http://www.smragan.com/2011/10/18/agave-spine-needle-and-thread/>)**

*Agave americana L.* leaf thorns were used to make pens, nails and needles by Indiana (Chattopadhyay & Khan, 2012:36). *Agave americana L.* fibre can be used to manufacture fabrics (even though it is difficult to spin) for medical end usages (Kandhasamy & Vasudevan, 2015:848). The *Agave americana L.* fibre is used to produce twine and ropes, paper, head bands, nets, rugs, cushion padding, doormats, saddle pads, bags, carpets, sandals, packing bags, brush brittles, fish stringers, baskets, bracelets, doilies and motifs, modern eco-friendly biocomposites (Rowell, 2008:4; Thamae *et al.*, 2009:96; Gao *et al.*, 2015:5476-5477; Tameem *et al.*, 2016:2). Underutilized *Agave americana L.* has the potential to supply innovative prospects for income generation if their sustainable fibre properties, processing and market potentials were fully renowned and established (Thies, 2000:3).

If softened into a more polished fibre, appropriate for producing refined lacework; figures 1.5 and 1.6 (Mara, 2013:1). The *Agave americana L.* leaf fibre was used by the Native Americans and Mexicans. Literature shows that the best quality fibre is obtained from the youngest leaves. *Agave americana L.* is also one of the most optimistic unconventional plants which can be utilized to produce biosynthetic fibres against petroleum-based synthetic fibres. It can also serve as a supplementary fibre for conventional natural cellulosic textile fibres (Isikgor & Becer, 2015:4498).

## **2.4.5 Limitations on the uses of *Agave americana L.* plant fibre**

### ***2.4.5.1 Relatively high lignin content***

*Agave americana L.* fibre makes better quality ropes and twine than bast fibres and it is to produce coarse and low-grade textiles usually requiring no spinning because of its coarseness and stiffness, due to its relatively high lignin content (Thamae *et al.*, 2009:96; Gao *et al.*, 2015:5476-5477, Tameem *et al.*, 2016:2).

### ***2.4.5.2 Lack of market infrastructure***

Like many underutilized fibre crops, *Agave americana L.* is excluded in both subsistence and commercial farming, regardless of its viable and potential market. Perhaps this happens because of the absence of production, processing and marketing infrastructure that is suitable for quality and amounts needed for potential customers (Thies, 2000:3).

### ***2.4.5.3 Lack of technologies***

*Agave americana L.* fibre processing is labour-intensive and time-consuming. To increase its production scale, effective, well-organised manufacturing, storage and processing technologies have to be invented so as to ensure good quality standards (Thies, 2000:3).

### ***2.4.5.4 Loss of cultural diversity and lack of information***

*Agave americana L.* plant has neglected potential fibre and by-products characteristics which should be explored by scientists and consumers in order to change the perspectives about local plant fibres. The adverse attitudes people have about, indigenous *Agave americana L.* plant fibre and its by-products result in loss of cultural diversity and Lack of understanding. Studying and improving *Agave americana L.* fibre can be updated and provide information for cultural diversity (Thies, 2000:3).

### ***2.4.5.5 Deficiency of political motivation***

The political motivation to produce and process indigenous plant fibres normally favours the green insurgency fibre crops and puts excessive emphasis on conventional fibre crops like cotton and flax. The neglected and underutilised fibre species like *Agave americana L.* fibre which can flourish even under adverse growing conditions are excluded. These policies inhibit research and market for unconventional, local plant fibre variations (Thies, 2000:3).

#### **2.4.6 The Potential Sustainability Impacts of *Agave americana* L. Fibre**

*Agave americana* L. fibre production and processing can be achieved through environmentally and socio-economically sustainable ways. From an ecological perspective, *Agave americana* L. fibre production and processing can minimize any undesirable eco-friendly effect and keep an ecological balance in nature because of its cheap means of planting, regional availability and to some extent the ease of collection which directly influences its suitability to become an alternative source of natural fibre (Asim *et al.*, 2015:1-2). The main responsibility of *Agave americana* L. fibre producers and textile science researchers is to advance the production, processing, supply and marketing performances and approaches towards best textile sustainability. *Agave americana* L. fibre plant is in this case regarded to be of "high-profit" potential. High-profit plant refers to the plant that enables a sustainable livelihood including the relationship between fibre plants and people as well as income (Takane *et al.*, 2010:1). *Agave americana* L. fibre as a natural fibre; has the advantage of being sustainable fashion fibre because it is biodegradable, noncarcinogenic and cost-effective (Asim *et al.*, 2015:1, Hayles, 2015:104).

*Agave americana* L. fibre plant has several functions that are of ecological, economic, social and social security, construction, textile and medicinal and pharmaceutical nature. If it is, environmentally, socially and economy promoted and processed to produce textile fibre, it can have a sustainable future of which, people are very conscious about (Msahli *et al.*, 2006:9; Singha & Rana, 2010b:1055-1056; Niinimäki & Hassi, 2011:1877, Pandey *et al.*, 2014a:82; Izijesu & Thamae, 2015:39).

Textile sustainability advocates for the use of textile resources at a speed that never surpasses the capability of the planet earth to renew them. *Agave americana* L. fibre production and processing sustainability measures should ensure unchanged or reduced water and land usage; resourceful and cautious use of textile inputs; reduced GHG productions; better-quality expected investment; supported resistance and decreased ecological influence (UNEP, 2011:33). As such the fibre is sustainable because it is made from organic, recyclable or degradable textile substances, that are relatively safe and non-toxic and it has a reasonable socio-economic impact (Rusu, 2011:2). However, the *Agave americana* L. fibre is sustainable to some extent because, the fresh *Agave americana* L. plant leaves have sap and marginal and terminal spikes that can be harmful to human beings if care is not effectively and efficiently engaged (BIC, 2012:18).



Sustainable textile intensification can consequently be defined as a process of effectively and efficiently producing textile products with no additional textile resources; for a long time so as to uphold its potential to satisfy present and future textile needs and targets, concurrently, while increasing environmental reparation and fabricating pliability and the eco-socio-economic currents of textile services (Basiago, 1999:155).

#### ***2.4.6.1 Agave americana L fibre is an organic fibre***

Uygur (2017:164-165) clearly defines organic textile fibre manufacturing as sustainable textile production and processing approaches which prevent the use of chemical fertilizers; pesticides and herbicides in order to decrease global warming, depletion of the natural resources, energy requirement, waste such as biological degradation and the socio-economic impacts (Biobased Industries Consortium (BIC), 2012:18; Hayles, 2015:104). The future of *Agave americana L.* fibre is determined by dimensions such as, its cultivation and textile processing approaches that are ecological, social and sensitive economically. The increase in *Agave americana L.* fibre consumption implies the increase in organic textile fibre cultivation possibilities and the positive eco-socio-economic textile impacts (BIC, 2012:18; Chattopadhyay & Khan, 2012:33; Murphy, 2012:15; Minderhoud, 2015:3-4; Roos *et al.*, 2015:19).

*Agave americana L.* fibre production qualities mostly qualify it to be an organic fibre; even though; it requires much more time to grow and it is labour intensive to extract but less cost value, little or no care and adapt to special cultivated growing conditions than conventional fibres. In Lesotho, *Agave americana L.* fibre production is generally wild and without the use synthetic fertilizers, herbicides and pesticides because pests and diseases are of less concern for its cultivation (Thamae, 2008:54) into useful textile fibre and has environmental- ecological, economic, social positive impacts (Chattopadhyay & Khan, 2012:33; Murphy, 2012:15; Minderhoud, 2015:3-4; Roos *et al.*, 2015:19).

During production processing, it engenders predominantly organic wastes and leaf remains that can be used to produce bioenergy, produce animal feed, biofertiliser and ecological construction material (Uygur, 2017:165). At the end of its life cycle, it is completely decomposable.



#### **2.4.6.2 Ecological Impact of *Agave americana* L. Plant and Fibre**

The *Agave americana* L. fibre plant production has minute ecological and economic effect (Hulle *et al.*, 2015c:73). This could be achieved due to the plant's widespread root system that contributes optimistically to the water crisis management economy. The leaves are cut during harvesting, a major portion of the roots remains within the soil (Ciechanska & Nousiainen, 2005:61). *Agave americana* L. fibre crop is a perennial fibre plant species, as such it provides year-round cover that can upsurge the water retaining capability of the soil. *Agave americana* L. plant can increase soil carbon stock through both roots and leaf litter. Its residues also left on the field prevent weed growth (Eisentrau, 2010:14).

The *Agave americana* L. fibre and their by-products after further processing maintain and improve land soil fertility because they are 100% biodegradable, thus, provide natural manure for other subsequent crops to be grown (Elsasser, 2010:41; BIC, 2012:18; Cushman *et al.*, 2015:4179; Kandhasamy &, Vasudevan, 2015:849; Mielenz *et al.*, 2015:1; Stewart, 2015:4; Uygur, 2017:164). *Agave americana* L. fibre plants have a natural tendency to grow on rocky and infertile soils as such it stabilizes the land and local environment and reduces soil erosion figure 2.21.



**Figure 2.21: *Agave americana* L. fibre planted in a donga for land reclamation**

*Agave americana* L. plants get rid of heavy metals ions from polluted soil and prevent desertification (Li *et al.*, 2014:1; Chen *et al.*, 2014:320; Lok Sanjh Foundation, 2016:3). *Agave americana* L. plants can increase soil carbon stock through both roots and leaf litter (Eisentrau, 2010:14) As such, it can also mitigate climate change (Lok Sanjh Foundation, 2016:3). *Agave americana* L. fibre crop has high biomass productivity with minimal inputs. *Agave americana* L. plant as a deep-rooted, perennial that uses CAM pathway of photosynthesis has three- and six-fold

more WUE than C4 and C3 crops, respectively. As a result; it has a highly positive impact on the WUE from catchments drought experiencing countries like Lesotho (Eisentrau, 2010:170; New Phytologist, 2014:738).

*Agave americana L.* plants produce fibre which has potential sustainable biotextile properties for semi-arid and arid lands because it uses CAM pathway thus, they can vitally fight against textile fibre insecurity (Singha & Rana, 2010b:1057; Chivenge *et al.*, 2015:5688). *Agave americana L.* plants to maintain balanced ecosystems. *Agave americana L.* fibre plant adapts well to the adverse plant growing conditions such as extreme weather and other abiotic stresses, and have enough resilience to ensure good and continuous yields (GFAR, 1999:5; Chambers & Holtum, 2010:20; Chattopadhyay & Khan, 2012:34; Barth & Carus, 2015:6; Baldermann *et al.*, 2016:107).

Scientific researchers claim that the fastest growing eucalyptus tree yields up to four times less cellulosic fibre than *Agave americana L.* plant which collects five times more CO<sub>2</sub> than the best-known carbon collecting bionetworks (Elsasser, 2010:41; New Phytologist, 2014:738). Thus, cuts on GHG discharge amounts and the use of petroleum-based fibre (Quarshie & Carruthers, 2014:44). Measured over its life-cycle, *Agave americana L.* plants absorb more carbon dioxide from the atmosphere than it produces. *Agave americana L.* plants can lower the indigenous adverse social, economic and ecological impacts and maintain the overall solution to climate change (Chambers & Holtum, 2010:20; Cushman *et al.*, 2015:4179; Eisentrau, 2010:197; Kandhasamy & Vasudevan, 2015:849; Uygur, 2017:166).

*Agave americana L.* plants are green renewable fibre sources that are par replaced automatically by nature and avoid environmental overloading to purify and renew themselves by natural processes and cannot be depleted easily because they reproduce in both sexual and asexual means. The *Agave americana L.* fibre production and processing are less likely to lead to natural resources' depletion as it is the case with their synthetic counterpart (Sharma and Satyanarayana, 2012:1). *Agave americana L.* plants are monocarpic; sustainable plants that are fully renewed with no or less use of chemicals but by nature (Barth & Carus, 2015:6; Uygur, 2017:164).

#### ***2.4.6.3 Economic Impact of Agave americana L. plants and fibre***

*Agave americana L.* Plant is an economically viable and sustainable source of textile biofibre (Borland *et al.*, 2009:2879; Angela 2011:40). The nutrients and water use efficiencies and the biomass productivity for *Agave americana L.* plant are very high. Furthermore, *Agave americana L.* Plant prevents desertification and removes heavy metals ions from polluted soil. These

attractive properties make it potentially treasured as a low-cost global biofibre source (Li *et al.*, 2014:1-2). *Agave americana L.* fibre production and upgrading have the potential to reduce reliance on international textile markets and price increase. With petroleum prices expected to increase, the competitive alternative production and processing of *Agave americana L.* biofibre can relatively stabilise market prices in the long run. These activities can advance technology for fibre production and processing, further decreasing prices and rendering them competitive against conventional fibre crops and synthetic fibres in the medium to long term. Poor communities producing *Agave americana L.* biofibre, however, stand the chance to benefit from the extra income they can acquire by selling the by-products (Eisentrau, 2010:143-144; Hourri & Machaka-Hourri, 2016:90).

*Agave americana L.* plant is a multi-purpose plant that possesses no-waste characteristics that can allow producers to extrapolate profits on investment through the use of value-added supplementary commodity supply of market prices during formal market recession (Damme, 2008:32-33; Uygur, 2017:166). Commercial exploration of *Agave americana L.* fibre has a promising value addition to raise household income. New employment prospects can also be generated even on marginal land for conventional and non-conventional fibre and non-fibre crop cultivation and the labour inputs. The collection, transport, pre-processing, and the biofibre and by-products production can create more jobs. Most Basotho in rural areas, do not access textile fibre services. The enhanced use of *Agave americana L.*, fibre can significantly be associated with poverty reductions because greater access to textile services ease women's basic survival activities and reduce deforestation and indoor pollution caused by non-biodegradable synthetic fibre use (Eisentrau, 2010:13 & 144). These activities can increase economic growth and reduce poverty. The quality and degree of job creation will depend on the level of involvement within the entire production and processing. This is one of the major motives behind upgrading *Agave americana L.* fibre (Eisentrau, 2010:182 & 144; Yang *et al.*, 2011:380).

#### **2.4.6.4 Social Impact of *Agave americana L.* Plants and Fibre**

Generally, the *Agave americana L.* Plants are of great significance for ornamental and landscaping, not only because of their beautiful leaf shapes and colours, long-leaf rosettes and eye-catching blossoms (Rahmani *et al.*, 2015:1). *Agave americana L.* fibre is a promising textile biofibre (Angela, 2011:35 & 40). Its production and processing can be socially essential because *Agave americana L.* plant has a multi-purpose character and has the potential to produce good

quality textile fibres which can be converted into a sustainable fibre source (Boguslavsky *et al.*, 2007:15; Hulle *et al.*, 2015b:73; Tameem *et al.*, 2016:2).

The *Agave americana L.* fibre is likely to become a prospective source of employment. The *Agave americana L.* fibre crops are hardy and adapted to the needs of people in negligible farming milieus. The cultivation of *Agave americana L.* crops and their integration into the social needs of people such as food, clothing and shelter and feed for livestock has possible advantages, including; improved fibre security, thus, decrease human reliance on only conventional fibre crops for textile uses and petroleum-based textile, providing a number of opportunities to solve unemployment and poverty problems among Basotho communities, increasing use of marginal or unsuitable lands for the conventional crops, promoting advanced textile technology and skills for underprivileged Basotho communities, intensifying small and medium-scale business opportunities in order to generate income and build capacity ones (Eisentrau, 2010:13 &187), for the Basotho communities, especially the underprivileged ones. However, it might be hazardous to small children since it has leaf marginal and terminal spines. Extra care must be taken when cultivating it in this regard (Uygur, 2017:166). The *Agave americana L.* fibre cultivation can sustain life in arid lands where no other food or water is available (UNIDO, 2007:4; Leal-Díaz *et al.*, 2015:3924). The *Agave americana L.* plant is among the most promising sustainable Source of bio-based textile fibre that can improve social economies (Boguslavsky *et al.*, 2007:15; El Oudiani *et al.*, 2015a:15).

#### **2.4.7 *Agave americana L.* plant, neglected and under-utilized fibre species (NUFS)**

Neglected and under-utilized fibre species (NUFS) are described with terms such as abandoned, alternative, forgotten, indigenous, local, lost, minor, neglected, new, niche, non-conventional, orphan, promising, traditional and underused fibres (Morrison, 1999:22; Thies, 2000:1; Ahmad & Javed. 2007:1436; Sastrapradja & Haryatmo, 2008:72; Polok *et al.*, 2008:36 & 38; Ravi *et al.*, 2010:110). NUFS currently occupy low levels of utilisation status. Historically, they have played a substantial part in providing textile fibre security (Manisha *et al.*, 2012:69; Chivenge *et al.*, 2015:5688). The *Agave americana L.* fibre is a well-known NUFS of which Steward (2015:1) anticipates that it is neglected and underutilized because of its comparatively lengthy development cycle. Conceivably, its monocarpic blossoming behaviour and distinctive morphology contribute tremendously too.

NUFS are gradually considered very important in textiles because they make textiles more sensitive and sustainable environmentally, economically and socially. As such NUFs are also given high priority particularly for tender biorefineries to susceptible to drought and desertification (Williams & Haq, 2002:2; Padulosi *et al.*, 2011:507). *Agave americana L.* fibre is definitely under-researched, unimproved, undervalued or unrecognized (Mabhaudhi *et al.*, 2016:13) and its potential is untapped and consequently, it is unable to compete with improved lignocellulosic fibres although it has potential to become viable textile fibre (Polok *et al.*, 2008:36; Padulosi, 2012:7).

It suffers from a negative image, despite its excellent basic textile fibre features and potential roles in maintaining fibre variability and security of local communities (Polok *et al.*, 2008:40; Sastrapradja and Haryatmo, 2008:73-74; Padulosi *et al.*, 2013:12). The post-2015 agenda framework indicated that underutilised fibres have the potential to address textile fibre security and their status as linked with some sustainable developments (Ahmad & Jave, 2007:1435). Information on *Agave americana L.* as fibre yielding plant is needed for maximum utilisation and thus, would assist in uplifting the socio-economic status of the native people by strengthening income generation and livelihood opportunities (Sahu *et al.*, 2013:339), for the Basotho nation.

#### **2.4.7.1 The NUFs characteristics of *Agave americana L.* fibre**

Like numerous neglected and underutilized fibre crop species (NUFCS), *Agave americana L.* fibre is sustainably rich (Chattopadhyay & Khan, 2012:33; Bouaziz *et al.*, 2014a:1). Its loss can have a negative impact on crop fibre status and fibre security in Lesotho. The enhanced use of *Agave americana L.* fibre can uplift the textile economy and the socially sustainable developments of the country (Singha & Rana, 2010b:1057; Dansi *et al.*, 2012:1-2, Chivenge *et al.*, 2015:5688).

*Agave americana L.* fibre crop has variable uses, but it is not so popular in terms of research and marketing. It is well adapted to marginal and stress situations such as moisture stress, barren soil and extreme adverse temperatures (Pandey *et al.*, 2014:80). Its indigenous potential may be well known to people, while, business and market values are unidentified to the public. Snowballing global inhabitants and fast diminution non-renewable natural resources oblige people, to explore the possibilities of effective and efficient uses of newer indigenous plant fibre resource like *Agave americana L.* fibre, lying unexplored and underexploited (Chen *et al.*, 2014:320).

*Agave americana L.* Plant is an emergent unconventional source textile fibre due to its loftier mechanical and physical properties and its abundance. As a NUFs, it has well-adapted to harsh,

marginal, local growing conditions (Davis *et al.*, 2017:315), of SSA countries, thus offers potential sustainable textile production and processing. *Agave americana L.* plant is particularly valuable in sustaining fertility on depleted or marginal soils and is highly efficient in producing the fibre. According to McKell, 2010:51, NUFS are those fibre plant species that grow in areas that have not been exploited for commercial development and export constitutes an opportunity for positive botanical development. *Agave americana L.* like other NUFS has desirable attributes and potential for intensive use. It holds great promise for meeting the expanding needs of society for fibre, fuel, food, feed, medicines, construction and improved land efficiency and other purposes on a sustained yield basis (Padulosi *et al.*, 2011:507).

Historically, *Agave americana L.* crop has been a good source of technical fibre used by the indigenous communities of its native states. It has potential, to reduce textile insecurity and alleviate poverty but its cultivation and usage had been uneconomical, unappealing, under-explored and under-promoted when compared to other conventional lignocellulosic fibres like flax, which are promoted even under unsuitable conditions (Padulosi *et al.*, 2011:507; Chivenge *et al.*, 2015:5688; Kant & Alagh, 2015:2547).

*Agave americana L.* plants like other NUFS need quite low agricultural inputs but yield high amounts of fibre in their lifecycle, thus, exhibits sustainable characteristics (Polok *et al.*, 2008:39). Exploration and utilisation of *Agave americana L.* fibre can contribute significantly to textile diversity and the improvement of human life. In the post–2015 program framework, a number of Sustainable Developments are interrelated with a subsection in the biodiversity system which shows the potential of neglected and underutilised fibre crops to address textile insecurity, generate income and improve individuals' lifestyle and status (Ahmad & Jave, 2007:1435).

*Agave americana L.* fibre crop is side-lined, if not utterly overlooked. It is an unrecognised fibre crop that can be domesticated, semi-domesticated or wild species. It is locally naturalised adaptable non-timber species. *Agave americana L.* fibre crop is a traditional fibre crop that is mainly used by women to extract fibre. Its fibre processing is tedious, traditional and used locally with no commercial purposes. Having been neglected for a long time, today *Agave americana L.* plant and fibre are gradually recognise for their potential roles in alleviating fibre insecurity, ecological, economic and social concerns (Thies, 2000:1, Padulosi *et al.*, 2013:9).

*Agave americana L.* plant has prospective textile options, regardless of its development status since it can acclimatise to the fluctuating weather conditions, so it can improve people's



livelihoods by strengthening the economy for all actors involved in the value chain activities. *Agave americana L.* fibre production, processing and utilisation had been carried out mainly by women as a way of empowering them. It is used to reaffirm people's identity by valuing a conventional textile fibre, the allied culture, and ensures fibre production and processing extra resistance during climate change (Padulosi *et al.*, 2013:12).

*Agave americana L.* plant has been marginalized globally like all other NUFs, despite its inherent important adaptive and pro-livelihood features. Global research support on *Agave americana L.* is generally weak rather provided only for conventional fibre crops such as cotton (Padulosi *et al.*, 2013:12-13, Mabhaudhi *et al.*, 2016:2). *Agave americana L.* and other NUFs are cultivated, processed and utilized relying on indigenous knowledge and they are hardly represented in ex situ collections and improvement (IPGRI, 2002:9 &12).

At the national level, *Agave americana L.* fibre as a NUF can improve fibre security and safeguard socio-economic impacts which are due to over-concentration on fewer conventional fibres. Generally, fibre farmers opt for and depend on NUFs whenever conventional fibre crops fail them. During strenuous periods, after disasters and other emergencies, *Agave americana L.* plant can safeguard fibre security and offer other safety nets. They have a comparative benefit in marginal lands because they can withstand stress and can contribute to sustainable production with minimal inputs (Padulosi *et al.*, 2013:12-13). *Agave americana L.* plant is the no-waste NUF because its leaves produce a lot of fibre and all other parts can be effectively used to produce viable and sustainable products (Thamae *et al.*, 2009:100; Hulle *et al.*, 2015c:65).

It is less important than conventional fibres in terms of global production and market value. It is neglected by science and development; as such it is also referred to as an "orphan" crop fibre. Like other underutilized crop fibres *Agave americana L.* fibre was once grown more widely or intensively in arid and semi-arid regions. *Agave americana L.* fibre is perhaps less utilized because it is in some way not competitive with other conventional textile fibres (IPGRI, 2002:12).

#### ***2.4.7.2 The NUFs potential roles of Agave americana L. plant***

*Agave americana L.* fibre plant has the potential to address the current challenges including climate change adaptation, textile fibre security, the environment and employment opportunities in poor rural communities (Mabhaudhi *et al.*, 2016:1). The fast diminution of resources and ever-increasing demands for textile fibre supply challenges enforce people to produce sustainable textile fibres, intensify the production, processing and upgrading of the natural lignocellulosic



fibres; as one way to achieve increased resilience to shocks and change caused by climate change (GFAR, 1999:1; Donald, 2010:1764; Ghosh, 2012:271). There is an increasing endorsement regarding the importance of less-used fibre crops including *Agave americana L.* plant (Williams and Haq, 2002:2).

*Agave americana L.* fibre remains relatively underexploited (Chattopadhyaya & Khan 2012:33), with respect to its alternative properties that enable it to participate to the global ever-increasing textile production and processing requirements due to its distinctiveness, little production inputs and fitness to surroundings in which it is planted (Mabhaudhi *et al.*, 2016:1). *Agave americana L.* fibre has the potential roles to play which can address textile insecurity, poverty and environmental degradation challenges (Mayes *et al.*, 2012:1075). Under-exploitation and neglect of *Agave americana L.* plant can lead to the genetic loss of its usefulness and diversity. It is predicted that a boost in production of underexploited and neglected *Agave americana L.* fibre will empower people to preserve and increase the local biological and cultural plant resources (IPGRI, 2002:10) and generate employment through the high labour demand for fibre processing (Sastrapradja and Haryatmo, 2008:83).

(a) Climate Change Resilience

It is projected that climate change and inconsistency will occur, thus intensify weather extremes in the SSA countries including Lesotho. The SSA countries are mainly in semi-arid to arid environments and socio-economic and agro-ecological margins. One means to achieve increased resilience to these shocks by increasing the production, exploration, use and marketing of *Agave americana L.* and other NUFS, through sustainable textile fibre intensification (UNIDO, 2007:4). The increased *Agave americana L.* fibre production can safeguard against crop production risk and provide a constant environmental protection and more sustainable farming practices, which reduce land degradation associated with rainfall inconsistency and poor agricultural practices because *Agave americana L. plants* adapt better than the conventional fibre crops (Padulosi *et al.*, 2011:507; Chevenge *et al.*, 2015:5687 & 5691; Baldermanna *et al.*, 2016:107).

(b) Greater textile fibre security

As a local neglected and underutilized fibre crop, *Agave americana L.* can increase textile fibre security, particularly because it is adapted to SSA specific low agricultural conditions, input and maintenance (Thies, 2000:2; Ravi *et al.*, 2010:111). Its wild, managed or cultivated use, could have positive impact on the textile fibre security and health of the poor local communities.

Diversification is a means of risk reduction. Risk distribution is an indispensable means to reduce susceptibility specifically for already vulnerable people (Thies, 2000:2; Christinck, 2014:2). Increasing the use of *Agave americana L.* could be one of the better safeguards to assist diversifying and sustaining textile green and economic security in times of change (Jaenicke & Pasiecznik, 2009:1).

Fibre crop diversification results in more resilient textile fibre production and processing that assist in enhancing textile security. Textile security occurs when at all times, almost all communities have sufficient and safe means to meet their basic textile needs and wants (IPGRI, 2002:5; Ahmad & Jave, 2007:1435; Mayes *et al.*, 2012:1075; Chivege *et al.*, 2015:5686; Jaenicke & Pasiecznik, 2009:11). Enhancing *Agave americana L.* as an alternative fibre crop that would be grown to produce fibres as the main product (Morrison, 1999:22) will not only biodiversity textile fibre plants, but will also provide textile resilience, and improve textile fibre (Ahmad & Javed, 2007:1435; Padulosi *et al.*, 2013:14-15).

(c) Production for income generation

*Agave americana L.* fibre though, neglected and underutilized is capable of supplying potential raw textile fibre materials, which will offer new market options if its textile market potential is acknowledged and established (IPGRI, 2002:6; Mayes *et al.*, 2012:1075; Christinck, 2014:2; Ravi *et al.*, 2010:111; Mabhaudhi *et al.*, 2017:1; UNIDO, 2007:4). Literature indicated the significance of the neglected and underutilised plant species (Ahmad & Javed, 2007:1435; Hedge, 2008:289; Rana & Sthapit, 2012:229; Padulosi *et al.*, 2013:17) including fibres in textile sustainability. Strengthening *Agave americana L.* fibre research studies could generate employment by empowering local communities mainly women, thus; could boost their social identity, status and self-confidence (Mabhaudhi *et al.*, 2016:7 & 8).

(d) Poverty reduction

Like many underutilized fibre plant species, *Agave americana L.* plant production relates well with green economy concept because it requires very few, external inputs and have characteristics that safeguard the water cycle, the ecological equilibrium versus diversification of fibre production, the carbon sequestration and its contribution towards climate change alleviation and soil enrichment. *Agave americana L.* plants have high yields even on negligible conditions and without additional agricultural chemical inputs (UNEP, 2011:17). Where there is no arable land,

*Agave americana L.* plants can still be grown wild (GFAR, 1999:1; Pradheep *et al.*, 2003:20; Mayes *et al.*, 2012:1075; Padulosi *et al.*, 2011:508; Christinck, 2014:2).

(e) Sustainable use

*Agave americana L.* is locally adapted fibre crops and thus, offers promising effective and efficient utilisation of non-conducive plant growing conditions. As such it a sustainable local fibre plant with ample fibre and by-product benefits which further restore soil richness (Sthapit & Padulosi, 2012:34; Christinck, 2014:2).

(f) Indigenous knowledge

*Agave americana L.* fibre investigation is another way of conserve and manage indigenous knowledge. The indigenous knowledge obtained from the local environment driven by the need to follow textile fibre social subsistence strategies and economic growth. The indigenous knowledge about *Agave americana L.* fibre is deep-rooted in social systems and believed to contribute to ethnic identities, customs, beliefs and global perspectives. It is wise to use this indigenous knowledge so that one can be better informed about the scientific behaviour of *Agave americana L.* fibre (IPGRI, 2002:6; Pradheep *et al.*, 2003:20; Christinck, 2014:3; Chivege *et al.*, 2015:5686).

(g) Cultural identity

The *Agave americana L.* plant and fibre are traditional resources that are entangled with the cultural identity of Basotho. Basotho have known *Agave americana L.* plant as a local source of fibre for decades. Conserving *Agave americana L.* plant is a sustainable way to contribute toward producing fibre from local, traditional, resources; thus saves local ecosystems (Padulosi *et al.*, 2013:18). Indigenous Basotho can retain knowledge embedded in historical continuity; land reclamation and poverty reduction, through the use of *Agave americana L.* plant and fibre resources. The cultural and traditional knowledge associated with *Agave americana L.* plant and fibre production and Basotho lifestyles can preserve the continuity of local culture and forfeit the loss of textile fibre legacy and its sense of association to the land; thus increase agricultural and fibre biodiversity and relationships between Basotho and their cultural plant fibre. Conducting *Agave americana L.* fibre upgrading research entails continuing efforts to document, the indigenous Basotho knowledge of a local *Agave americana L.* fibre, before its loss (Ahmad & Javed, 2007:1436; Padulosi *et al.*, 2013:19; Chivege *et al.*, 2015:5690).

(h) Cultural diversity

Diversity is the key to coping with climate change, for sustaining livelihoods and planet ecology. Historically, the *Agave americana L.* plant products have been used for Basotho cultural and traditional construction, artefacts, laundry materials, food and beverage purposes. *Agave americana L.* fibre production can enormously contribute in keeping the cultural diversity of Basotho booming. Even though it has been neglected and underutilized. To attain the vast diversity, the *Agave americana L.* plant should be utilised as a textile fibre source and its by-products. The *Agave americana L.* plant indicates its highest cultural significance at the local level, where it acquires better attention and association to cultural diversity. The primary challenge is conserving neglected and underutilized *Agave americana L.* plant for fibre e production is to secure its survival and environmental adaptation at the same time to provide increased incomes for the poor. (IPGRI, 2002:6 & 14; Padulosi *et al.*, 2011:507; Ahmad & Javed, 2007:1435; Chivege *et al.*, 2015:5686).

(i) Ecosystem stability

Climate change, land the degradation and scarcity of fibre resources mount curiosity about NUFS like *Agave americana L.* fibre. *Agave americana L.* plant can acclimatise to difficult environments and occupies specialized environments because it bears stresses well. Thus, *Agave americana L.* fibre can increase the general efficiency and constancy of plant fibre ecosystem sustainability and diversity (GFAR, 1999:1; IPGRI, 2002:6 & 14; Padulosi *et al.*, 2011:508).

(j) Biodiversity

African fibre biodiversity has emerged few years back as a strategy for sustainable development. It is the basis on which the substantial economic, aesthetic, health and cultural welfares are founded on. *Agave americana L.* fibre upgrading embraces the concept of African fibre biodiversity which uses valued resource inputs to adapt and improve goods and services towards a sustainable working environment, that affords human economies (van Damme, 2008:27; Pradheep *et al.*, 2003:20, Ahmad & Javed, 2007:1436; Mayes *et al.*, 2012:1076; Padulosi *et al.*, 2011:508). Processing of *Agave americana L.* fibre has the potential to produce new products for diversification of livelihoods and rural small business opportunities and to generate new market prospects with employment at various levels (UNIDO, 2007:4; Hedge, 2008:289) because, *Agave americana L.* species is, therefore, an important element of a green economy which is a multi-

purpose species that can provide textile fibre and the products, non-textile fibre products for subsistence and income generation (UNEP, 2011:13).

#### **2.4.7.3 Improving public awareness**

Raising awareness about the potential sustainable benefits of *Agave americana L.* fibre upgrading creates basic and valuable conditions for *Agave americana L.* plant production intensification, textile fibre utilization and by-products diversification. All textile fibre stakeholders should be aware of the practical paybacks that are obtained from effective and efficient upgrading and use of this fibre and should be encouraged to research and share information (IPGRI, 2002:25; Chivenge *et al.*, 2015:5688). One way to achieve increased textile fibre resilience to shocks and change is by increasing the study publications about textile fibre biosoftening and upgrading of the underutilised *Agave americana L.* that is naturalized to the extent that is considered indigenous with traditional uses and cultural links with local people (Jaenicke & Pasiecznik, 2009:11).

If all textile stakeholders are willing to participate in the production, upgrading, publicising and usage of *Agave americana L.* fibre, they can significantly impact the livelihood of the poor rural population. Promotion and raising awareness for *Agave americana L.* fibre potential benefits increases demands and the supply as well as to improve technologies for textile production, processing and exploitation. These efforts will afford and improve the livelihood and well-being of the communities (Sastrapradja and Haryatmo, 2008:83).

Currently, there is inadequate literature describing the potential benefits of traditional and indigenous *Agave americana L.* fibre. Such information exists, as indigenous knowledge which is not publicly revealed or it is in ancient literature which is not easily available. On top of that, there is limited research and development that verify the knowledge about the neglected and underutilised *Agave americana L.* fibre, both regionally and globally (Chivenge *et al.*, 2015:5688).

#### **2.4.7.4 Future trends of *Agave americana L.* fibre**

*Agave americana L.* fibre has increasing potential even though to a restricted extent since there is a rearrangement of the entire textile industry because of the sustainability concept, the improving research on quality and usage of the fibre due to diminishing major inorganic chemical resources. Fast mounting environmental movements and consciousness, the valuable new bioproducts and by-bioproducts such as reinforced plant polymers, plant base biocomponents which are 100%

biodegradable, Possibility of cultivation of the *Agave americana L.* plant and on soils polluted with heavy metals as alternative crop for non-food purposes and soil reclamation (Koztowski & Mackiewicz-Talarczyk, 2012:104).

## **2.5 PRE-TREATMENT OF *AGAVE AMERICANA L. (LIGNOCELLULOSE) FIBRE***

Lignocellulosic fibre is mostly of crystalline structure; predominantly consists of cellulose, hemicellulose, lignin and pectin. Lignin envelops and protects cellulose and hemicellulose from degradation. The crystalline lignocellulosic structure is extremely impermeable to most forms of degradation and complicates the industrial use of the fibre in textile processes. Pre-treatment which interrupts the lignocellulosic structures and improves the enzymatic accessibility into functional soft biofibre (Vega *et al.*, 2012:1; Silva *et al.*, 2014:1068). It does that through solubilising the cementing constituents of cell wall mostly hemicellulose and alter the structure of lignin, in order to reduce *Agave americana L.* fibre recalcitrance, disposing cellulose to enzymatic action (Hu & Ragauskas, 2012:1052; Grimaldi *et al.*, 2015:2). There are different techniques of lignocellulosic biofibre pre-treatment existing. The pre-treatment process is to disrupt the lignocellulose cell wall structure, so that *Agave americana L.* fibre is accessible to hydrolytic softening and upgrading enzymes (Singh, 2016:2).

### **2.5.1 Pre-treatment of *Agave americana L.* fibre**

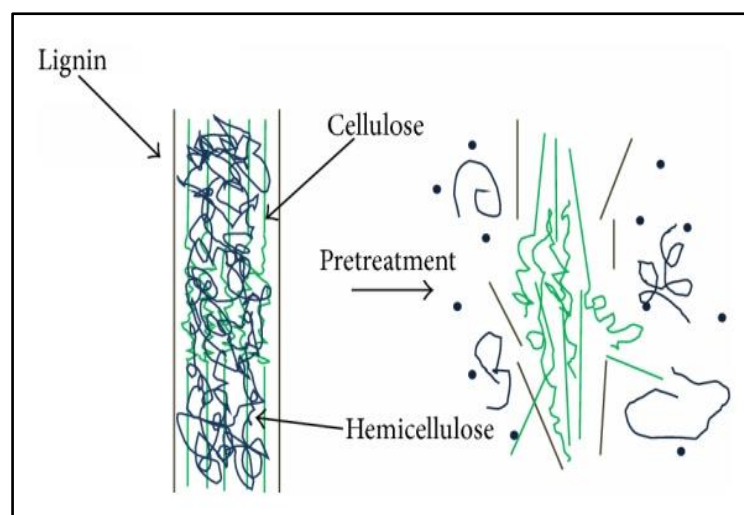
The lignocellulosic fibres which have not been pre-treated are difficult to enzymatically process because the structure is highly crystalline and cross-linked with non-cellulosic fibre constituents that include lignin and hemicellulose (Taherzadeh & Karimi, 2008:1621). However, there are various pretreatment techniques that can be applied to effectively disrupt the linkages between cellulose and non-cellulosic fibre constituents to effectively disturb the stabilised structure of lignocelluloses to remove lignin and hemicelluloses (Kumar *et al.*, 2016:152, Li *et al.*, 2014:1174). The pre-treatment process must be cost-effective and be able to eliminate lignin and hemicellulose to some extent in order to soften cellulose microfibrils (Kumar *et al.*, 2009:18). Ever-increasing international energy demand, unbalanced and high-priced petroleum resources and concerns over the international climate changes have forced the development and popularity of renewable fibre sources that displace substantial amounts of fossil fibre sources (Hu & Ragauskas, 2012:1043). The potential alternative biofibre resources such as *Agave americana L.* plants are gaining popularity because of their high availability and positive sustainability effects on society, environment and economy (Hu & Ragauskas, 2012:1044; Christy & Kavitha,

2014:26). However, the potential textile applications of the *Agave americana L.* fibre are limited by a complex cellulose, hemicellulose and lignin structure in which they are and other minor constituents, making it resistant to enzymatic softening processes (Kumar & Sharma, 2017:1; Dien & Bothast, 2009:79). *Agave americana L.* fibres are extracted from the plant parts by different fibre extraction processes. Some plant component debris and impurities have to be removed through various pre-treatment processes (Šimić *et al.*, 2015:50). In addition to that the hemicellulose-lignin complex cross-links must be damaged (Balan, 2014:7; Isikgor & Becer, 2015:4500) so as to dislocate the structure of the multi-component matrix, of lignocellulose complex (Isikgor & Becer, 2015:4500) as illustrated in figure 2.23.

Pre-treatment is an essential step in a number of applications mainly for biofibre upgrading (Mohieldin, 2014:85). Pre-treatment dispels away the physical and chemical obstructions that make natural biomass obstinate. Thus, makes the enzymatic biosoftening of lignocellulosic fibre easy (Kucharska *et al.*, 2018:4-5). This makes it possible to soften and upgrade the *Agave americana L.* fibres to textile fibre for the production of apparel and other valuable textile products. The primary function of the pre-treatment is to dislocate the structure of the multi-component matrix, of lignocellulose complex in order to make cellulose fibres more accessible to the biocatalysts and improve hydrolytic upgrading of lignocellulose fibre. Pre-treatment consequently, becomes an essential and indispensable step to follow prior to bioprocessing of lignocellulosic *Agave americana L.* fibre. The pre-treatment affects the quality of the fibre (Saritha *et al.*, 2012:123). The research and development have the power to reveal potential values for pre-treatment to effectively and efficiently produce high yield biofibres at low cost (Isikgor & Becer, 2015:4500).

The goal of pre-treatment is mainly intended to break up the lignocellulosic structure of the fibre to ease enzymatic softening of the fibre. This can be achieved by removing the lignin layer and to de-crystallize cellulose and increase porosity, for removal of the surrounding hemicellulose, swelling of the microcrystalline cellulose fibres and separate fibres into individual microfibrils. These directly relate to allowing the fibre softening enzymes ready access to the individual cellulose strands. To date, a fair number of readily available pre-treatment techniques are reported in the literature (Dien & Bothast, 2009:79).





**Figure 2.22: Separation of lignocelluloses into cellulose, hemicellulose, and lignin (Lee et al., 2014:6; Myat & Ryu, 2016:180)**

Pre-treatment of lignocellulosic *Agave americana L.* is critical to overcome structural obstinacy and increase the rate of upgrading the fibre. The physical, mechanical, chemical and organic pre-treatment processes can be performed to transform the structural, mechanical and chemical properties of *Agave americana L.* fibre. Physical pre-treatment reduces the size but raises the surface area of the fibre biomass. The working environment needs to be controlled carefully because the physico-chemical methods take place at high temperature and pressure. Pre-treatment methods partially confiscate and/or disrupt hemicelluloses and lignin and thus, releasing the structure of lignin-hemicellulose complex to some extent as illustrated by figure 2.22. The fibre biotechnological pre-processing procedures are necessary for fibre delignification. Biodelignification can also brighten and soften the fibre or be followed by softening and brightening processes with their respective upgrading and softening enzymes (Maurya et al., 2015:599).

The pre-treatment of the biofibre has to be well controlled for competence and cost-effective reasons. Understanding the natural cellulosic polymer chemistry and pre-treatment is essential due to their impacts on lignocellulosic processing and biofibre transformation (Hu & Ragauskas, 2012:1044). Pre-treatment can drastically change the properties of the pre-treated *Agave americana L.* fibre properties such as degree of polymerization, crystallinity index, specific surface area, lignin content, acetyl content and many others can drastically be changed by effective pre-treatment. Enzyme hydrolytic upgrading can be catalysed by efficient pre-treatment processes and the quantity of enzymes needed to soften the fibre biomass into a usable textile

product can also be significantly decreased. The ease of fibre biosoftening is influenced by the lignin content because lignin is recalcitrant and is responsible for unproductive enzyme activity. The removal of ample amounts of lignin during the pre-treatment process will facilitate enzyme use efficiency and cost-effectiveness of the process (Balan, 2014:8).

## **2.5.2 Parameters for effective pre-treatment of *Agave americana* L. fibre**

The efficiency and effectiveness of enzymatic upgrading of *Agave americana* L. fibres can be influenced by a number of factors. The factors are as follows: lignin–carbohydrate complexes (LCCs), the recalcitrant acetylated hemicelluloses, structure and content of lignin, pore-volume, specific surface area of cellulose cellulose crystallinity and DP (Maurya *et al.*, 2015:599).

### ***2.5.2.1 The recalcitrant structure and effect of lignin***

The presence of lignin provides structural rigor and integrity and prevents the bulge of lignocellulosic fibre. Lignin also envelops the cellulose and hemicellulose in the lignocellulosic cell wall framework. Thus shields them against enzyme softening. The cross-link between lignin, hemicellulose and cellulose, the organisational structure and dispersion of lignin in fibres hinder the enzymatic biocatalysis of fibre polymers, thus decreasing the efficiency of the hydrolytic separation into individual constituents. Lignin is the most recalcitrant lignocellulosic constituent. Therefore, the successful delignification process indicates the improved rate and extent of lignin degradation and hydrolytic upgrading of lignocellulosic fibre (Maurya *et al.*, 2015:599). There are times during enzymatic delignification, when lignin attracts enzymes through hydrophobic interactions to form an irreversible complex that inactivates enzymes and requires the use of more enzymes than necessary under normal circumstances (Saritha *et al.*, 2012:124).

### ***2.5.2.2 Effect of hemicellulose***

The cellulose is further encased and protected by hemicellulose in the lignocellulosic cell walls of the plant fibre. Hemicellulose as a physical barrier protects cellulose from the enzymatic hydrolytic degrading. Whenever hemicellulose is removed, cellulose is a cellulolytic degrading agent that speeds up cellulose hydrolytic hydrolysis. The rate of hemicellulose acetylation also influences *Agave americana* L. fibre upgrading since; acetyl groups and lignin are both connected to the hemicellulose biomatrix (Maurya *et al.*, 2015:599).

### **2.5.2.3 Acetylated hemicelluloses and lignin–carbohydrate complexes (LCCs)**

Cellulose micro fibrils are enclosed by hemicelluloses which impede enzyme softening. Acetylated hemicelluloses and lignin–carbohydrate complexes (LCCs) are the cross-links formed between hemicelluloses, cellulose and lignin with most bonds such as hydrogen, phenyl glycoside bonds, covalent, esters, and benzyl ethers: hydrogen bonds join the surface of cellulose fibrils with unbranched hemicelluloses; lignin is covalently joined to hemicelluloses and the lateral chains of diverged hemicelluloses to form enzyme-impermeable cross-links. These cross-links resist enzymes to freely catalyse cellulose. The lignocellulosic fibre softening has to start with hydrolysis of hemicellulose and breakdown of LCCs bonds to open the plant cell wall structure. It is recommended that reasonable hemicellulose (>50 %) be removed to considerably upsurge the enzymatic softening of cellulose fibres (Hu & Ragauskas, 2012:1045; Maurya *et al.*, 2015:599).

### **2.5.2.4 Pore volume, and specific surface area**

Moreover, the average specific surface area and pore size of cellulose fibres are increased by removing some hemicelluloses that sheath cellulose and are linked to lignin. This increase influences the easy adsorption and catalysis of enzymes on the cellulose surface to upgrade lignocellulosic fibre since enzymes must bind to the surface of cellulose fibrils before hydrolytically softening and upgrading them (Saritha *et al.*, 2012:124; Maurya *et al.*, 2015:599; Ravindran & Jaiswal, 2016:4). The main aim of pre-treatment is to increase pore sizes and surface area so as to improve enzyme permeation into fibre biomass, thus intensifies the enzymatic upgrading rate of cellulose fibrils (Saritha *et al.*, 2012:124; Hu & Ragauskas, 2012:1045; Ravindran & Jaiswal, 2016:4).

### **2.5.2.5 Fibre crystallinity**

The crystallinity of the cellulosic fibre is highly associated to its pre-treatment reactivity. When it has a lower degree of polymerization and crystallinity pre-treatment efficiency is greater than when it is highly polymerised and crystalline. The degree or speed of pre-treatment and enzymatic hydrolytic biosoftening of cellulose deteriorates with increasing cellulose crystallinity. The highly crystalline cellulose (cellulose I) is slowly hydrolysed because of the presence of the sturdy inter-chain hydrogen bonds that lead to densely packed and crystalline fibre that intensely resists biocatalysed degradation (Ansell & Mwalkambo 2009:73-74).

In contrast, the increased pre-treatment and enzymatic upgrading rate of cellulose fibrils occur because of lower crystallinity and longer distances between hydrophobic surfaces and the packed

density in cellulose II. The pre-treatment rate of cellulose declines linearly as crystallinity increases (Saritha *et al.*, 2012:124; Maurya *et al.*, 2015:598; Ravindran & Jaiswal, 2016:4). Thus, enzymes adsorption properties on crystalline and amorphous cellulose are comparable and associated to the reactivity difference concerning crystalline and amorphous cellulose structure (Hu & Ragauskas, 2012:1046; Maurya *et al.*, 2015:599).

#### **2.5.2.6 The degree of polymerization (DP)**

Degree of polymerization (DP) is uttered as a measure of the length and branching of cellulose chains and it is among the most essential physico-chemical properties of lignocellulosic fibre. It can affect the mechanical properties of the fibre, the solubility of lignocellulosic fibre in a pre-treatment solvent, and the enzymatic hydrolysis efficiency of lignocellulosic biomass. The DP decrease by a pre-treatment process is conveyed by the formation of more cellulose ends available to the enzymes (Hu & Ragauskas, 2012:1047; Karimi & Taherzadeh, 2016:1015; Ravindran & Jaiswal, 2016:4).

#### **2.5.2.7 Humidity**

The role of the water in the pre-treatment processes should be overvalued. The presence of water facilitates partial deacetylation, depolymerisation, degradation and removal of lignin from the fibres. Moreover, the cleavage of the glycosidic bonds in the lignocellulosic fibre is brought about by high temperatures that end up in a reduced pH medium. For instance, the medium pH 5.6 is reached at 220°C and the medium pH 7.0 is reached at room temperature. This effect implies that the role of the water at high temperatures is more efficient and relevant than the role performed by the free organic acids (Maurya *et al.*, 2015:599, Ravindran & Jaiswal, 2016:4).

## **2.6 CLASSIFICATION OF PRE-TREATMENT PROCESSES**

Pre-treatment processes are basically, classified into two main categories: non-organic and organic. Non-organic pre-treatment procedures never include any microorganisms in their treatments and employ methods that are harsh and cost/energy-intensive. They are categorised into physical, chemical and physico-chemical means (Isikgor & Becer, 2015: 4500; Kumar & Sharma, 2017:2). Organic pre-treatment processes, on the other hand, employ micro-organisms, are mild and environment-friendly (Saritha *et al.*, 2012:126; Maurya *et al.*, 2015:599).

*Agave americana L.* fibre is a compound structure of biopolymers; mainly cellulose, hemicellulose, and lignin. The fibre, however, remains underdeveloped, to some extent due to the problems faced in the characterisation of its native structure and effective and efficient separation of the structural components. The great challenge for the pre-treatment is to find an effective and cost-effective strategy to disturb the lignocellulosic compound structure so that enzymatic bioprocessing can occur, with low enzyme concentrations and processing times, in an economic and environmentally sustainable manner (Grimaldi *et al.*, 2015:2).

### **2.6.1 Hydrothermal pre-treatment**

Hydrothermal pre-treatment, also referred to as liquid hot water pre-treatment, has commercial benefits and is eco-friendly because it uses pure water, with no added chemicals; as a reaction medium, it does not need special non-eroding apparatus and does not produce significant amounts of unwanted degrading substances like furfural. The pre-treatment is mainly intended to solubilise hemicellulose and change the structure of lignin, thus, reduces *Agave americana L.* fibre enzymatic softening recalcitrance. *Agave americana L.* fibre is sustainable and can substitute fossil fibre. However, *Agave americana L.* fibre has not been widely utilized because of the complicated and stable structure of the plant cell wall. Hydrothermal pre-treatment of *Agave americana L.* is an efficient method to disrupt lignocellulosic structural complex. Contemporary, literature demonstrates the effects of hydrothermal pre-treatment and the change of lignocellulosic structural complex (consists mainly of hemicellulose cellulose, and lignin) are illustrated as in figure 2.22 (Chen *et al.*, 2018:2).

## **2.7 AGAVE AMERICANA L. FIBRE VARIABILITY**

Natural fibre properties tend to vary a lot when compared to the man-made fibres. They vary considerably depending on individual fibre maturity, age, location, source, chemical composition and structure, related to fibre type, developing situations, harvesting time, extraction technique, pre-treatment and storage processes (Everitt *et al.*, 2013:1; Pickering *et al.*, 2016:99). The cellulosic fibres have different characteristics that include the tensile strength, length, texture, and water absorbency to mention a few. Fibre variability may be attributed to the type of the fibre, the part of the plant from which the fibre is acquired, structural and chemical composition (Ayele *et al.*, 2018:47-48). The cellulosic fibres such as cotton and ramie fibres are normally stronger than the lignocellulosic fibres, like *Agave americana L.* fibre. The fibre strength is mainly due to the

pureness of cellulose, the thickness and assembling of the cell walls (Pandey & Gupta, 2003:194-195).

Lignocellulosic fibres are divergent in their mechanical properties because of their different environmental and climate conditions as well as the chemical and structural arrangements. The fibre crops' taxonomic classes also bring about the distinctive dissimilarities in fibre composition that can lead to the easy identification of the origin of some species. Within the same plant, morphologies often differ due to climate and seasonal growth or age differences (Dungani *et al.*, 2014:20; Asim *et al.*, 2015:2; Hulle *et al.*, 2015c:65; Sorieul *et al.*, 2016:18).

*Agave americana L.* fibre species has not been extensively explored equally from different phyto-geographical regions of the world for its upgrading and use as a textile fibre (Hulle *et al.*, 2015:65a). *Agave americana L.* fibre variability is considered greater than man-made fibres. The morphological properties of these fibres are extremely variable among themselves and along the length of the single fibre. *Agave americana L.* fibres greatly differ in terms of their sizes and properties different plant and even within the same crop growing as well as within the same leaf. Thus, it is not only the difference between different plant fibres of the same species but also the intra-fibre cell walls variations that have to be measured (Hulle *et al.*, 2015c:65; Msahli *et al.*, 2015:2b).

Variability in *Agave americana L.* plant fibres is caused by several factors that are interior and exterior to the plant. Interior factors entail developmental phase and period, the plant leaves from which the fibre is obtained, the leaf morphology and structural level of harvested leaves and fibre microstructure. Exterior factors include ecological or growth environments, the fibre extraction, softening and testing methods (Lowe *et al.*, 2010:2158; Everitt *et al.*, 2013:1; Eder & Burgert, 2010:33; Pickering *et al.*, 2016:99). *Agave americana L.* fibre diverges broadly in both its physical and mechanical performance properties, because of inherited, ecological, collecting, extraction and upgrading factors (Msahli *et al.*, 2015b:2 &13).

The properties of *Agave americana L.* fibre bundles differ quite substantially from each other. This implies that within a single plant species and also within the same plant, fibre properties often differ owing to age differences, period, climate and growing conditions like availability of nutrients. Concurrently growing *Agave americana L.* leaves that are at different growing positions, in a plant can also produce fibre with different morphological properties. Differences in fibre structure and morphology are so evident between different types of *Agave americana L.*

plants and within the same species, due to climatic conditions, seasonal growth or age differences. Plants employ various theories to adapt and alter their fibre mechanical properties. The *Agave americana L.* fibre mechanical properties are also age-related. An on-continuing growth fibre stiffness, tensile strength and buckling resistance, are directly related to plant aging lignification (Eder & Burgert, 2010:32; Msahli 2015b:2; Lowe *et al.*, 2010:2158; Sorieul *et al.*, 2016:18).

The *Agave americana L.* plant fibres dissimilarities are inter-plants and intra-fibre cell walls even in the same plant. Dissimilarities in lignocellulosic microfibril angles alongside the fibre are related to structural imperfections, referred to as dislocations or character designs that can determine some characteristics of the fibre (Eder & Burgert 2010:34; Mussing & Tanja, 2010:58).

*Agave americana L.* fibre is a natural lignocellulosic fibre and has fibre properties comparable to that of other natural lignocellulosic fibres such as sisal, banana fibre and jute. *Agave americana L.* fibres vary in their properties with grade and quality range (also called variability in inter-intra plant fibres) (Hulle *et al.*, 2015c:65). EI Oudiani *et al.*, 2015a:15) conducted experiments on variability in the fineness parameter of *Agave americana L.* fibre; they found that fibres extracted from diverse individual leaves harvested from the same level on a plant have comparable properties. Conversely, fibres extracted from diverse locations on the same leaf (tip, middle or base) have different properties. Fibres obtained from the oldest leaves of the plants have unlike properties with those obtained from the youngest leaves (EI Oudiani *et al.*, 2015a:91).

## **2.8 BIOSOFTENING OF AGAVE AMERICANA L. FIBRE**

Currently, there is an upsurge in the demand for sustainability in textile fibre production and processing techniques. pollution but produce textiles of high quality under save and comfortable working standards. The enzymatic biotechnology in textiles satisfies these demands and rapidly gains popularity because of its environmentally friendly and non-safety compromising qualities which overcome those of its counterpart; chemical technology (Shaikh, 2010:48, Radhakrishnan & Preeti, 2015:10501, Sumi & Unnikrishnan, 2015:262). Chemical technology in textiles is a traditional, successful and ancient practice. However, it uses plenty of resources like water and energy. It is also harsh, harmful and non-environmentally-friendly. It is therefore advisable to investigate intensively on emergent alternative, save and eco-friendly enzymatic biotechnology. The biotechnology is the use of microbes and their constituents to invent new and unconventional products (Shroff *et al.*, 2015:445).



The rapid economic growth and modernisation have improved the standard of living for mankind. These have a positive influence on the lifestyles of people. However, many textile manufacturing production processes have a negative influence on the milieu, like the exhaustion of natural assets, global warming, ecological pollution, the utilisation and discharge of detrimental and dangerous substances. Thus, to prevent insistent damages to nature, textile production and manufacturing technologies have to become milieu friendly (Dungani *et al.*, 2014:14; Kalim, 2015:2; Rameshaiah & Reddy, 2015:1).

The *Agave americana L.* fibre upgrading can improve the fibre defects through bioprocessing. Thus, improve fibre quality value-added impact and the diversification of its products which could provide textile fibre resilience and technical back-up to increase the fibre production, treatment and consumption of value-added and good quality *Agave americana L.* fibre. Textile biosoftening is the bioprocessing technology used in the textile industry. The textile biosoftening is the use of active microbes and/or their by-products to manufacture textile processes and products, which are mainly based on enzymes. Enzymatic biosoftening of *Agave americana L.* fibre has the potential for new textile fibre. Enzymatic delignification, enzymatic bioscouring, biobleaching and biopolishing are few examples of biosoftening lignocellulosic fibres for textiles (Kumari *et al.*, 2013:1388, Kumar *et al.* 2016:152). The key benefit of this enzymatic bioprocessing of textile fibres is that lignocellulosic fibres do not damage the environment, people become safe and water, as well as energy, are also saved (Etters; 1999:1, Gurung, 2013:2; Teli & Adere, 2016b:256).

With ever-increasing consumer demands for quality, reliability, sustainability, and process optimization (Kopania *et al.*, 2012:167), the textile enzymatic bioprocessing is increasingly gaining momentum due to the fact that enzymes are eco-friendly, non-toxic and reduce pollution in textile production. It also shortens processing times, energy and water consumption. Thus, they save a lot of money (Polaina & MacCabe, 2007: ix; Rehman, 2013:1). Biosoftening is physico-chemical treatment that changes the surface of the fibre so as to minimize pollution. It is a process by which specific micro-organisms and/or enzymes, are used to bio-process the textile fibres; commonly, the natural plant. With the biosoftening, the following characteristics softening, thinning, and bleaching of the fibre can be achieved. This process is carried out without using caustic chemicals, thus it will minimize pollution (Kumar *et al.*, 2016:152). Biosoftening; of *Agave americana L.* fibre is physico-chemical treatment, that ensures smoothness and softness of the lignocellulosic fibre to get the improved fibre quality (Desai *et al.*, 2016:30).

### **2.8.1 Enzymatic biotechnology in textile**

Textile processing has advanced greatly in both eco-friendly and product quality aspects through the use of enzymes (Bhardwaj *et al.*, 2017:832). Enzyme biotechnology in textiles is a dominant enabling mechanism for attaining uncontaminated engineering processes and products that are a basis for textile industrial sustainability (Bano *et al.*, 2017:2). It is regarded as a potential biocatalyst tool in textile technology and is gaining popularity because of increasing demands for sustainable textile catalysts. It develops sustainable textile strategies for efficient utilisation of the natural lignocellulosic textile resources, to assure that the resources benefit the current and future generations (Onofre *et al.*, 2014:276; Muhammad *et al.*, 2016:11133-11134; Teli & Adere, 2016b:256).

It is the bio-economy that assures the eco-socio-economic textile resource balance (Singh *et al.*, 2016b:1 & 9). The renewable raw resources are imperative for textile sustainability in order to improve the ecological stability of industrial production and processes (Bano *et al.*, 2017:1). The enzymatic biotechnological alternative explores the natural power of enzymes as biocatalysts (Uddin, 2015:1). The textile renewable raw materials, bioproduction and bioprocesses through the use of Enzymes are currently receiving greater emphasis, in order to effectively and efficiently improve the environmental balance (BMBF & BMEL, 2015:74-75).

The enzymes in textile processing have brought a major contribution to textile processing, especially textile wet processing. Enzymes are harmless unconventional tools in textile bioprocessing that can substitute harsh chemicals, which when disposed of without pre-treatment pose ecological distresses. The enzymatic textile processing applications are potentially broad (Mojsov, 2011:233-234; Tavčer, 2011:387; Rehman, 2013:1; Hoque & Azim, 2016:167). Research and development in Textile science necessitate improvements in new and sustainable technologies that increase the responsible use and reuse of raw natural resources that match specific eco-friendly requirements and possess unique properties (Polaina & MacCabe, 2007:x, Teli & Adere, 2016a:209-210). Potential and specific fibre applications such as single or mixtures; have been explored due to advances in enzymatic biotechnology use in the textile industry. There is a still substantial possibility for new and upgraded enzymatic applications in textiles (Mojsov, 2011:230-234).

The enzymatic biotechnology in the textile industry is an environmentally friendly and social sustainable technology that can improve the quality of the physico-mechanical properties of

*Agave americana* L. fibre (Poonam, 2013:597). It does not pollute the environment, conserves energy and natural resources, sustainably feasible, non-violent and healthy for the workforces (Singh *et al.*, 2015:2). Enzymatic biotechnology processes are more specific, efficient and green and less health harmful like the conservative physico-chemical or chemical-founded processing methods. However, the enzymes are expensive and their stability is low (Kotzia *et al.*, 2012:126). Therefore, for the last few years, research scientists are interested in enzymatic softening of lignocellulosic fibres. Biosoftening of *Agave americana* L. fibre is multi-stepped processes like pre-treatment and enzymatic upgrading so that the modified or pre-treated biomass becomes more agreeable to enzymatic biotechnology. Enzymatic biosoftening and brightening is an operative method to attain upgraded lignocellulosic fibre for textile products, under controlled, mild and eco-friendly reaction settings from the pre-treated *Agave americana* L. fibre (Anwar *et al.*, 2014:168; Usluoğlu & Arabaci, 2015:3280; Shrimali & Dedhia 2016:674). Enzymatic biotechnology fast-tracks substrate-enzyme complex reactions through the reduced energy activation process (Bharathi & Kanaka, 2015:4857; Shroff *et al.*, 2015:445; Šimić *et al.*, 2015:50; Shrimali & Dedhia, 2016:674; Sarma & Deka, 2016:998).

Safe handling, storage, use and disposal make enzymes the best choice catalysts for the textile bioprocessing. Enzymes are generally efficient in the textile biosoftening processes such as delignification, scouring, bleaching and polishing of lignocellulosic textiles to mention a few (Bharathi & Kanaka, 2015:4857-4859). Enzymes are biodegradable sustainable catalysts that originate from natural organisms implying that they readily return to nature to be recycled as organic manure (Ezeonu *et al.*, 2012:16; ACS, 2013:7; Novozymes, 2013:9; Silva *et al.*, 2010:78). The enzymatic biotechnology is sustainably risk-free and eco-friendly important in the textile chemical processing. Thus it can decrease pollution in textile production and processing (Hossain & Uddin, 2011:14; Jothi, 2013:2970).

### **2.8.2 Nomenclatural classification of enzymes**

The International Union of Biochemistry (IUB) and the International Union of Pure and Applied Chemistry (IUPAC) established the International Commission on Enzymes (IEC) in 1956. IEC developed a nomenclatural classification of enzymes that were well-known by then and designed a standardized technique that could be employed to scientifically name newly recognised enzymes (Shaikh, 2010:48). Based on the types of reactions they catalyse, enzymes are divided into six broad clusters such as hydrolytic, synthesising, transferring, oxidising and reducing, lytic and isomerising reactions (Shrimali & Dedhia, 2016:674).

Enzyme Commission (EC) number is an arrangement of four numbers (with periods in between each digit) that come after “EC” for example EC 3.2.1.4, signifies cellulase. The first character of the EC taxonomic code designates the overall type of reaction catalysed by the enzyme and ranges from one to six. Most enzyme names like cellulose pectinase ligninase and many others are generic and have many types; so the EC numbers and /or EC-accepted names specify the exact enzymes which are being referred to. According to enzymatic nomenclature system, each EC number is related to a proposed name for the respective enzyme. Classification starts with EC 1 symbolises Oxidoreductases: enzymes which either oxidise or reduce reactions, EC 2; Transferases: enzymes which relocate functional groups in a molecule, EC 3 Hydrolases: enzymes which use water to catalyse the chemical bonds, EC 4 Lyases enzymes which break chemical bonds without adding water, EC 5 Isomerases: rearranges atoms within a molecule and EC 6 Ligases are enzymes which covalently bind two molecules. The consecutive three numbers that follow the first digit further express and refine details of the reaction type, with regard to the composite, assemblage, link or product involved in the reaction. The EC name for an enzyme is normally devised from the name of the enzyme-substrate but end with the suffix “-ase”. For example, Lignin is the enzyme-substrate catalysed with the enzymes referred to as ligninases. However, there are some exceptions (Mojsov, 2011:232; Novozymes, 2013:7-8, Mojsov, 2014:37).

### **2.8.3 The origin and properties of enzymes**

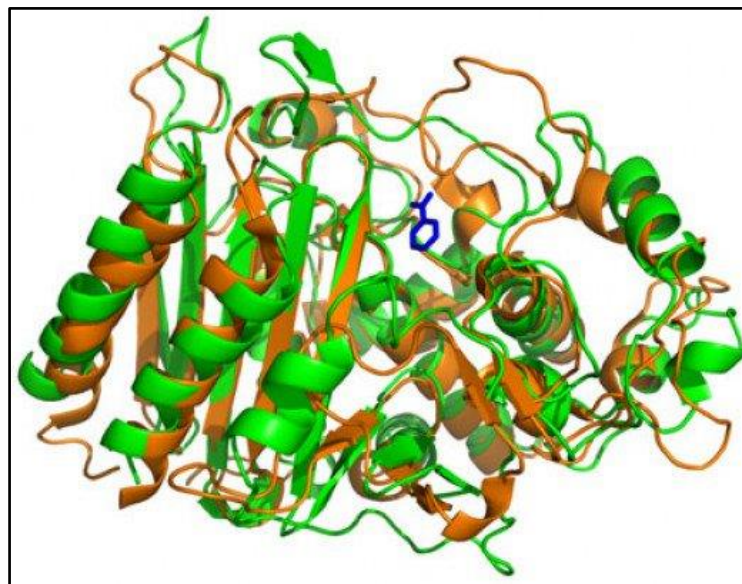
#### ***2.8.3.1 The origin of the enzymes***

Enzymes are basically derived from animal tissues, plants and micro-organisms. They are produced by living organisms but they are not alive. These enzymes are usually not produced in adequate amounts for textile applications. Conversely, commercial amounts can be attained by separating microbial strains to generate the anticipated enzyme and optimising the conditions for growth, through the fermentation process (Mojsov, 2014:35).

#### ***2.8.3.2 Enzymes as biocatalysts***

Enzymes are well-organized biological catalysts that fast-track organic reactions (Bhardwaj *et al.*, 2017:829). They are mostly globular structured (figure 2.23), high biomolecular weight proteins comprising of elongated lined amino acid chains that multifold into three-dimensions, making specific structures with unique properties (Bernava and Skaidrite, 2013:131; Rehman & Imran 2014:92). They have a complicated tertiary (figure 2.23) and at times quaternary structure and

speed up the reaction by creating an enzyme-substrate complex (Shaikh, 2010:48; Sarma & Deka, 2016:998). Enzymes increase the responsiveness of a specific matter or group of matters designated substrates (Gurung *et al.*, 2013:1; Odelade *et al.*, 2016:39).



*Figure 2.23: The typical globular structure of an enzyme (<https://hubpages.com/education/what-are-enzymes-where-do-they-work>)*

An enzyme is a specific biocatalyst with unique properties produced by an individual amino acid arrangement (Mojsov, 2014:135). The enzymes' presence accelerates the immense and diverse sets of chemical reactions which would otherwise react extremely sluggish and are never being consumed in the process. Enzymes provide a high degree of catalytic specificity unsurpassed with manmade catalysts. Enzymes biocatalyse the chemical reactions by reducing the activation energy. Activation energy is the energy needed to breakdown bonds. (Li *et al.*, 2012:1; Vigneswaran *et al.*, 2013:121; Khan & Barate, 2016:853).

The enzyme acts on an initial substance which is referred to as enzyme-substrate, which is transformed into a product or products. After the completion of the reaction, the enzyme is unbound and all set to begin a new reaction (Mojsov, 2014:36; Rehman & Imran, 2014:92-93). Enzymes stability is limited and can cause them to lose their activity over a period of time (Shaikh, 2010:48; Khan & Barate, 2016:853). The enzyme biocatalysts accelerate the reactions inside and outside the cell (Buchholz *et al.*, 2005:2; Gurung *et al.*, 2013:2).

### **2.8.3.3 Enzymes specificity**

Unlike inorganic catalysts, enzymes are extremely specific to the reactions they catalyse (Kalim, 2015:2). This means an enzyme catalyses only one particular substrate which has a particular variety of chemical bonds or functional groups and corresponding geometric shapes that perfectly fit into each another (Bhardwaj *et al.*, 2017:829). This is often beckoned with “the lock and key” model which explains enzyme specificity very well, the enzyme stabilization during transition state which is explained by the induced fit model (Silva *et al.*, 2010:78). Enzymes are so specific that they catalyse only the renewable raw materials that include textiles because they function under mild conditions, are harmless and tranquil to handle thus; can substitute harsh chemicals, act only on specific substrates to speed up reactions, enzymes are renewable and biodegradable (Gupta *et al.*, 2015:11).

### **2.8.3.4 Enzymes as efficient catalysts**

Enzymes are, highly energized protein molecules that have extraordinary catalytic power to accelerate chemical reactions tremendously, often far more than inanimate catalysts (Kalim, 2015:2). Some enzymes do not need co-factors to activate enzymes since they are self-regulating. Co-factors are non-protein molecules that stimulate enzymes to accelerate or reduce the activity. Co-factors are either inorganic like metal ions or organic co-enzymes (Šimić *et al.*, 2015:48). Enzyme catalysed reactions are normally faster than the corresponding not catalysed reactions, with a manifold range of 10<sup>6</sup> to 10<sup>13</sup> times (Rehman & Imran, 2014:93).

## **2.8.4 Enzyme activity in wet textile processing**

In the chemical reactions, enzymes catalyse substrates which are transformed into end products. During a chemical reaction, the enzyme active site temporarily binds with the substrate, to lower the molecular activation energy and hasten the reaction. When the reaction is complete, freshly produced product(s) are released from the enzymatic active site and the enzyme retains its original unique shape. The same enzyme is ready for use in another specific reaction (Mojsov, 2011:232).

### **2.8.4.1 Factors that influence enzyme activity**

Enzyme denaturation occurs when enzymes unfold and lose their secondary or tertiary or quaternary structure; thus interrupts its three-dimensional structure caused by a number of denaturants. The denaturation inactivates enzymes either reversibly or irreversibly (Gurung, 2013:3). There are essential factors that can influence enzyme activities which need to be



carefully considered when using enzymes in textile upgrading (Nierstrasz, 2009:140; Deshmukh & Bhat; 2011:36, Hasan *et al.*, 2015:17). The most imperative factors that affect enzyme activity are the type of enzyme and its specific parts, substrate and enzyme concentrations, ecological conditions such as pH values, temperature, activators and inhibitors' presence, additives including surfactants and chelators and mechanical strain influence the activities of the enzymes (Ammayappan, 2013:3; Gupta, et al., 2015:11; Mohan *et al.*, 2013:8; Uddin, 2015:2).

### 2.8.5 Components of an active enzyme

The active enzyme has an active site. The active site is a three-dimensional small polypeptide chained catalytic crevice (figure 2.24), also referred to as an enzyme binding site, that reacts with the specific substrate-binding site. All enzymes are proteins, but some of them are inactive and need other molecules to activate them to work. An inactive enzyme consists of a protein called an apoenzyme and an active non-protein chemical catalytic compound termed cofactor. An active enzyme consists of an apoenzyme combined with a cofactor is referred to as a holoenzyme (figure 2.24). Cofactors are classified into two types: cofactors of inorganic ions which are atoms with a net electric charge such as copper, iron magnesium, manganese, zinc, calcium or cobalt and compound biological molecules referred to as coenzymes, for example, CoA, NAD<sup>+</sup>, NADP<sup>+</sup>, FMN and FAD. Biological molecules simply mean the molecules that contain element, carbon. Coenzymes are comprised partially or entirely of vitamins (Patel *et al.*, 2016:387-388).

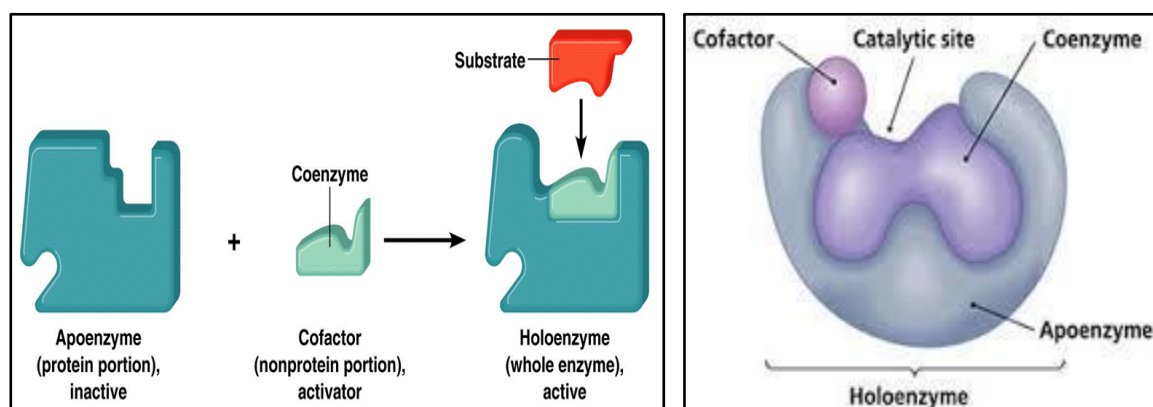
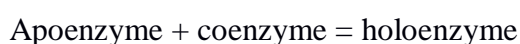


Figure 2.24: Specific enzyme components (Kumar, 2014 s.p; Patel *et al.*, 2016:387 respectively)

#### 2.8.5.1 Enzyme concentration

Enzyme concentration influences the action of the enzyme in textile wet processing. An increase in enzyme concentration leads to the enhanced enzymatic activity until when the enzyme reaches



its optimum concentration where the activity becomes constant too. This implies that the speed of the reaction is partly proportionate to the quantity of enzyme available for it. An increase of enzyme concentration; in textile wet bioprocessing; however, increases fibre strength and weight losses and reduces fibre thickness (Nelson, 1998:111).

#### **2.8.5.2 The potential of hydrogen (pH) value**

The pH is an acidic or alkaline concentration in a solution. The pH value runs from 0 to 14. The pH value of 0 indicates that the solution has very high amounts of hydrogen molecules dissolved and has the highest concentration of acid and pH 14 signifies the most alkaline or basic solution with the lowest pH concentration. The pH value 7 indicated neither acidic nor alkaline and pure water is a good example of a neutral solution. When an enzyme pH is reduced H<sup>+</sup> ions are increased, and eventually, enough side chains are impacted and the enzyme shape is distorted. Similarly, when the pH is elevated, the enzyme H<sup>+</sup> ions are raised and the enzyme ultimately changes its active site which consequently, does not fit well with the substrate and the enzyme activity is negatively affected. The enzymes are sensitive to environmental pH values and have specific ranges of activity. Each enzyme has its optimum pH value. When the pH value goes to an extreme for the specific enzyme, then the basic structure of the enzyme denatures (Nelson, 1998:111; Ekinici & Şentürk, 2010:385). Majority of enzymes function best between pH ranges of 6 to 8. Nonetheless, some specific enzymes function well only in acidic or basic environments. The optimal pH for a particular enzyme depends upon its origin and the type of buffer used (Ekinici & Şentürk, 2010:385; El-Yassin, 2012:6).

#### **2.8.5.3 Temperature**

Each enzyme requires a favourable temperature to work best. Enzymes have an optimum temperature range for the maximum activity rates (Hasan *et al.*, 2015:17). An upsurge in temperature mostly leads to an upsurge in reaction rates until it reaches optimum temperature range, thereafter leads to a sharp decrease in reaction rate; due to enzyme denaturation. Contrary to that, low-temperature conditions slow down the reaction. The extreme temperatures are not good for the enzyme activity. However, there are some distinctive enzymes that function well in extreme temperatures (El-Yassin, 2012:6; Uddin, 2012:301).

#### **2.8.5.4 Time of treatment**

Treatment time as well, influences enzyme action. Enzyme reaction time is shortened at the optimal treatment conditions. Fibre damage is greatest when treatment durations are long. The

internal structure is affected when treatment times are elongated even if there is no agitation. Likewise, short treatment times with agitation mostly impact fibre surface. Generally, reduced treatment time results in less weight loss and strength loss, and a slight decrease in DP (El-Yassin, 2012:6; Ammayappan, 2013:3).

#### ***2.8.5.5 Extent of agitation***

Mechanical agitation can affect the lignocellulosic fibre upgrading hydrolysis reaction. Both the adsorption and desorption activities of enzymes depend on the agitation to eliminate fibre debris and foreign matter in order to enzymatically biosoften the cellulosic fibres. This is mostly important when biopolishing cellulosic fibres; whereby cellulase adsorption is followed by fibres and fibrils cutting which are debilitated by the enzyme activity. Excessive agitation usually damages fibre surface (Anuradha & Nachiyar, 2012:12).

#### ***2.8.5.6 Substrate saturation***

Substrate concentration is proportionate to other factors that affect enzyme activity. This implies that it can sometimes function as a limiting or boosting factor (Uddin, 2012:300). Increasing the substrate concentration while enzyme concentration and other factors are high enough increases the rate of reaction. Understandably, the higher the substrate concentration, the larger the substrate molecules numbers are involved within the enzyme-catalysed reaction and vice versa. Enzyme saturation is achieved when the rate of reaction reaches a maximal point where no more increase occurs at a particular substrate concentration. At this point in time, there are no free enzyme active-sites to be engaged in forming enzyme-substrate complex; all the enzymes molecules are restricted to further bind substrate. The reaction rate remains constant; at the saturation point. The reaction cannot increase the pace even if the additional substrate is added. (El-Yassin, 2012:6; Uddin, 2012:301).

#### ***2.8.5.7 Salt concentration***

Most enzymes do not bear extreme salt concentrations. When salt concentration is too high the charged amino acid side chains of the enzyme molecules repel each other and the normal enzyme-substrate interface is prohibited, thus, prevents the chemical enzyme-substrate reaction. Similarly, when the concentration is too low the enzyme molecules' charged amino acid side chains, strongly attract each other and denature enzyme and inactivate its active-site. The typical enzyme-substrate interaction is prohibited. The medium salt concentration brings about the optimum enzyme activity. Normally enzymes function well in salt concentrations range of 1-500 mM.

However, there are exceptions such as the halophilic enzymes which need higher salt concentrations (Deniz & Murat, 2010:383; Ekinçi & Şentürk, 2010:385; El-Yassin, 2012:6).

#### **2.8.5.8 Level of macromolecular crowding**

The presence of high concentrations of macromolecules in a solution is termed macromolecular crowding. Macromolecular crowding reduces the diffusion rate, shifts the equilibrium of enzyme-substrate interactions, changes enzyme structure and properties thus, affect enzyme catalysis (El-Yassin, 2012:6).

#### **2.8.5.9 Concentration of reaction products**

The greater saturation of reaction products can decrease the enzyme speed of reaction. This can be caused by the equilibrium condition of the enzyme-product more than the enzyme-substrate complex which blocks the active sites in certain portions of enzymes (Ammayappan, 2013:3).

#### **2.8.5.10 Modulators**

Enzymes interrelate with various molecules other than the substrates that regulate how fast the enzyme acts. Any substance /molecule that increases the rate of the enzyme-substrate reaction when interrelated with an enzyme other than a substrate is an activator but if it reduces the response rate it is an inhibitor. Modulators can also control the speed with which the enzyme works (Ekinçi & Şentürk, 2010:386; Mohan *et al.*, 2013:10).

### **2.8.6 Enzyme reaction mechanism**

In most cases, enzymes are bigger than substrates they catalyse. The active site of the enzyme (figure 2.25) is the only part in which there is contact between the enzyme and substrate molecule to create an enzyme-substrate complex. The enzyme-substrate complex formation is signified by the equation:  $E + S \rightleftharpoons ES \rightleftharpoons E + P$ , and also illustrated in figure 2.27.

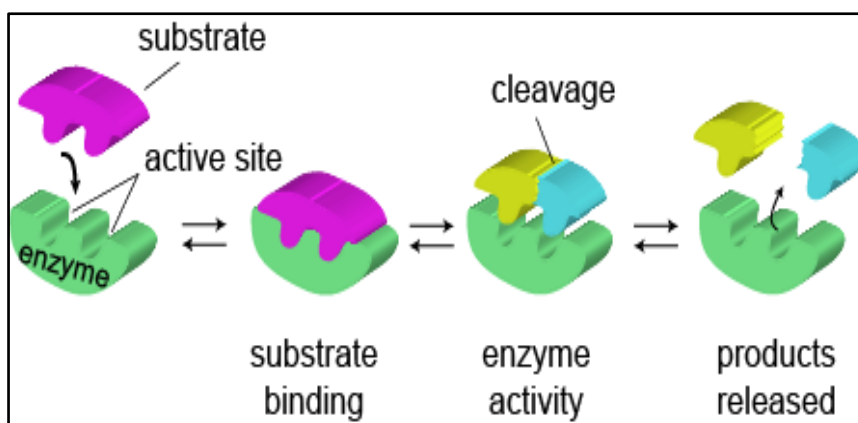


Figure 2.25: An enzyme-substrate complex (<https://www.shmoop.com/energy-flow-enzymes/enzymes.html>)

The mechanism of enzyme activity starts with enzyme activation by co-factor and then followed by enzyme-substrate complex formation at the active site and converting them into different molecules, called the products. The active site and substrate molecule shapes are specifically complementary and fit into each other each like puzzle pieces (Ammayappan, 2013:2-3). The enzyme-substrate complex is a transient molecule with an activated form of shape held by weak non-covalent bonds formed, by the time an enzyme comes across its substrate at an active site. This shape change can link substrate molecules together or divide individual molecules into smaller parts referred to as product(s) (Šimić *et al.*, 2015:48; Mohan *et al.*, 2013:4; Gurung, 2013:3; Ekinici & Şentürk, 2010 383-384).

### 2.8.6.1 Lock and key model

The Lock and Key analogy was first posited in 1894 by Emil Fischer (Tripathi & Bankaitis, 2017:2). It explains how well an enzyme interacts specifically with a single substrate to release products. The lock-and-key model, explains a theory which states that an enzyme is a rigid framework that interfaces only with a specific substrate which also possesses a fixed and tailor-made shape to fit precisely into that particular shape of its active site to create a catalytic enzyme-substrate complex to release products (Vigneswaran *et al.*, 2011:3-4). It also infers how some other small molecule(s) that inhibit or reduce substrates-enzyme reaction work(s), thus unlock or start the reactivity (Ringe & Petsko, 2008:1428). In this prototype, the enzyme is represented by the lock and the substrate by the key. Only the correctly sized, shaped and set key fits into the keyhole (active site) of the particular lock (enzyme) to bolt and unlock as illustrated in figure 2.26 (Shaikh, 2010:48; Šimić *et al.*, 2015:48).

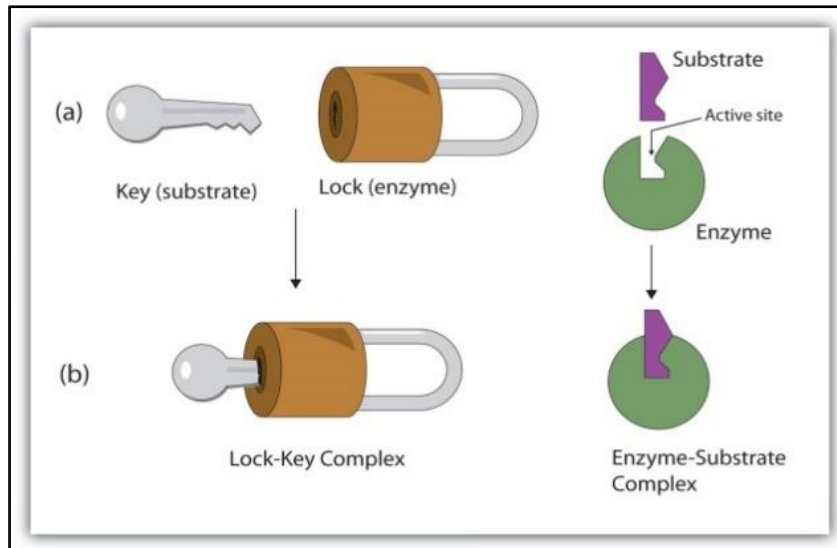


Figure 2.26: Lock and key model (Ball et al., 2011: figure 18.11)

### 2.8.6.2 Koshland's induced fit model

The lock and key model was modified in 1958 by Daniel Koshland, who entitled the new model; the induced fit theory. The induced-fit theory indicates that the enzyme sometimes has to change its shape to accommodate the substrate and that this shape change could be part of the catalytic reaction. This then opposes the lock and key theory which states that the enzyme surface is inflexible and that only the specific substrate would induce the accurate enzyme-substrate complex that steers catalysis. The enzymes can transform their shape to precisely fit the substrate into its catalytic site to initiate the reactions (Ringe & Petsko, 2008:1428; Šimić et al., 2015:48) as illustrated in figure 2.27.

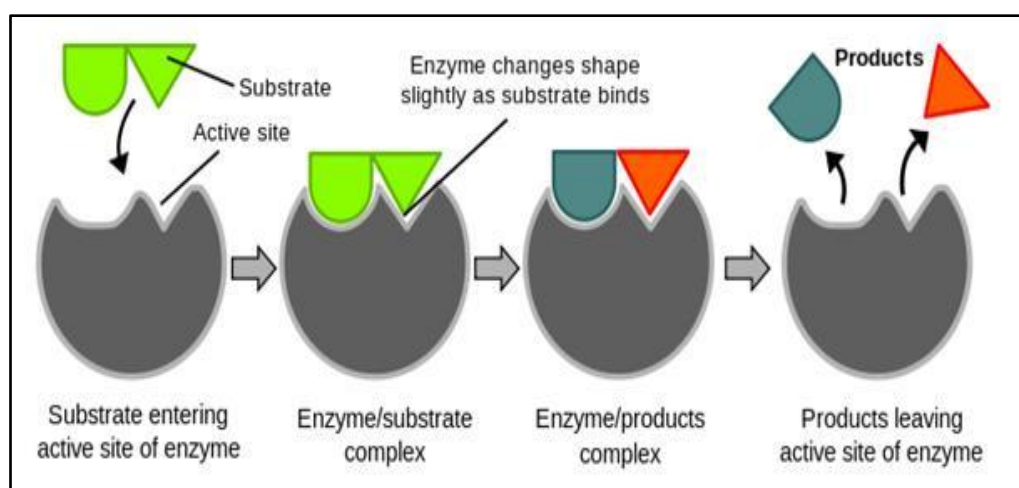


Figure 2.27: Induced fit Model for enzyme-substrate complex formation (Sheikh et al., 2010:48)

The active site is repeatedly remodelled to form enzyme-substrate complex when coming in contact with the substrate when the substrate shape and/or size does not fit perfectly into the enzyme's active site. It is also believed that sometimes, the substrate molecule likewise modifies its shape to some extent as it approaches the enzyme active site. They both keep changing until they form a perfect and functional enzyme-substrate complex. This elucidates why certain substances bind with enzymes but form non-reactive compounds. Sometime the substance molecules may be too large or small to prompt the perfect fit and consequently do not react. Only the suitable substrate can bring about the perfect fit into the active site (Shaikh, 2010:48; Hossain & Uddin, 2011:16).

### **2.8.7 Enzyme inactivation**

Enzyme deactivation is a state of environment which destroys the characteristics of a biomolecule by distorting its molecular conformation. Enzymes are three-dimensional protein structures which are sustained only under limited definite environmental conditions. Enzymes are denatured and inactivated outside these conditions. Thus, changes in the shape of the enzyme active sites and, the substrate molecules that are meant to react, cannot bind to the active sites (Gupta, *et al.*, 2015:11). To prevent excessive fibre weight, length and strength losses that can lead to unpredictable and irreproducible results, it is very essential that effective methods of controlling and ending the enzymes' activities are employed during and at the end of treatment respectively. Enzyme deactivation can be reversible or irreversible depending on a variety of conditions (Shaikh, 2010:48).

### **2.8.8 Sustainability benefits for the application of enzymes in textiles**

The reduction of pollution is one of the requirements for sustainable textile production, processing and consumption. The utilisation of enzymes in textile fibre softening is increasingly becoming famous because enzymes are environmentally sustainable since they are (and their products) best function under moderate reaction conditions (pH and temperature); highly efficient, specific, non-toxic, non-pollutants that require minimal water consumption (Vigneswaran *et al.*, 2011:2-3). They also originate from natural systems thus, biodegradable: readily return back into nature and biofertilise the soil. It is also possible to produce industrial enzymes which can be a healthily way to recycle enzymatic reaction by-products as fertilizer too (Koztowski, 2012:74; Mojsov, 2014:39; Jothi, 2015:38; Singh *et al.*, 2016:25). Enzymes can be effectively used in textile fibre softening since they are less risky when compared to the traditional lignocellulosic fibre softening methods (Vigneswaran *et al.*, 2013:121; Uddin, 2015:1).

It is highly encouraged worldwide, to prevent persistent destructions of natural assets, the environment and environmentally friendly technologies (Kalim, 2015:2). The tools of enzyme technology are sustainably essential to diminish the use of destructive chemicals in the textile industry to prevent environmental pollution and safeguard biovariety, in order to bring economic benefits but this is not highly recognised by the public. There is urgent need to improve productivity and efficiency through valuable environmentally friendly alternative processes to traditional chemical practices for most textile processes as means to drive toward textile sustainability through commercially successful applications such as bioscouring, biobleaching, biodelignification and biopolishing using enzymes (Araujo *et al.*, 2010:21; Silva *et al.*, 2010:90; Tahir *et al.*, 2011:5268-5270; Van Dam, 2015:12). Enzymes are successfully applied in lignocellulosic fibre softening because they are large molecules; their activity is constrained to the surface of the fibre, leaving fibres maintaining their favourable bulk properties (Silva *et al.*, 2010:78).

Traditionally, the effective textile production technologies for softening and brightening the lignocellulosic fibres consume large quantities of water and energy and they need costly treatment of effluent before disposal (Teli & Adere, 2016b:256). Thus, they are not sustainable since they are not economic, social and ecologically friendly. They are also detrimental to the strength of the textile fibre. Softening of the *Agave americana L.* fibre devoid of affecting the strength will be of utmost importance and a considerable challenge. Enzymatic biotechnology is regarded as sustainable and eco-friendly technology which has no significant adverse effects on fibre strength and thus, the promising alternative to conventional textile processing (Nelson, 1998:111; Van Dam, 2002:17; Miettinen-Oinonen, 2007:51; Shaikh, 2010:51; El-Sayed, 2015:806; Kandhasamy & Vasudevan, 2015:849).

Enzymatic biotechnology has the potential to trigger new and developments and better quality processes, goods and services to satisfy human needs from alternative underutilised raw textile plant materials such as lignocellulosic fibres (Buchholz *et al.*, 2005:1). The E3 which stands for energy and environmental conserving and economical paybacks are benefits of enzymatic biotechnology when compared to traditional wet processes in textile processing (Cavaco-Paulo, 1998:186; Adrio & Demain, 2005:8; Auterinen, 2006:1; Arja, 2007:52; Shaikh, 2010:48; Mojsov, 2014:135; Šimić *et al.*, 2015:48). Furthermore, the enzymes are in general easy to use since they do not need special equipment and can be used effectively with normal equipment at moderate



heat and non-corrosive and acceptable pressure conditions at any stage of textile wet processing (Bernava & Skaidrite, 2013:131).

Consumers are increasingly becoming aware of global demands for sustainable textile fibre production and processing which reduce the exploitation of resources and excessive waste production (Vega *et al.*, 2015:161). The use of enzymes has currently become an indispensable part of the textile technology (Mojsov, 2014:36). The enzymes save chemicals and energy with the capability to reduce processing time and improve the aesthetic and hand properties of lignocellulosic fibres without processing damage. They can optimize the production process through raw material efficiency and by combining enzymes and production processes (Araujo *et al.*, 2008:332; Gupta, *et al.*, 2015:11; Skaidrite, 2013:131; Bharathi & Kanaka, 2015:4857; Shrimali & Dedhia, 2016:674).

The use of enzymes in textile technology is social, cost and ecological efficient since bulk buying of enzymes can be cheaper than chemicals, enzyme utilisation also saves energy and water, produces less waste, results in shorter processing time, has higher efficiency and is more specific (Adrio & Demain, 2005:8). It has contributed to approximately 60% cleaner textile fibre production. Contrarily, very harsh chemical softening of lignocellulosic fibre needs extremely elevated temperatures, pressures and pH. This chemical disposal causes environmental problems (Mojsov, 2014:35; Rehman & Imran, 2014:93). Enzyme technology is regarded as a potential sustainable textile fibre production and processing alternative to hazardous conventional chemical use in textile processing to improve lignocellulosic fibre quality (Garg *et al.*, 2016:6).

Conversely, enzyme biotechnology does not create harmful discharge, because enzymes can easily be deactivated before they are disposed of (Satyanarayana *et al.*, 2011:222). Enzyme biotechnology lucratively unlocks up new textile prospects for use in formerly unidentified application areas rather than only improvement of innovative yields for conservative textile industries (Nierstrasz, 2009:141; Hardin, 2010:142; Deshmukh & Bhat, 2011:36; Yang *et al.*, 2011:421; Jagannathan & Nielsen, 2013:228-229; Gabrič & Pohleven, 2014:50).

## **2.8.9 The enzymatic biotechnology in textile technology**

### ***2.8.9.1 Alkaliphilic enzymes in wet processing textile technology***

Alkaline enzymes are nowadays regarded to be the most appropriate enzymes for lignocellulosic fibre scouring by many textile researchers and scientist, because; of their ability to degrade and

eliminate non-cellulosic components of plant cell wall; thus; facilitates biosoftening which is adopted as a clean technology in textile fibre manufacturing (Qureshi *et al.*; 2012:12563; Garg *et al.*; 2016:6; Kubra *et al.*, 2018:23). Alkaline (alkaliphilic) enzymes are currently utilised with great success in the bioprocessing of lignocellulosic fibre textiles (Bhardwaj *et al.*; 2017:832). The alkaliphilic enzymes are usually more stable, energy and cost-effective (because they use lower temperatures), produce a speedier and more trustworthy product, decrease waste disposal hitches (Moubasher, 2016b:103 -104). The Alkaliphilic enzymes catalyse reactions between neutral and alkaline pH value range at the elevated temperatures and these enzymes are stable at high reaction temperatures, have short reacting time, do not compromise the strength of fibre, and have good finishing properties. Enzyme treatment of fibres is an eco-friendly way of upgrading the following fibre properties: desirable appearance and soft handle (Jabasingh & Nachiyar, 2012:12; Kubra *et al.*, 2018:23). Commercial enzymes have for a long time been found as acidic and neutral enzymes, categorised with the pH ranges at which they are highly active and user-friendly (Uddin, 2016:2). Alkaliphilic enzymes are considered very important as they are recently accessible in biotextile technology. The alkaliphilic enzymes are those that are very active under alkaline conditions (Vega *et al.*, 2015:160).

Alkaliphilic enzymes have unique properties, which make them suitable to close up the gap that exists between biological and chemical textile fibre processing of lignocelluloses (Moubasher *et al.*, 2016b:104). Alkaline enzymes are secreted by alkaliphilic bacteria, with pH optima for growth ranging from 8.0 to 11.0, but mostly pH 9.0, defined as alkaliphilic microorganisms. Alkalophiles are unique microorganisms with great potential for microbiological and biotechnological exploration. Alkaliphilic microorganisms are sources of many new extracellular enzymes which reveal distinctive specificities in many respects (Patel *et al.*, 2011:116). The optimal activity of alkaliphilic enzymes from neutral to alkaline pH, and are increasingly being involved in textile fibre treatment processes because the alkalinity or neutrality in pH lessens textile back- staining (Hardin, 2010:141; Zhou *et al.*, 2015:5714; Vega *et al.*, 2015:161; Uddin, 2016:2).

#### **2.8.9.2 The lignocellulolytic enzymes in textile wet processing**

The Lignocellulose, also referred to as xylano-pectino-lignocellulose, is globally the most plentiful renewable biomaterials. It is the main substrates of lignocellulose-degrading enzymes that are referred to as either the xylano-pectino-lignocellulolytic enzymes or lignocellulases (Nierstrasz, 2009:141; Deshmukh & Bhat, 2011:36; Singh *et al.*, 2015:1). Lignocellulolytic

enzymes are enzymes capable of hydrolysing tough lignocellulose complex networks from the plant biomass in order to extract and upgrade the lignocellulosic fibres. Various microbes that include bacteria and fungi are good sources of lignocellulosic enzymes (Mahabub *et al.*, 2015:17, Saini *et al.*, 2015:1). Lignocellulolytic enzymes are utilised in textile biosoftening processes to effectively substitute the conventional chemical processes. Enzymatic biosoftening technology is more eco-socio-economic sustainable and energy-effective than its counterpart (Tavčer, 2013:100; Singh *et al.*, 2016:25). The lignocellulolytic enzymes are used extensively in lignocellulosic textile processing because they are able to transform the lignocellulosic fibres in structured and desirable ways so as to upgrade plant fibre quality and appearance. Bioscouring, biodelignification, biobleaching and biopolishing are well-identified modern textile applications of Lignocellulolytic enzymes on lignocellulosic fibre (Kalia *et al.*, 2011:9; Anwar *et al.*, 2014:168; Singh *et al.*, 2015:1.).

The xylan, pectin and lignin are imperative lignocellulosic biocomponents of plant fibre which are efficiently degraded by lignocellulolytic enzymes (figure 30) (Singh *et al.*, 2015:1). The commonly used xylano-pectino-lignocellulolytic enzymes in the textile industry are cellulases, hemicellulases, ligninases and pectinases which are used in diverse applications, hence why the name xylano-pectino-lignocellulolytic enzymes (Radhakrishnan & Preeti, 2015:10501; Saini *et al.*, 2015:1). The resistance of plant cell walls to breakdown has been reduced by transforming plant cell walls through biotechnology. The reduction of recalcitrance is accomplished through dislocating lignin-carbohydrate complexes linkages which assist to decrease lignin content and change its structural configuration and manipulate hemicellulose bioseparation using enzymes in plant fibre upgrading (Li *et al.*, 2014:1174).

Enzymatic treatment of lignocellulosic fibres has been established to upgrade a number of physico-mechanical properties of lignocellulosic fibres, such as softness, smoothness and tenacity (Backgrounder, 2013:1; Karolia & Bhoj, 2016:18). Enzymes that are used mainly in the textile processing are traditionally referred to as technical enzymes (Nierstrasz, 2009:141; Deshmukh & Bhat, 2011:36). Enzymatic treatment of lignocellulosic fibres using different enzymes is feasible. The relevant lignocellulolytic commercial enzymes for biofibre softening are a collection of hydrolytic enzymes accountable for cellulolytic and xylanolytic biosoftening undertakings. They are mostly cellulase, pectinase, hemicellulase and ligninases (Mtui, 2012:1548) as illustrated in figure 2.28. Commercial enzymes are not pure and comprise of unstated other enzymes with

other activities. For example, xylanase preparations contain cellulases and considerable hemicellulase activities (Dien & Bothast, 2009:82).

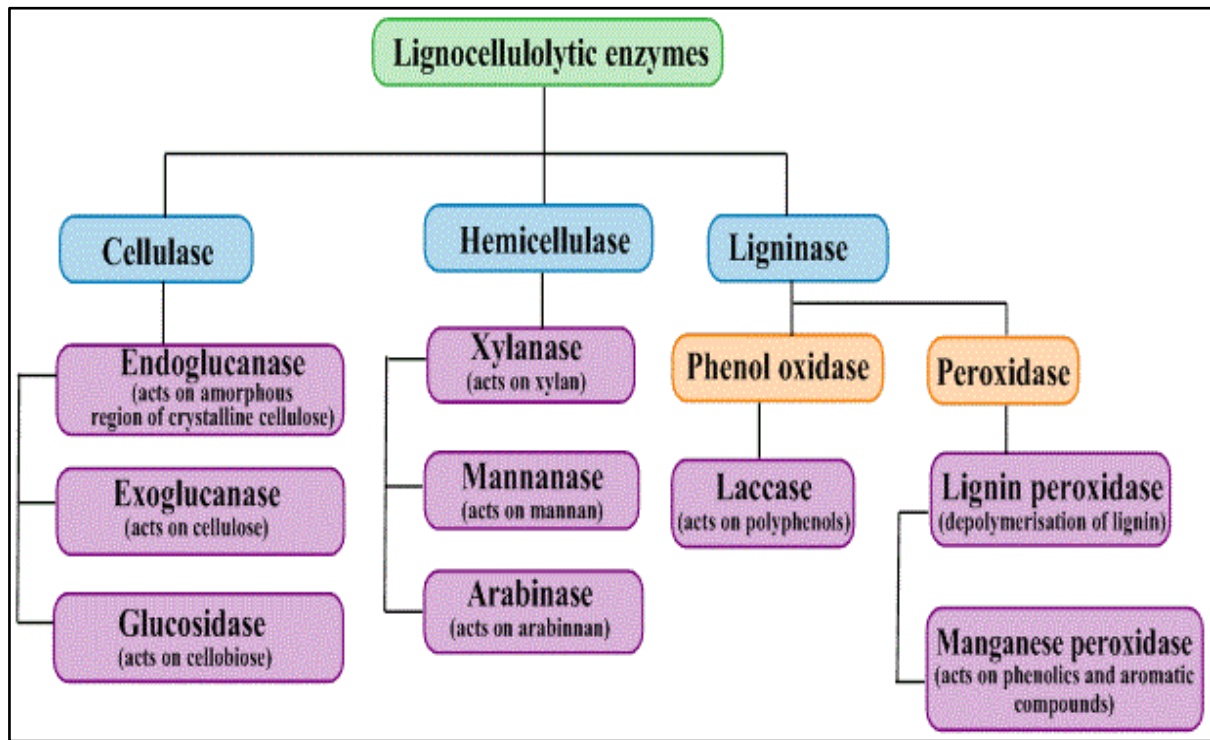


Figure 2.28: Diagram illustration of lignocellulolytic enzymes (Sajith *et al.*, 2016:3)

(a) Cellulases

Cellulases are often utilised successfully in the textile fibre processing of cellulose-containing materials. Cellulases are also referred to as cellulolytic enzymes (Shaikh, 2010:50; Tavčer, 2013:100; Onofre *et al.*, 2014:276; Maki *et al.*, 2014:165; Bharathi & Kanaka, 2015:4858; Saini *et al.*, 2015:3; Vega *et al.*, 2015:161). Cellulase is a complicated enzyme that is composed of multi-component enzyme systems (Bahtiyari *et al.*; 2011:3) which are substrate-specific, have different structures and mechanisms. They include endo- $\beta$ -glucanase (EC 3.2.1.4), exo- $\beta$ -glucanase (EC 3.2.1.9.19) and  $\beta$ -glucosidases (EC 3.2.1.21) which work together at the same time (Miettinen-Oinonen, 2007:51; Zhang *et al.*, 2013:133; Mishra & Suseela, 2016:65; Hossen, *et al.*, 2017:587). Cellulases are hydrolytic enzymes produced predominantly by different species of microbes. But, plants and animals; to some extent, also produce some cellulases (Nierstrasz, 2009:142; Singh *et al.*, 2016:24; Uddin, 2016:1).

Biosoftening of denim garment, biopreparation, biopolishing, and biosoftening of lignocellulosic textile fibres and their products are mainly achieved through the use of cellulases (Imran *et al.* 2016:44 & 49, Behera *et al.*, 2017:205), with an intention to upgrade their tactile texture and

visual look (Datta *et al.*, 2016:26). Thus, the improvement and upgrading of the textile quality through controlled modification of lignocellulosic fibres. The fibre surface is improved through the removal of the protruding fibrils and microfibrils; thus transforming lignocellulosic fibres into smooth, soft, cool, bright coloured, pliable and better pilling resistant fibres that can be used for further textile applications (Konczewic & Koztowski, 2012:168; Uddin, 2015:1; Moubasher *et al.*, 2016:104).

#### (b) Hemicellulases

Hemicellulases or xylanolytic enzymes are the enzymes which catalyse hemicelluloses (Kantharaj *et al.*, 2017:3). Xylanase and galactanase are examples of enzymes that belong to the hemicellulase group (Backgrounder 2013:1). Xylanases (glycosidases which hydrolyse xylan) are largely used in lignocellulosic fibre biosoftening processes (Kalim, 2015:2). The major function of xylanases is to break down fibre by converting one of its components, a gummy polysaccharide (beta 1, 4) xylan, into simple and soluble substances such as xylose and xylobiose (Qureshi *et al.*, 2012:12563; Hossen, *et al.*, 2017:586).

The use of xylanase increased its importance in textile sustainability and enzymatic upgrading of lignocellulosic fibres. The effective biosoftening of lignocellulosic fibre involving xylanase enzyme system consists of EC 3.1.1.72 (acetyl xylan esterases), EC 3.1.1.73 (ferulic/coumaric acid esterases), EC 3.2.1.37 ( $\beta$ -D-xylosidases), EC 3.2.1.8 (endo- 1, 4- $\beta$ -xylanases), EC 3.2.1.55 ( $\alpha$ -L-arabinofuranosidases) and EC 3.2.1.139 ( $\alpha$ -glucuronidases. Xylanases (E.C. 3.2.1.8) are prospective enzymes for effective use in textile industries where they are largely used in lignocellulosic biosoftening processes and in waste treatment (Kalim, 2015:2; Moubasher *et al.*, 2016b:104; Hossen, *et al.*, 2017:586).

Xylanases hydrolyse the hemicellulose in the lignocellulosic fibres and biobleach them with no or minimal effect on strength rather enhances the physical properties (Burlacu *et al.*, 2016:339; Walia *et al.*, 2017:8). The use of xylanases also unstiffens the fibres, letting them be cohesive and spinable. Xylanase has great potential to revolutionize fibre bioprocessing. The xylanase can be used to improve biomass pre-treatment economics by removing or reducing the need for fibre pre-treatment process (Corral & Villaseñor-Ortega, 2006:310; Saini *et al.*, 2015:5). Beyond these benefits, xylanase is also able to boost textile fibre bioprocessing efficiency and, more generally, make bioprocessing more economical. Xylanase utilisation can also reduce the need to use harsh peroxide (Shaikh, 2010:51; Pastor *et al.*, 2007:67; Burlacu *et al.*, 2016:339). The thermostable and

cellulose-free xylanases are favoured for biobleaching of *Agave americana* L. fibre (Thakur *et al.*, 2012:2221).

### **2.8.9.3 Lignolytic Enzymes**

Lignin is the carbohydrate with an extremely complex structure comprising of different biologically stable linkages. Ligninolytic enzymes are lignin-degrading enzymes also termed ligninases because they hydrolyse polymer lignin; which is complex and recalcitrant. Lignolytic enzymes are multipurpose in nature (Niladevi, 2009:397). The ligninolytic enzymes include three eminent oxidative enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Aarti *et al.*, 2015:123). Ligninolytic enzymes biobleach lignocellulosic fibres (Kirk and Jeffries, 1996:2, Niladevi, 2009:398; Maciel *et al.*, 2010:4). However, the lignin hydrolysis of lignocellulosic fibres with the eco-environmentally friendly enzymatic procedure is a global challenge for textile researchers and developers. The adversative effects of lignocellulose fibre on the environment can be reduced by economical, eco-friendly and sustainable approach (Konczewic & Koztowski, 2012:168; Aarti *et al.*, 2015:122).

#### **(a) Laccases**

Laccases are extracellular, blue multi-copper oxidoreductases (BMCO) which are glycosylated (Kantharaj *et al.*, 2017:4). They use molecular oxygen to oxidise phenols, the principal recalcitrant component in the lignocellulose and a variety of aromatic and non-aromatic compounds through a radical-catalysed reaction mechanism (Dashtban *et al.*, 2010:39; Hasan *et al.*, 2015:18). Water is produced as a by-product by laccases. Laccases have distinctive characteristics of reactive radical production (Kalim, 2015:1). The laccase is produced by different types of organisms such as plants, fungi and bacteria (Imran *et al.*, 2012:1; Aarti *et al.*, 2015:123). Various complex substances are hydrolysed by laccases because of their low substrate specificity. They can display ligninolytic and polymerizing capabilities when acting on lignin in plant fibre. This lateral-networking property is essential for them to gain speedy sustainable application in lignocellulosic textile fibre biosoftening processes (Gabrič and Pohleven, 2014:49-50). Of all ligninases, laccase is the most popular enzyme; however, combinations of more than one ligninolytic enzymes are often synergistically efficient for biodelignification of lignocellulosic fibres (Abdel-Hamid *et al.*, 2013:1; Plácido & Sergio, 2015:3).

#### **(b) Heme peroxidases**



Lignin and manganese peroxidases are collectively termed heme peroxidases because they contain protoporphyrin IX as a prosthetic group (Kantharaj *et al.*, 2017:3). They are extracellular enzymes that catalyse lignin which is found outside the cell due its large and complex structure which cannot allow it to enter the cell for intracellular action (Dashtban, 2010:40-41). Lignin peroxidases can specially catalyse elevated redox potential phenolic or nonphenolic aromatic compounds which comprise more or less 90% of lignin. They require H<sub>2</sub>O<sub>2</sub> for activating their reactions. Veratryl alcohol is the LiP substrate, which acts as redox mediator for subsidiary oxidation of other substrates. Manganese peroxidases require H<sub>2</sub>O<sub>2</sub> to be activated since they are low redox potential heme peroxidases (Shaikh, 2010:51; Bholay *et al.*, 2012:59; Aarti *et al.*, 2015:123; Saini *et al.*, 2015:5; Hossen, *et al.*, 2017:588).

#### **2.8.9.4 Pectinases**

Bioscouring of lignocellulosic fibres is attained through the use of pectinases (Bhardwaj *et al.*, 2017:832). Pectinases are a diverse collection of enzymes which degum pectic substances in the natural lignocellulosic fibres (Kusuma & Reddy, 2014:162; El-Sayed, 2015:805; Sarma & Deka, 2016:997; Sethi *et al.*, 2016:1-2; Kubra *et al.*, 2018:23). They are also referred to as pectinolytic enzymes and can be classified into the following three main groups; pectinesterase (PE), polygalacturonase (PG) and pectin lyase (PL) based on their mode of action on the pectin containing substrate (Tariq & Latif, 2012:7259; Khatri *et al.*, 2015:2). Pectinases are considered as the most effective enzymes for bioscouring of lignocellulosic fibre (Ravindran & Jaiswal, 2016:4; Sethi *et al.*, 2016:2). During pectinase bioscouring the middle lamella disintegrates and release fibre easily during fibre separation (Gummadi *et al.*, 2007:98; Sarma & Deka, 2016:998; Hossen, *et al.*, 2017:588).

Alkaline pectinolytic enzymes have great biotechnological potential as an eco-friendly treatment on lignocellulosic fibre upgrading and are currently an essential part of textile industries (Hardin, 2010:14; Garg *et al.*, 2016:6; Sarma & Deka, 2016:998). Pectinase treatments can efficiently eliminate the shave and epidermal tissues of the lignocellulosic fibres (Raj *et al.*, 2011:2). These indicate that *Agave americana L.* fibre can be softened successfully with pectinases.

Lignocellulosic fibre bioscouring was traditionally performed with harsh alkaline chemicals such as sodium hydroxide at elevated heat. Pectinolytic bioscouring effectively and eco-friendly substitutes sodium hydroxide with pectinases to hydrolyse pectin but does not impart negative effects on fibre strength, because pectinases have little or no cellulase activity which could affect



cellulose integrity and are selective to pectin hydrolysis (Pedrolli *et al.*, 2009:15; Ramakanth *et al.*, 2014:1; Sarma & Deka, 2016:997). They also contribute to the natural recycling of carbon in the environment (Tavčer, 2013:100; Radhakrishnan & Preeti, 2015:10501). Commercial enzyme preparations have synergistic catalytic activities mostly of pectinase, and to some extent hemicellulases and cellulases. Enzymes containing high amounts of pectinase and reduced amount of cellulase are highly preferable to prevent lignocellulosic fibre damage (Raj *et al.*, 2011:2).

#### **2.8.10 Biosoftening of *Agave americana* L. fibres**

Textile fibre softness is an essential property that determines the end-use and selection criteria of a textile product. In order to reduce fibre prickliness on the skin and improve softness and lustre, enzymes are currently used to treat textile fibres as per their end-uses (Ammayappan, 2013:8). Biosoftening of *Agave americana* L. fibre is physico-chemical biotechnology, used to transform the surface of the fibre so as to minimize pollution. Enzymatic biosoftening is a bioprocess in which the effect of the natural *Agave americana* L. fibre is achieved with the use of specific enzymes, particularly toward surface cell-wall components. In this lignin; which causes, the brittleness to the *Agave americana* L. fibre is partly removed from the surface. With this removal of impurities, the fibre components that include cellulose and hemicellulose to become more compact, and increase the fibre strength and flexibility of the. Thus, the quality after biosoftening is expected to stay more or less the same (Silva *et al.*, 2014:1068; Desai *et al.*, 2016:30).

The growing world population along with modern developments led to a scarcity of non-renewable textile fibre resources, which pushes human beings to explore alternative, understudied and unutilized fibre sources. *Agave americana* L. can become a source of biotextile fibres for the production of textile items (Almeida & Cavoco-Paulo, 1993:185; Chen *et al.*, 2014:478). *Agave americana* L. fibres is a lignocellulose fibre that is coarse, stiff and has relatively high lignin content (Gao *et al.*, 2015:5476-5477; Hulle *et al.*, 2015:67). The fibre softening processes are key to taking advantage of *Agave americana* L. fibre resources. The economic processing of *Agave americana* L. fibres is mostly dependent on the development of efficient and sustainable upgrading biotechnologies (Sarma & Deka, 2016:998), such as enzymatic processing (Michelle & You-Lo, 1998:213).

*Agave americana* L. fibres can be softened, rendered clean and brightened eco-friendly with minimal fibre damage by the enzymatic bioprocessing. The enzymatic biodelignification, bioscouring, biobleaching, and biofinishing, are save where the alternative chemical methods are

hazardous and performed at high temperatures. The disposal of these harsh used chemicals into the environment causes pollution and produce toxic by-products. Mechanical methods on their own do not degum adhesive substances sufficiently from the lignocellulosic fibre surface and require high energy input. Biosoftening aims to remove mainly lignin and other gummy substances networking lignocellulosic fibre cells no or little negative effects on fibre strength. Lignin is stiff as a result it impairs fibre flexibility and cohesion need for spinning (Bezazi *et al.*, 2014:1; Mojsov, 2014:135; Kandhasamy & Vasudevan, 2015:2; Shaikh, 2010:48; Gao & Yu, 2015:1305; Singh *et al.*, 2015:1).

The presence of lignin darkens colour and imparts rigidity of lignocellulosic fibre. Biosoftening of the *Agave americana L.* fibre can be achieved through biodelignification, bioscouring, biopolishing and biobleaching treatment. The use of these treatments is potentially sustainable and favourable alternatives for upgrading physico-mechanical properties of lignocellulosic fibres (Ales & Sumanasiri, 2010:2; Hernández-Hernández *et al.*, 2014). The aim is to achieve *Agave americana L.* fibre bio-processing effect with the use of selected and controlled specific commercial, enzymes so as to obtain the softer, fuller, less stiff, and less crisp fibre. A long treatment time apart from the high damage of the fibre has a negative effect on handle (Almeida & Cavoco-Paulo, 1993:185).

#### **2.8.10.1 Biodelignification with enzymes**

Enzymatic biodelignification is biotechnology whereby purified or semi-purified ligninases and enzymatic extracts are used to hydrolyse and degrade lignin in the fibre (van Dam, 2002:11, Plácido & Capareda, 2015:4). The effective conventional chemical delignification involves degradation of the non-cellulosic fibre binding components but causes environmental pollution and reduces fibre strength (Saxena *et al.*, 2017:56). Mechanical methods need higher energy inputs to soften the fibre on the other side usually do not completely remove the adhesive biomass from the surface of the fibre bundle with reduced energy input. Biodelignification is the preferred alternative due to controls and disadvantages of existing chemical treatment technologies (Gao *et al.*, 2015:5477). Biodelignification can be performed effectively and efficiently through the use of ligninolytic enzymes or microbial use without affecting the integrity of cellulose (Rameshaiah & Reddy, 2015:3; Sarma & Deka, 2016:998).

### **2.8.10.2      *Bioscouring with enzymes***

Scouring is the removal of non-cellulosic constituents from the lignocellulosic fibre and thus directly transforms the fibre structure (Shrimali & Dedhia, 2016:675). The hydrophobic properties of raw lignocellulosic fibres are mainly instigated by the pectins (Sarma & Deka, 2016:998). The removal of pectin through the use of scouring chemicals results in more hydrophilic, brighter, softer and smoother lignocellulosic fibre than enzymatic biotechnology; but with beyond the limits; reduced strength and causes environmental hazards (Hardin *et al.*, 1998:190, Saxena *et al.*, 2017:56). Contrary to that, it is eco-friendlier and better retains fibre strength while at the same time removes the non-cellulosic scums from the lignocellulosic fibres to soften and brighten the fibre surface. Bioscouring also reduces high water and chemicals' utilisation and waste production and discharge (Garg *et al.*, 2013:43; Anwar *et al.*, 2014:168; Garg *et al.*, 2016:6). Thus, sustainable quality lignocellulosic fibre is obtained (Tavčer, 2011:391; Konczewic & Koztowski, 2012:172).

Bioscouring is an eco-friendly process that has a great future (Sharma 2013:769). Pectinases proved to be the most effective, and economic alternative enzymes for lignocellulosic fibre bioscouring that do not adversely affect the cellulose (Hasan *et al.*, 2015:16, Nisha, 2016:293). Alkaline pectinases are especially recommended and commercially appealing for imparting water absorbability in lignocellulosic fibres (Hardin, 2010:141; Tavčer, 2011:391; Teli & Adere, 2016a:210). The new enzymatic bioscouring function is a good, innovative and eco-friendly alternative to decrease pollution and does not cause excessive fibre damage. It further minimises the demand for high energy, a lot of water, corrosive and concentrated chemicals and the costs (Mojsov, 2012:24; Novozymes, 2013:20; Shrimali & Dedhia, 2015:675; Sarma & Deka, 2016:997). In general, pectinases are mostly used for lignocellulosic fibre bioscouring mostly uses pectinases to hydrolyse the lignocellulosic fibre cell wall structure to release pectin. The Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) of enzymatic bioscouring process are less than that of alkaline scouring (Cavaco-Paulo & Almeida, 1994:353; Pedrolli, 2009:15; Garg *et al.*, 2013:43; Novozymes, 2013:20; Mojsov, 2014:136; Bharathi & Kanaka, 2015:4857 & 4859).

### **2.8.10.3      *Biobleaching with enzymes***

A major objective of bleaching the lignocellulosic fibres is to confiscate the remaining lignin and improve the fibre colour and absorbency. The bleached lignocellulosic fibres are brighter, more commercially attractive and produce value-added products (Rameshaiah & Reddy, 2015:3).

Bleaching whitens and increase fibre brightness. The *Agave americana L.* fibre is bleached can produce a number of colour hues and pastel shades (Van Dam, 2002:8; Usluoğlu & Arabaci, 2015:3281). The usual chemical bleaching of lignocellulosic fibres involves highly concentrated and large quantities of chlorine and subsequent, large amounts of water for rinsing. The bleaching lignin-chlorine products tend to be are mutagenic and pose waste effluent treatment problems since they are toxic and dark coloured (Saxena *et al.*, 2017:56). Therefore, environmental concerns become a pushing factor to explore for alternative cleaner ways to at least reduce or abolish the use of chlorinated bleaching chemicals (Selvam & Arungandhi, 2013:96). Moreover, chlorinated bleaching chemicals react with the fibre and decrease fibre DP and consequently, cause serious loss of fibre strength (Bharathi & Kanaka, 2015:4857; Singh *et al.*, 2013:3).

Adopting eco-friendly biobleaching technology for lignocellulosic fibre is an alternate way to abolish the use of harmful chemical wet processing procedures (Shrimali & Dedhia, 2016:675). Bleaching using enzymes is of interest to explore new ecological, social and economic techniques for softening and brightening the fibres (Van Dam, 2002:11, Sharma 2013:769). Thermostable commercial enzymes are active under alkaline conditions of fibre are generally preferred for biobleaching. Enzymatic bleaching modifies and improves lignocellulosic fibre properties and sometimes creates completely new fibre properties. The basis of these enzymes must be economically feasible for the *Agave americana L.* fibre biobleaching with a view to diversify the end-uses of the fibre (Usluoglu & Arabaci, 2014:364). The enzymatic biobleaching technique is normally carried out with oxygen-based enzymes which about the colour-creating mixtures in the cell wall lignin component of the fibre. The enzymatic biobleaching techniques, are the considered to be one of the desirable ways, to reduce absorbable organic halide (AOX) levels in the discharged wastewaters, thus reduce the need for chlorine during processing and enhance fibre quality gaining intensity and the decrease in Post Colour Number (PC number) (Thakur *et al.*, 2012:2220-2221).

#### **2.8.10.4      *Biopolishing with enzymes***

The enzymatic biopolishing also called biofinishing is a biological process in which the enzymes mostly cellulases act on the surface of the fibre (Schindler & Hauser, 2004:181; Rehman & Imran, 2014:94), to improve the softness and the efficiency of the subsequent textile fibre finishing process either as pre-treatment and enzyme-assisted textile fibre finishing or biofinishing. Chemicals used for wet fibre polishing are harsh and can cause fibre damage and environmental pollution (Saxena *et al.*, 2017:56). The enzymatic biopolishing of lignocellulosic fibre has often

been reported environmentally safe and successful without significant negative effects on the strength of the fibre (Schindler & Hauser, 2004:181; Rehman & Imran, 2014:94). With proper control, enzymatic biopolishing is absolutely risk-free and effective because it is conducted under mild chemical and physical conditions (Eklahare, 2011:43; Anuradha & Nachiyar, 2012:12; Chinnamma & Antony, 2015:1607).

The cellulase biopolishing of the lignocellulosic fibre occurs only on the surface because cellulase cannot infiltrate the fibre interior since its molecules are bigger, than water molecules (Uddin, 2015:1-2). Thus, fibre strength is not adversely affected by cellulase biopolishing rather smoothens and provides a permanent effect on fibre surface. Contrary to that the conventional singeing and chemical polishing imparts temporary polishing effect; the textiles fuzz and pill; shortly after use and care. Thus loses colour and beauty (Konczewic & Koztowski, 2012:172; Rehman & Imran, 2014:94; Behera *et al.*, 2017:205). Cellulase biosoftening of lignocellulosic fibres brings about reduced pilling, increased softness and amelioration on handle, surface structure and fibre appearance (Heine & Hoecker, 2001:264; Vigneswaran *et al.*, 2011:9-10; Sharada *et al.*, 2014:428).

Enzymatic biopolishing is sustainably potential wet processing to upgrade and facilitate aesthetic and easier fibre spinning qualities of lignocellulosic fibres without or with limited use of harsh chemicals in the textile industry (Shanthi & Preeti, 2015:10501; Uddin, 2016:1). The biopolishing of *Agave americana L.* fibre with cellulases is expected to reduce brittleness and stiffness and promotes its affinity to readily accept dyes, as it has been the case with sisal (Manyam *et al.*, 2015:1748).

## CHAPTER 3: RESEARCH METHODOLOGY

### 3.1 INTRODUCTION

The natural fibres are prospective alternate to synthetic fibres. The ever-increasing demand for natural fibres in textiles is progressively compelling textile researchers to further research on sustainable and suitable textile fibre sources. The *Agave americana L.* leaves were harvested and extracted for investigation of the innovative and sustainable enzymatic techniques to soften *Agave americana L.* fibre for textile uses. Research design, plant sampling and harvesting, fibre extraction process, fibre biosoftening processes, fibre testing and fibre testing procedures form the basis of this chapter. Fibre extraction, biosoftening and evaluation of the physico-mechanical properties were compiled to form germane data to be statistically analysed. Enzymes were procured and a laboratory and equipment were prepared according to proposed plan. Various fibre extraction, softening and evaluation procedures were prudently observed and the sustainable and most appropriate choice was done on bases of logical and critical thinking based on literature reviewed.

Harvesting, fibre extraction and delignification experiments were conducted and reported in detail. The mechanical properties of the extracted fibre were tested before and after the enzymatic biosoftening treatment. The effects of the various enzymatic processing of *Agave americana L.* fibre was documented and the physico-mechanical properties such as colour, texture, tensile maximum load, tensile displacement at maximum load and initial Young's modulus were evaluated. The methods used were described and statistical data analysed and subsequently conclusions were drawn.

#### 3.1.1 Research Design

The research design is a methodical strategy through which the research questions or hypotheses are investigated and data collected and analysed in order to interpret the results, draw conclusions and suggest what and how to proceed with further research (Brink *et al.*, 2012:96; Maree & Pietersen, 2016:96). There are two main methods of conducting research, the qualitative and the quantitative. Qualitative research approach offers an in-depth consideration of the issue (Ivankova *et al.*, 2016:307). In this research study; quantitative research method was used in its two forms: experimental design and non-experimental design. Quantitative research method is a scientifically structured approach; with clear objectives to investigate the possible conceptual source and outcome using statistical measurements to collect and analyse data to test hypotheses or respond

to the research questions (Ivankova *et al.*, 2016:307). It is conducted with a small, specified group within a population. It assists the researcher to find a rapport amongst the variables; in order to generalise the results to the entire population (Brink *et al.*, 2012: 112; Maree & Pietersen, 2016:162).

The experimental quantitative method is the method whereby the researcher manipulates the behaviour of the independent variables to come up with the interventions by testing the hypotheses explicitly called the cause and effect questions and establishing the relationship between the independent and dependent variables to produce an evidence research (Brink *et al.*, 2012:102-103; Maree & Pietersen, 2016:171). Non-experimental method describes certain issues, explores and explains the relationship between relevant variables without manipulation of independent variables. There is neither an involvement nor experimental control conducted by the research (Brink *et al.*, 2012:112; Maree & Pietersen 2016:171).

As a result, the choice of this method was influenced by the nature of the topic and the main objectives of this study. Information pertaining to this study was gathered through review of existing literature and experimental work on inter-and- intra plant fibre variability, sustainable and eco-friendly methods of harvesting, extracting, softening and brightening Lesotho blue *Agave americana L.* fibre with the aim to determine the fibre physical and mechanical textile properties and to improve the physical properties. Since the research design is part of a research plan, the researcher adhered to all procedures in collecting data as described in this chapter. The research design consisted of the following three phases:

- Phase I is the selection of *Agave americana L.* plants; leaf harvesting; preparation of leaves for water retting and fibre extraction.
- Phase II consisted of enzyme procurement and the enzymatic biosoftening of *Agave americana L.* fibre.
- Phase III consisted of physical and mechanical evaluation of both non-biosoftened and biosoftened *Agave americana L.* fibre.

### **3.1.2 Sampling for plant leaf harvesting**

A sample is a small number of observations taken from the total number that makes up the population. The group is chosen for its characteristics that can allow the researcher to test the hypotheses and/or respond to research questions (Maree & Pietersen 2016:192). For the purposes



of this study, the researcher however, used human beings in data collection (subjective observation of the hand of the fibre due to lack of an objective method but in minimal scale. *Agave americana L.* fibre which is a natural fibre, obtained from the leaves of *Agave americana L.* plant and which is vastly available as wild plant in both urban and rural areas in Lesotho was selected for this study. In this study; intra-and-inter *Agave americana L.* plant variability in fibre physical and mechanical characteristics was explored based on experimental designs, techniques and statistical analyses. Three fleshy and mature wild *Agave americana L.* plants were selected randomly at Baruting, Sehlabeng-sa-Thuoathe, Berea, Lesotho. Six leaves were selected from each plant depending on the three different levels of development. Thus two leaves from the same level on the same plant were harvested: two mature leaves from the lower level of the plant, at an angle greater than 45 degrees to the vertical leaf cross-section stand, subjected to down-bending forces by spring balance perpendicular to the leaf axis the point of leaf bole attachment with a typical knife blade falling vertically through the cross-sections. Two leaves from the middle level of the same plant and two from the top level, denoting young leaves but not the innermost four leaves at the apex because cutting them is likely to impact negatively on the plant growth and production. The total of 18 leaves was selected for this study.

### **3.1.3 Materials and methods**

#### ***3.1.3.1 Materials and equipment***

The research work was conducted using the following materials and equipment: *Agave americana L.* plants, heavy duty knives or sickles, protective rubber hand gloves, protective eye goggles, and laboratory coat.

#### ***3.1.3.2 Safety***

Like animals which have protective mechanisms; plants have means to protect themselves. Sometimes they have sharp-edged leaves or thorns or toxins which work when ingested or even touched. *Agave americana L.* plant is an irritant plant that has leaves with both spiny margins and tips and the irritating sap that causes skin dermatitis. This has irritant properties due to its calcium oxalate crystals, oxalic acid, and saponins. The researcher wore protective clothing, including gardening eye protection, heavy duty rubber gloves, long sleeves garments and/or laboratory coat, long pants and closed shoes when harvesting and preparing *Agave americana L.* plant leaves for treatment to avoid being stung and skin irritations by the leaf sap. Hands were washed after handling the partially degraded *Agave americana L.* fibres.

### 3.1.3.3 Plant Leaf harvesting Procedure

The plant leaves were hand harvested by cutting them close to the stalk base; with a large heavy duty sharp knife and /or sickle and bundled for transport to the processing fibre extraction factory for fibre extraction.

### 3.1.3.4 The preparation and morphological parameters of the cut leaves

In the extraction plant, the marginal and terminal spikes of harvested leaves were removed.



**Figure 3.1:** Harvested *Agave americana L.* leaves ready for triangulation water retting

*Agave americana L.* leaves were manually stripped lengthwise starting from the base and ended closest to the tip but still intact towards tips, to accelerate the retting process and improve the quality of fibres, because the retting process is normally slow figure 3.2 These strips followed the lines of the fibre bundles and subsequently tapering towards the tips of the leaves. The huge full lengthened green freshly *Agave americana L.* leaves were half stripped into two ribbon sections (as illustrated in figure 3.2). The ribbons were mechanically stripped from the leaf base section toward the leaf tip but left still intact at the tip. The leaf ribbons were bent halfway lengthwise into the drums, with the base and tip sections at the bottom of the drums and the middle part of the leaves on the top most surface of the drums. The drums containing the leaves were then filled with clean tap water to the brims to immerse the leaves. The drums were then closed with lightly fitting lids and led to ret under water.



*Figure 3.2: Stripped Agave americana L. leaves*

## **3.2 FIBRE EXTRACTION**

In this research the fibre was extracted from the non-cellulosic components through water retting of *Agave americana L.* leaves in the drums; then followed by hand scraping of the cuticle, the outer layer and washing away of the pulpy biomass. The choice of appropriate method of fibre extraction is of utmost importance since it could result in plant fibre with good and required properties such as length, fineness, strength, high cellulose content, optimal value and homogeneity (Konczewicz *et al.*, 2013:118). Water retting of *Agave americana L.* leaves was considered to be an efficient and feasible way for extraction of good quality fibre which allows greater control (Zawani *et al.*, 2015:314).

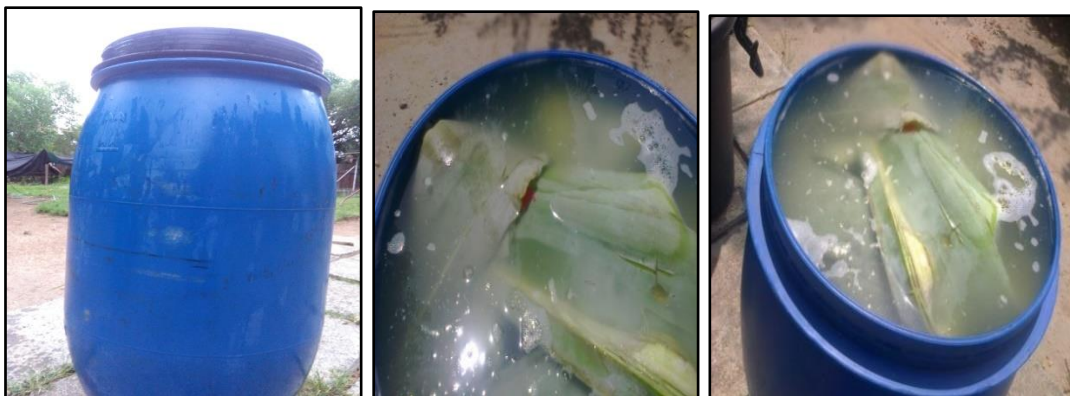
### **3.2.1 Triangulation Water Retting of *Agave americana L.* leaves**

In this research *Agave americana L.* fibres was extracted according to the water retting method conducted by Mafaesa, 2006:69-70, with some modifications; in accordance with the principles used by Sarkar & Sengupta (2015:171). The stripped leaves were divided into young, mid matured and fully matured leaves but still fleshy and fresh; sets. Three barrels were used; each barrel was used to water ret the six leaves of the same plant. The leaves were arranged vertically but bent in halves; and the base and tip sections of the leaves at the bottom and middle sections were on the surface of the barrels. Clean tap water was used to submerge the *Agave americana L.* leave in a barrel of water for up to 24 days at mostly rainy weather conditions. The length of retting time was determined by the efficiency rate of fibre production in *Agave americana L.*

leaves. This research was conducted to investigate the optimum retting for *Agave americana L.* leaves for fibre production. Retting process was frequently monitored after every two days.

The two leaves of each level from each plant were marked with coloured insulation tape to differentiate the other six leaves from each plant which were instead, vertically bent in half in an individual drum of about one hundred and twenty litres capacity. Clean cold tap water was then filled in the drum to submerge the leaves (figure 3.3). After two days, the need to low down the leaves was apparent. Flat stones were used to low down the retting *Agave americana L.* leaves in submerged condition. The submerged condition is explained by Sarkar & Sengupta (2015:171) as a strategy to avoid the production of the ferrous-tannin that can discolour the fibre. The retting drum was then closed with its lid.

*Agave americana L.* fibre can successfully be extracted by conventional water retting, from the *Agave americana L.* leaves but cause environmental pollution. Water retting is the process whereby *Agave americana L.* leaves are immersed in water for a while to rot through the chemical action of water and microbes in order to separate with ease the *Agave americana L.* fibre bundles from non-fibrous matter. Retting research section was conducted to investigate the optimum retting for *Agave americana L.* leaves and extract fibre for enzymatic biosoftening process. In this research study there were no added activators used to fast-track the retting process. Retting occurred naturally in the closed barrels.



**Figure 3.3: Triangulation water retting of *Agave americana L.* fibre in drums**

The use of a closed drum was employed as a form of tank retting method which used stagnant water because it is feasible at any season and weather of the year. It allows greater control and produces more uniform quality (Parida *et al.*, 2011:5269, Bezazi *et al.*, 2014:3). It was aimed to accelerate leaf matrix retting process which is brought about by the bacterial rapid development due to warmer temperature increased by closing the drums. Literature has confirmed the issue of

accelerating *Agave americana L.* leaves retting through the use of closed barrels (Manimekalai & Kavitha, 2017:374). The leaves were then monitored frequently: after every two days, to a day when heading towards retting completion; when the outer cuticle and waxes on the covering layer of leaves were easily removed from the leaf biomass when rubbed with individual's fingers. Periodic checking of retting *Agave americana L.* leaves was done frequently to ensure adequate retting for extraction of fibre in proper time and to avoid under retting or over retting. When the fibre slipped out with ease from the leaf biomass on pressure between the thumb and forefingers, adequate retting was achieved.

### **3.2.2 Manual decortication of *Agave americana L.* Fibre**

The leaves were then hauled out from the drums and fibre was extracted manually; one leaf at a time. The cuticle and waxes on the covering layer of leaves were then scrapped off with the knife using its blunt side. The fibre was then washed first in the retting water; (figure 3.4); it was further cleaned prudently in clean water once, to remove the non-cellulose cementing leaf biomass and other impurities. However; the non-cellulose cementing leaf biomass was not fully degraded to release the leaf fibre bundles; especially from lower and thicker parts of the leaves. The fibre bundles were then slightly pounded with a hammer (figure 3.5), to further soften and release binding components from the cellulosic leaf fibre.



*Figure 3.4: Agave americana L. fibre washed in the retting water which formed a high foam*





*Figure 3.5: Agave americana L. fibre lightly hammered to degrade the binding components*

The fibres were then washed with clean water twice and then hanged vertically on line and air dried for six hours (figure 3.6) to dry. The bundles fibre was collected for further investigation.



*Figure 3.6: Extracted Agave americana L. fibre hung to air dry*

### **3.3 ENZYMATIC BIOSOFTENING OF AGAVE AMERICANA L. FIBRE**

#### **3.3.1 Design of fibre biosoftening Experiments**

The enzymatic biosoftening of the lignocellulosic polymer fibres is currently among the most substantial; eco-friendly alternatives to non-environmental friendly harsh chemical modification methods. It is more innocuous and more advantageous when compared to its counterpart chemical technique for the fact that enzymes are specific to substrate(s) to catalyse, have colossal reaction rates, function under milder reaction conditions and leniently transform the surface lignocellulosic polymer fibres (Kalia *et al.*, 2013:101). The *Agave americana L.* fibre was extracted from *Agave americana L.* plants obtained and harvested from Sehlabeng-sa-Thuoathe (Thuoathe plateau)

Berea, Lesotho. The *Agave americana L.* fibre was cleaned by hand to remove impurities, such as debris and weighed on a sensitive balance scale. Three (3) grams *Agave americana L.* fibre was first boiled for 15 minutes (figure 3.7); before enzymatic biosoftening. After pre-treatment time the fibre samples were rinsed with distilled water. The commercial lignocellulolytic enzymes and other chemicals were bought from Sigma-Aldrich and Merck companies. The enzymes were employed in individual and sequence combinations. The following enzymatic biosoftening experiments were conducted: biodelignification, bioscouring, biobleaching and biopolishing.

**Table 3.1: Enzymatic biosoftening processes and conditions**

<b>Bioprocess</b>	<b>Enzyme type</b>	<b>pH</b>	<b>Temperature (°C)</b>	<b>Treatment time (hours.)</b>
Biodelignification	Manganese peroxidase	8.7	50	72
Bioscouring	Pectinase	8.5	55	1
Biobleaching	Xylanase	8.5	60	8
Biopolishing	Cellulase	8.5	50	2

The processing parameters such as temperature in degree Celsius, time in minutes and hours and enzyme concentration in percentages were chosen and adapted based on, literature review and amounts available. The experiments were conducted in laboratory scale. After each enzymatic biosoftening processes, the fibre was dried.

### **3.3.2 Materials**

- *Agave americana L.* fibre extracted from *Agave americana L.* plant leaves through triangulation water retting.
- Lignocellulolytic enzymes:
  - Manganese peroxidase was obtained from *Phanerochaete chrysosporium*, procured from Sigma-Aldrich; Germany and and it was intended to delignify the fibre.
  - Pectinase was obtained from *Rhizopus* species, procured from Sigma-Aldrich; Germany and it was intended to bioscour the fibre.
  - Xylanase obtained from *Trichoderma viride*, procured from Sigma-Aldrich; Germany, and it was intended to biobleach the fibre and



- Cellulase obtained from *Trichoderma viride*, procured from Merck New York USA; and it was intended to biopolish the fibre.
- Water (tap and distilled)
- Chemicals: Tris (0.2 M), Hydrogen chloride (HCl; 0.2 M), Manganese sulphate (MnSO<sub>4</sub>), Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>; 2 mM), Glycine (0.2M) and Sodium hydroxide (NaOH; 0.2 M)

### 3.3.3 Tris – HCl buffer preparation

The Tris amounting to 24.2 g was dissolved in 1 litre distilled water. The 19.76 ml HCl (0.2 M) was measured and distilled water was added into HCl to fill up 1 litre. A mixture was shaken to mix well. The 5 ml HCl solution was added to Tris 50 ml solution. The distilled water was added into Tri-HCl solution to fill up 200 ml. MnSo<sub>4</sub> of 0.089g was dissolved in 1 ml of 0.5% albumin and 0,014 ml = 140 microlitre H<sub>2</sub>O<sub>2</sub>. (2 mM). MnSo<sub>4</sub> solution and Tris-HCl solution were mixed to complete Tris-HCl buffer.

### 3.3.4 Sodium hydroxide (NaOH)-glycine buffer preparation

The NaOH (0.2 M) of 8g was dissolved in 1 litre distilled water. The glycine of 15g (0.2 M) was dissolved in 1 litre distilled water. Glycine solution of 50 ml and 8.8 ml NaOH solution were mixed and distilled water was added to fill up to 200 ml. Glycine solution amounting to, 250 ml and 44 ml NaOH solution were mixed in 1 litre beaker and with distilled water was poured in to make 1 litre ready to use buffer.

### 3.3.5 Preliminary experimental procedures

#### 3.3.5.1 Individual enzymatic biosoftening of *Agave americana L. fibre*

The three grams of *Agave americana L.* fibre was weighed and boiled for fifteen minutes (figure 3.7) to wet the sample. Manganese Peroxidase enzyme was weighed to 0.002g. Tris–HCl buffer (pH 8.7) of 22.5 ml was measured. The enzyme-buffer solution was prepared. The boiled wet fibre and enzyme-buffer solution were added in 250 ml conical beaker which was shaken to mix all the reagents. The fibre was then incubated for 72 hours (3 days) at 50°C bath water. During incubation the beaker was shaken at 12 hours intervals to distribute mix and wet the fibres. At the end of processing time, the enzyme was deactivated by boiling the fibre in water for 10 minutes. The enzyme treated fibre sample was then washed thoroughly with tap water and rinsed once with distilled water. The fibre was dried at room temperature for 12 hours and then re-weighed.



*Figure 3.7: Agave americana L. fibre boiled in water as a pre-treatment process*

### **3.3.5.2 Bioscouring with pectinase enzyme**

Three grams of *Agave americana L.* fibre was boiled for 15 minutes in a cooking pan to wet it. Pectinase enzyme weighing 0.33g was dissolved in 60 ml sodium hydroxide (NaOH)-glycine buffer. The fibre was put into 250 ml conical beaker and enzyme-buffer solution was poured over the fibre. The beaker was shaken and fibre was then incubated at the controlled temperature of 50°C for 60 minutes in water bath (figure 3.8). The beaker was shaken at 20 minutes intervals. After the treatment time the enzyme was deactivated by boiling in water for 10 minutes, then washed twice with cold tap water and finally with cold distilled water. The fibre was dried at room temperature for 12 hours and then re-weighed.

### **3.3.5.3 Enzymatic biobleaching with xylanase enzyme**

Three grams raw *Agave americana L.* fibre was boiled for 15 minutes. Xylanase enzyme of 0.01g was dissolved in 60 ml NaOH-glycine buffer. The measured enzyme was dissolved in measured buffer to make enzyme-buffer solution. The fibre was placed into the 250 ml conical beaker and the enzyme-buffer solution was poured over the fibre, shaken and incubated in a water bath at the optimal temperature (60°C) (figure 3.8) for 8 hours. During incubation; beaker contents were shaken after every 2 hours. After the incubation time the fibre was boiled in water (figure 3.8) for 10 minutes to inactivate the bleaching enzyme, then washed with cold tap water twice and finally rinsed with clean distilled water, dried at room temperature for 12 hours and re-weighed.



**Figure 3.8: Enzymatic biosoftening of *Agave americana L.* fibre incubated in water bath**

#### **3.3.5.4 Biopolishing of *Agave americana L.* fibres with cellulase**

Three grams of *Agave americana L.* fibre was weighed and boiled in water for 15 minutes. The boiled fibre was put in to a 250 ml conical flask. The 0.005g commercial cellulase was dissolved in 60 ml NaOH-glycine buffer. The enzyme–buffer solution was poured into the beaker over the fibre. The beaker was then shaken and fibre was then incubated at controlled 50°C temperature in water bath for two hours with occasional shaking at 30 minutes intervals. The enzyme activity was deactivated after treatment time by boiling the fibre in water for 10 minutes. The enzyme treated samples were then removed from the source of heat and washed twice with cold tap water, and subsequently with distilled water. The fibre was air-dried for 12 hours at room temperature and then re-weighed.

#### **3.3.6 Sequential enzymatic biosoftening of *Agave americana L.* fibre**

The 3 grams of *Agave americana L.* fibre was weighed and boiled for 15 minutes. Manganese peroxidase of 0.002 g was dissolved in 22.5 ml of Tris–HCl buffer (pH 8.7). The fibre and enzyme-buffer solution were added in 250 ml conical beaker which was shaken to dissolve the enzyme and mix the reagents. The beaker was covered with para-film paper and foil (figure 3.9) to prevent enzyme–buffer solution to evaporate. Weight lids were put on top of the foil. The fibre was then incubated for 72 hours at controlled temperature of 50°C in a water bath. During incubation the beaker was shaken at 12 hours intervals. At the end of incubation time the enzyme was deactivated by boiling the sample in water for 10 minutes. The sample was then washed twice in cold tap water and rinsed once with distilled water and dried for 12 hours.

The dried fibre sample was boiled for 15 minutes. The boiled fibre sample was then put into 250 ml conical beaker. The 0.33 g pectinase was dissolved in 60ml NaOH-glycine buffer The enzyme-

buffer solution was poured over the fibre and shaken. The beaker was covered with para film paper, foil and the weight lids. The fibre in the beaker was then incubated in bath water set at 50°C for 60 minutes. The beaker was shaken for 30 seconds at 20 minutes intervals. After incubation time the sample was boiled in water for 10 minutes to deactivate the enzyme activity, then washed twice with cold tap water and finally with cold distilled water. The wet fibre sample was put into the 250 ml conical glass beaker. The 0.01g xylanase enzyme was dissolved in 60 ml NaOH-glycine buffer of pH 8.5. The enzyme-buffer solution was poured over the fibre, shaken and incubated water bath at 50°C for 8 hours. During incubation the sample was shaken after every 2 hours. After incubation time the fibre was boiled in water for 10 minutes to deactivate the enzyme, then washed with cold tap water twice and finally rinsed with clean distilled water.

The wet fibre was put into a 250 ml beaker. The 0.005 g cellulase was dissolved in 60 ml NaOH-glycine buffer. The enzyme –buffer solution was poured over the fibre to immerse it. The beaker was then shaken to distribute the solution over the fibre. The fibre was then incubated at 50°C in the water bath for 2 hours. The sample was shaken after every 30 minutes. When the incubation time was over, the sample was boiled for 10 min to deactivate the enzyme. The sample was then washed twice in cold tap water, and subsequently by distilled water. The fibre was then air-dried for 12 hours at room temperature and then re-weighed.

### **3.3.7 Research experimental procedures**

There were lessons learned during preliminary testing which led to some changes in research experimental procedure: some sample containers in water bath tilted upside down and disturbed the experiment. The water evaporation was too high because it was not easy to cover the bath so became a challenge during the night. These force a change from water bath to convection oven (figure 3.9). The enzyme concentrations were also found inadequate hence they were increased in the experimental procedures. The experimental design was still the same as the preliminary testing.



*Figure 3.9: Outside feature of a convection oven used for incubation of *Agave americana* L. fibre*

### ***3.3.7.1 Individual enzymatic biosoftening of *Agave americana* L. fibres***

Tris–HCl and Sodium hydroxide (NaOH)-glycine buffers were prepared as per preliminary experiments.

### ***3.3.7.2 Biodelignification with manganese peroxidase***

The three grams of *Agave americana* L. fibre was weighed and boiled for 15 minutes. Manganese Peroxidase weighing 0.02g was dissolved in 22.5 ml of Tris–HCl buffer (pH 8.7) to make enzyme-buffer solution. The boiled wet fibre and enzyme-buffer solution were added respectively in 250 ml conical beaker which was then shaken to mix all the reagents. The beaker was covered with para-film paper and foil (as illustrated in figure 3.10) to prevent enzyme –buffer solution to evaporate. Weight lids were also put on top of the foil. The fibre was then incubated for 72 hours (3 days) at controlled temperature of 50°C in a convection oven (figure 3.11).



*Figure 3.10: *Agave americana* L. fibre with enzyme solution before incubated in the convection oven*

During incubation the beaker was shaken after every 12 hours. At the end of the treatment time the enzyme was deactivated by boiling the sample in water for 10 minutes and then washed twice in cold tap water and rinsed once with distilled water and dried. The fibre was then re-weighed.

#### **3.3.7.3 Bioscouring with pectinase**

Three grams of *Agave americana L.* fibre and 0.5g pectinase enzyme were weighed. The fibre was boiled for 15 minutes. The enzyme was then dissolved in 60 ml sodium hydroxide (NaOH)-glycine buffer. The fibre was put into 250 ml glass beaker and enzyme-buffer solution was poured over the fibre. The contents were shaken to further and the beaker was then covered with para film paper and the foil. Weight lids were put on top of the foil. The sample was then incubated in convection oven at the temperature of 50°C for 60 minutes. During incubation the sample was shaken at 20 minutes intervals. After the enzyme treatment time the fibre was boiled in water for 10 minutes, then washed twice successively with cold tap water and finally with cold distilled water and then, dried for 12 hours at room temperature. The fibre was then re-weighed.

#### **3.3.7.4 Biobleaching with xylanase**

Three grams of raw *Agave americana L.* fibre water boiled for 15 minutes. Xylanase enzyme of 0.1g was dissolved in 60 ml NaOH-glycine buffer of (pH 8.5). The enzyme-buffer solution was made. The fibre was placed into the 250 ml glass beaker and the enzyme-buffer solution was poured over the fibre. The contents of the beaker were shaken to distribute the reagents evenly. The beaker was then covered with para film paper and the foil to prevent enzyme–buffer solution to evaporate. Weight lids were also put on top of the foil. The fibre was then incubated for 8 hours in convection oven at the optimum temperature of 50°C. During incubation the beaker was shaken after every 2 hours. After 8 hours Incubation fibre was boiled in water for 10 minutes and, then washed with cold tap water twice and finally rinsed with clean distilled water and dried for 12 hours; at room temperature. The fibre was then re-weighed.





*Figure 3.11: Agave americana L. fibre incubated in the convection oven*

### **3.3.7.5 Biopolishing with cellulase**

The 3g fibre was boiled in water for 15 minutes and put in a 250 ml conical beaker. In enzyme the biopolishing solution was prepared by dissolving cellulase enzyme weighing 0.01g in 60ml NaOH-glycine buffer. The enzyme–buffer solution was poured into the beaker over the sample and contents were then shaken. The sample was then incubated at 50°C in the convection oven for 2 hours with occasional shaking after every 30 minutes. The enzyme activity was deactivated after treatment by boiling the sample in water for 10 minutes and washed twice in cold tap water, and subsequently by distilled water. The fibre was then air-dried for 12 hour and then re-weighed.

#### **(a) Sequential enzymatic biosoftening of *Agave americana L.* fibre**

There were three sequential enzymatic biosoftening combinations conducted in this section: pectinase and xylanase, pectinase, xylanase and cellulase and MnP, pectinase, xylanase and cellulase.

#### **(b) The pectinase and xylanase sequential biosoftening**

The procedure for an individual pectinase biosoftening 3.9.6.1.2 above was first followed but the fibre sample was never dried and re-weighed. The xylanase biosoftening procedure 3.9.6.1.3 was then pursued but omitting first two steps of the weighing and boiling the fibre sample.

#### **(c) The pectinase, xylanase and cellulase sequential biosoftening**

The procedure for sequential pectinase and xylanase biosoftening 3.9.6.2.1 above was first followed but the fibre sample was never dried and re-weighed. The cellulase biosoftening



procedure 3.9.6.1.4 was then pursued but omitting first two steps of the weighing and boiling the fibre as well.

(d) MnP, pectinase, xylanase and cellulase sequential biosoftening

The procedure for an individual manganese peroxidase biodelignification (3.9.6.1.1.) above was followed. Subsequently, the pectinase, xylanase and cellulase sequential biosoftening procedure (3.9.6.2.3.) was followed including the second steps of boiling the fibre for 15 minutes.

### **3.3.8 Weight loss percentage (WL%) computation**

At the end of each enzymatic biosoftening process the weight loss of each sample was calculated with the difference between the fibre before and after bioprocessing. The weight loss percentage ( $W_L$ ) was computed; as per the equation (1):  $WL\% = W_1 - W_2 \div W_1 \times 100$ . Where,  $W_1$  was the weight of fibre samples before bioprocessing and  $W_2$  was the weight of the fibre samples after bioprocessing.

## **3.4 BOILING WATER PRE-TREATMENT OF *AGAVE AMERICANA L.* FIBRE**

### **3.4.1 Preliminary water boiling pre-treatment**

The *Agave americana L.* fibre was cleaned by hand to remove impurities, such as debris and weighed on a sensitive balance scale. The five grams *Agave americana L.* fibre was first boiled as per the above pre-treatment for enzymatic biosoftening but time was extended to 30 minutes. After that the fibre was washed twice in cold tap water and subsequently by distilled water. The fibre was then air-dried for 12 hour and re-weighed.

### **3.4.2 Experimental water boiling pre-treatment**

The experimental water boiling pre-treatment was conducted the same way as for preliminary test but the fibre weight was reduced to three grams. There were two sets of pre-treatment conducted a 30 minutes set and a one hour set.

### **3.4.3 Weight loss percentage (WL%) computation**

At the end of the water boiling process the weight loss of each sample was calculated with the difference between the fibre before and after processing. The weight loss percentage ( $W_L$ ) was

computed; as per the equation (1):  $W_L\% = W_1 - W_2 \div W_1 \times 100$ . Where,  $W_1$  was the weight of fibre samples before processing and  $W_2$  was the weight of the fibre samples after processing.

### **3.5 THE AGAVE AMERICANA L. FIBRE EVALUATION**

Knowing the physical properties of *Agave americana L.* fibre assists individuals to grasp its maximum potential in textiles. The physical properties of *Agave americana L.* fibre such as defects, strength, fibre dimensions, variability, texture, colour and structure can affect fibre performance properties and it is imperative to analyse them in order to attain its full potential importance as textile fibre. The *Agave americana L.* fibre quality and grading depends on many parameters including fibre length and diameter, tensile strength and elongation, fibre softness and stiffness, cleanliness, colour, lustre to mention a few. Since there is no standardized fibre grading system currently in use in Lesotho, to determine fibre quality, the general two methods of fibre evaluation namely, the instrument and visual-and-hand evaluation (Das *et al.*, 2013:755), were used for *Agave americana L.* fibre exploration for potential textile use. Microscopy observations, tensile strength, texture, colour and brightness indexes, fibre weight loss were conducted on raw *Agave americana L.* fibre and bioprocessed *Agave americana L.* fibre to both qualitatively and quantitatively determined the nature of changes on the fibre surfaces caused by the enzymatic biosoftening.

#### **3.5.1 Fibre evaluation general design**

Evaluation of *Agave americana L.* fibre was conducted according to methods described by Uddin (2015:2-3) with some modification. For the fibre samples to reach moisture balance, they were weighed and conditioned for the experimental treatments at  $21 \pm 2^\circ\text{C}$  temperatures and  $65 \pm 2\%$  relative humidity to warrant accurateness and dependability of results.

#### **3.5.2 Instrumental Evaluation**

Fibre testing instruments are used to evaluate the essential fibre physico-mechanical properties that determine fibre quality. The instrumental evaluation is more accurate and unbiased for fibre quality. To reduce personal errors that are due to subjective judgement of fibre quality it is advisable to use, instrumental method. Instruments such as Instron tensile tester, scanning electron microscope (SEM), light microscope and Shirley stiffness tester were used to test *Agave americana L.* fibre tensile properties and surface characteristics. The physico-mechanical

properties of the raw, water boiled and enzyme biosoftened *Agave americana L.* fibre were tested and analysed.

### **3.5.3 The fibre tensile testing method**

Naidu *et al.* (2017:2084) explained the mechanical properties as ways by which a composite fibre indicates its capacity to endure forces that pull it apart and how much it can stretch before its failure. The tensile tests of raw (untreated), water boiled and enzymatic biosoftened of *Agave americana L.* fibre samples of which each was comprised of 12 fibres; were performed with Instron universal automated material testing machine type 4200/4300/4400 as per the standards specifications of American Standard Testing and Measurement (ASTM) D 3822-01) method. Tensile test was conducted at a ramp rate of 20.00 kN/min and the data rate of 6.667 pts/sec. Each tensile specimen was placed in the Instron universal tester and then the tensile load was applied and the computer generated tensile stress-strain graph was automatically plotted, as the specimen stretched. The required parameters were then recorded as individual and average values; until the specimen ruptured. The following mechanical properties of *Agave americana L.* fibre were tested: the tensile strength in the form of maximum load (N), elongation displacement at maximum load mm and Young's modulus (MPa) and specific work of rupture.

### **3.5.4 Scanning electron microscopic (SEM) morphological evaluation**

The SEM was used to assess the morphological modifications of *Agave americana L.* fibre. Lengthwise views indicated the surface morphology and size of untreated (raw) and enzyme biosoftened *Agave americana L.* fibres. The morphology of the *Agave americana L.* fibre before and after enzymatic biosoftening were conducted.

#### **3.5.4.1 SEM evaluation equipment**

The SEM, Microscope slides, permanent marker, slide plates or containers, cover glasses, water in a small container, mineral oil and its container, sharp tweezers, eye dropper, sharp small scissors, notepad, pen or pencil.

#### **3.5.4.2 SEM evaluation procedure: Longitudinal view**

The *Agave americana L.* fibre samples to be tested were mounted on the microscope slide which has been lubricated with one drop of water and one drop of mineral oil. Water facilitates easier observation of the surface features; mineral oil improves the internal observation of the fibres. The pair of tweezers was used to centre the fibre bundles on a glass slide, which is free of foreign

bodies including other fibres. With the tweezers tips fibre bundles were separated in such a way that each does not come in contact or overlap with other fibre bundles. A drop of mounting medium was applied on the slide. Microscope cover glass was carefully placed over fibres. The sample was then assessed under the Scanning Electron Microscope (SEM).

#### **3.5.4.3 Light microscopic evaluation: transverse view with plate method**

##### (a) Light microscopic evaluation materials and equipment

Light microscopic evaluation materials and equipment: microscopic slide, flat two holed microscopic plate of equal size and shape of a microscopic slide, a tuft of *Agave americana L.* fibre to test, coloured acetate thread, nylon pull through thread, microscopic slides, microscopic cover glasses, a sharp razor blade, microscopic slide containers, water in a small container or mineral oil in the container, sharp tweezers, eye dropper, sharp small scissors, notepad, pen or pencil.

##### (b) Light microscopic evaluation procedure

To identify the transverse view of the *Agave americana L.* fibre; a small holed perforated metal plate from Shirley Institute kit which is of the same size as a microscope slide was used. The tightly packed tuft of coloured acetate fibre was used for the background and one or two *Agave americana L.* fibre samples for test was made. The tuft was then looped with a strong nylon thread and carefully pulled through into a hole in the metal plate with a loop. The tuft was then cut off with care on top and bottom of the plate, using the sharp razor blade ensuring that a tuft slice is safely retained in the hole subsequent to cutting. The slide plate was then lubricated with a drop of oil and protected with the cover slide and the cross-sectional images were examined with light microscope (Nikon) fitted with camera Nikon DS-fi1 10x enlargement –optip hot-2 and photographed to evaluate the cross-section, and the micrographs (AATCC, 1990:53).

#### **3.5.4.4 Bending length evaluation with Shirley Stiffness Tester**

##### (a) Hypothesis

A conditioned; individual *Agave americana L.* fibre sample was placed on a horizontal platform on Shirley stiffness tester in such a way that it bent downwards like a cantilever until it was reflected. The Shirley stiffness tester has a platform that works with two plastic pieces there is a mirror attached on the side of the tester which facilitates the operator to conveniently observe both

index lines. The tester's scale is graduated in centimetres of bending length of maximum 8 cm which can also be used as a cutting template

(b) Equipment and materials

Shirley Stiffness Tester, scissors, measuring ruler and tape, 30 cm loose single *Agave americana* L. fibre as samples.

(c) Experimental conditions and test procedure

The experiment was conducted under  $\pm 21^{\circ}\text{C}$  atmospheric temperature and  $\pm 65$  relative humidity. The bending length of raw, boiled and selectively enzyme biosoftened *Agave americana* L. fibre was measured using Shirley stiffness tester in accordance with BS 3356:1990. The *Agave americana* L. fibre was first straightened. A single fibre was positioned between a fixed horizontal platform and a mobile grip that was steadily moved forward in a manner that allows the fibre to hang over like a cantilever and bent downwards to produce an ideal bending behaviour. Then, the bending length was instantly read off from the scale mark opposite a zero line carved on the side of the platform. Each fibre was measured eight times: four times, from each end (Base end and tip end) in different directions, and their mean values were calculated and analysed.

### **3.5.5 Visual-and-hand evaluation**

Professional and non-professional evaluators can evaluate the physical characteristics such as fibre fineness, softness, smoothness, colour, lustre and many more through visual-and –hand evaluation. However, the visual-and-hand evaluation method is bias and results analysed with it greatly differ as per individual evaluator (Das *et al.*, 2013:755) but can provide useful information where instrumental tests are not available.

#### ***3.5.5.1 Visual-and-hand evaluation experiments***

The visual-and-hand properties are usually determined by a panel of assessors to come up with reliable results that can be measured. The visual-and-hand evaluation is a subjective assessment. The answers must be converted to numerical values and data be analysed statistically to find relationship between objective measurements (Hurren *et al.*, 2002:5, Kayseri *et al.*, 2012: 247). The following physical *Agave americana* L. fibre properties; biosoftening rate, fineness, softness, smoothness, lustre, colour, density, uniformity, flexibility and suitability for textile use were assessed with the visual and hand evaluation method adapted from Hurren *et al.* (2002:4), Kayseri

*et al.* (2012: 247). Thirty-five fibre samples were arranged in rows on the tables and numerically labelled 1 to 35. All were compared to a control raw sample of the fibre obtained from the lower leaves that was labelled 0. Ranking values on a scale of 1-5 were assigned to the alternative descriptions of each property. A panel of 11 Consumer Science workers and students was asked to look, touch and assign to each anonymous but labelled fibre sample; a number on a scale of 1 to 5 that corresponds with a feature that best described the fibre property to be selected (Table 3.2).

**Table 3.2: The visual and hand evaluated *Agave americana* L. fibre physical properties**

<b>Ranking</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Fibre property</b>	<b>(most satisfying property)</b>	<b>(Satisfying Property)</b>	<b>(neutral)</b>	<b>(Not satisfying)</b>	<b>(Not at all satisfying)</b>
Biosoftening rate	Over-softened	Softened	Average	Under-softened	Extremely under-softened
Fineness	Very fine	Fine	Average	Thick	Very thick
Softness	Very soft	Soft	Average	Harsh	Very harsh
Smoothness	Very smooth	Smooth	Average	coarse	Very coarse
Lustre	Very shiny	Shiny	Average	Dull	Very dull
Colour	White	Off-white	Greenish	Brown	Dark brown
Density	Very light	Light	Medium heavy	Heavy	Very heavy
Flexibility	Very flexible	Flexible	Average	Stiff	Very stiff
Uniformity	Very even	Even	Average	Uneven	Extremely uneven
Suitability for textile use	Most suitable	Suitable	Average	Unsuitable	Most unsuitable

## CHAPTER 4: DISCUSSION, ANALYSIS AND INTERPRETATION OF RESULTS

### 4.1 INTRODUCTION

The aim of this research study was to extract fibre from the *Agave americana L.* leaves with triangulation water retting, biosoften the fibre with lignocellulolytic enzymes and investigate the fibre physico-mechanical properties and variability of raw, water boiled and enzymatic biosoftened fibre. The enzymatic biosoftening removed some lignin, hemicellulose and cementing non-cellulosic fibre impurities. However, this treatment reduced the fibre diameter and tensile strength.

The wild *Agave americana L.* fibre was harvested and extracted, enzyme biosoftened and tested as aggregated fibre bundles for textile fibre prerequisite. The physico-mechanical properties of the untreated (raw), boiled and enzyme bio-softened fibre were tested under the standard conditions. Improvised closed tank retting was found faster and more environment-friendly than open conventional retting. Literature shows that *Agave americana L.* fibre is a natural, long and strong, but stiff, coarse and hard-surfaced, lignocellulosic fibre (Mafaesa, 2006:101,105 & 126, Hulle *et al.*, 2015:69). The *Agave americana L.* plant is an emergent; alternate source of fibre that has potential to become a sustainable textile fibre due to its physico-mechanical properties and its abundance and organic origin.

*Agave americana L.* fibre exists as fibre bundles consisting of numerous single micro-fibrils referred to as ultimate fibres or just ultimates. *Agave americana L.* fibre is lengthy with different sized diameter which is determined by a number of ultimates across the section. The *Agave americana L.* fibre is approximately cylindrical in shape. It is lengthy and relatively thick, irregular, narrowing to a point, with one thicker side; that is from the base side of the leaf. The enzymatically biosoftened *Agave americana L.* fibre has improved textile qualities when compared to untreated *Agave americana L.* fibre. Nonetheless, it has reduced strength. The overall aim of this research is to explore the potential sustainable extraction, viability, enzymatic biosoftening and textile physico-mechanical properties of *Agave americana L.* fibre, in order to add value, diversify its end uses and increase textile fibre security in Lesotho.



## 4.2 TRIANGULATION WATER RETTING OF *AGAVE AMERICANA L.* FIBRE

Triangulation water retting of *Agave americana L.* leaves is an innovative variation of water retting fibre extraction method. It is a multifaceted retting process that involves a number of theoretical, methodical, and empirical perspectives to overcome the inadequacies of conventional whole *Agave americana L.* leaves open water retting (Nøkleby 2011:147, Ndanu. & Syombua, 2015:47, Honorene 2017:92). It facilitates an innovative, improved, fast-tracked removal of non-cellulosic constituents of *Agave americana L.* leaf biomass through degradation catalysis with enzymes produced naturally by aquatic microbes (Devi *et al.*, 2017:53). Retting is a preliminary; essential step forward in fibre extraction process; that determines the rate of natural plant fibres production efficiency (Omenna *et al.*, 2016:275). Water retting is a natural biological process of engaging the microbial action by immersing the *Agave americana L.* leaves in water for some weeks to dissolve and/or rot away the cementing non-cellulosic leaf components to facilitate ease separation of the *Agave americana L.* fibre without damaging (Hulle *et al.*, 2015c:67).

### 4.2.1 Retting reactions and morphological changes of *Agave americana L.* leaves



**Figure 4.1: Retting process started with leaching and a gaseous fermentation**

The triangulation water retting of *Agave americana L.* fibre was accomplished by submerging the harvested; lengthwise stripped leaves in water in the closed drums. Closing of drums was intended to accelerate the fermentation process to separate with ease the cellulosic fibre from non-fibrous leaf biomass. The retting of *Agave americana L.* fibre observed analogous to retting of other plant fibres that include banana pseudo-stem fibre; as it occurred in two consecutive stages (Subagyó & Chafidz, 2018:5). The first physical stage; was recognized as the stage where leaf biomass absorbed water and got swollen and easily released the fibre soluble constituents into the water which was no longer clear but cloudy (figure 4.1). Sarkar & Sengupta, (2015:171) described the water colour change situation to be a result of the process called leaching; whereby dirt and colouring matter from the leaves are absorbed into water to ensure fibre cleanliness. The

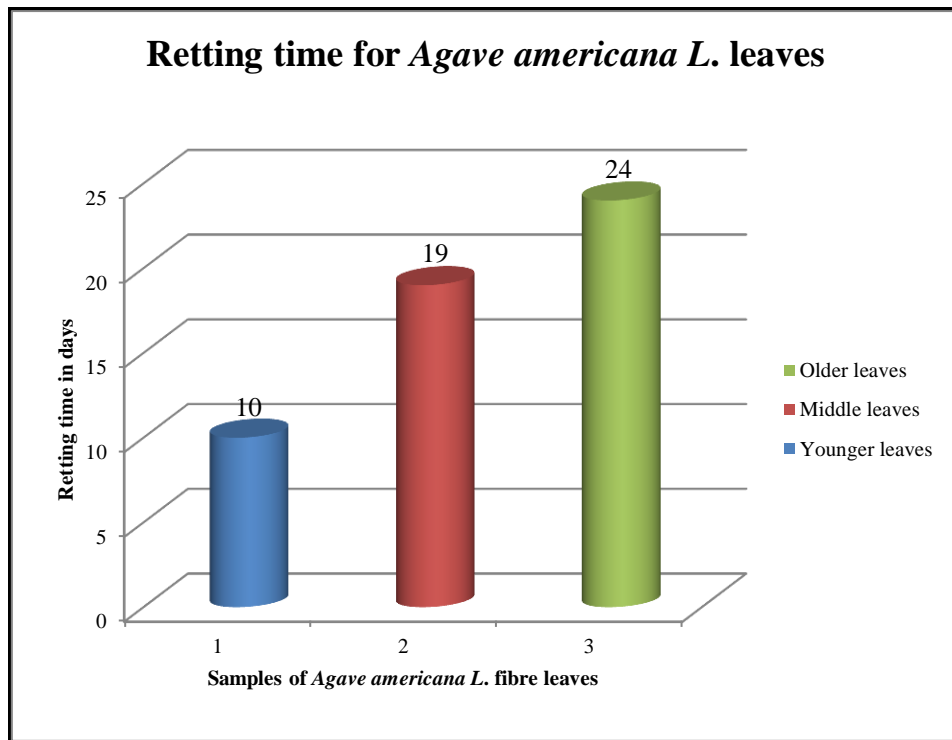
subsequent stage is microbial development and activities. The natural microbes which can either be aerobic or anaerobic were thought to have developed, and produced the gas bubbles as evidenced from figure 4.1. Das *et al.* (2015:43), described the gas bubbles formed, as due to, various organic acids and gases formed from microbial biochemical activities which end up in turbid medium.

It was also observed that the cuticle (outer layer of the leaf) becomes soft and a number of substances are solubilized in the water and the water gradually became turbid with the progression of gas formations and the subsequent, lathering bubbles (figure 4.1) and ever-increasing pungent alcoholic odour developed. This is an indication that microbes acted upon non-fibrous leaf biomass and resulted in fermentation that occurs because of the presence of the pectin from the middle lamella of the plant cell wall which gently softens to provide food for microbial growth (Gowda, 2007:25).

Di Candilo *et al.* (2009:195) and Das *et al.* (2014:272-273), described this situation as transitory water pollution that is due to gradual increase of microbial load and gradual depletion of O<sub>2</sub> levels which eventually result in an anaerobic milieu for the anaerobic bacteria to develop. However, retting products and by-products are non-toxic and are entirely decomposable. As retting progressed towards the end the rate of the evolution of gases and the consequent frothing slowed down and the water becomes clear with less pungent smell. Das *et al.* (2015:43), assumed that the condition was an indicative; that retting reached completion. At this stage, the non-fibrous biomass was softened and retted leaves were removed from retting water for fibre separation. Sarkar & Sengupta (2015:171), claimed that good timing and judgment are important for best results. Under-retted leaves could end up with the fibre that was not adequately softened to separate with ease from the other leaf biomass contents. Over-retting, could also be detrimental to the fibre quality and quantity; since microbes can continue to break down the plant tissues including the fibre bundles consequently negatively affecting the fibre strength.

#### **4.2.2 Retting Duration**

Eighteen *Agave americana L.* fibre leaves of the length range of 1.50 to 2.00 meters were harvested from three plants and water retted in 3 polythene drums for more or less three weeks. Figure 4.2 presents retting time required in days for the *Agave americana L.* fibre leaves against their level of maturity which is determined by their position in the parent plant.



**Figure 4.2: Retting time for younger, medium & older *Agave americana L.* fibre leaves**

After ten days of retting; the youngest leaves were adequately retted; with the cuticle easy to remove and the fibre mesh was easier and faster to remove from the fibre than that of older plant leaves which were under-retted and difficult to separate fibre from the biomass mesh by then. This could be explained as the age factor: the fact that the older leaves; would have accumulated more of lignin by then and as the plant leaves grow older, the fleshy tissues turn to be highly matured; as the leaves mature the cell wall structural matters become more abundant and resistant to decompose (Das *et al.*, 2014:272; Sarkar & Sengupta, 2015:171). Thus, it takes longer time for microorganisms to ret the leaves. The retting process of the middle-aged leaves was completed after 19 days. The retting process of the older, lower leaves was completed after 24 days. The varying retting times are likely due to the fact that the older leaves; would have accumulated more of lignin, and other cementing fibre components such as hemicelluloses and pectin.

The retting time could have been shortened more when compared to the sizes and number of leaves retted simultaneously in a drum. The depth of the drums is believed to have contributed to lengthening the retting time especially for the base and tip sections of the leaves. Hence, why fibre obtained from the sections deeper than 35 cm, under the surface of retting water, was not easy to extract and had rough surface texture which is likely due to adhesive leaf components debris. The retting time for *Agave americana L.* fibre leaves was comparable to that of most plant fibres like jute, kenaf without any added additional activators; regardless of the leaves' volume during which

retting occurred. It is reasonable therefore to think that triangulation retting techniques used for retting the *Agave americana L.* leaves improved the speed with which it happened. Majumdar *et al.* (2013:26), reported that the retting process is delayed if retting temperature deviates from the maximum aforementioned temperature ranges of 34-36°C, on either side and during the heavy rainfall time due to abrupt fall of retting water temperature.

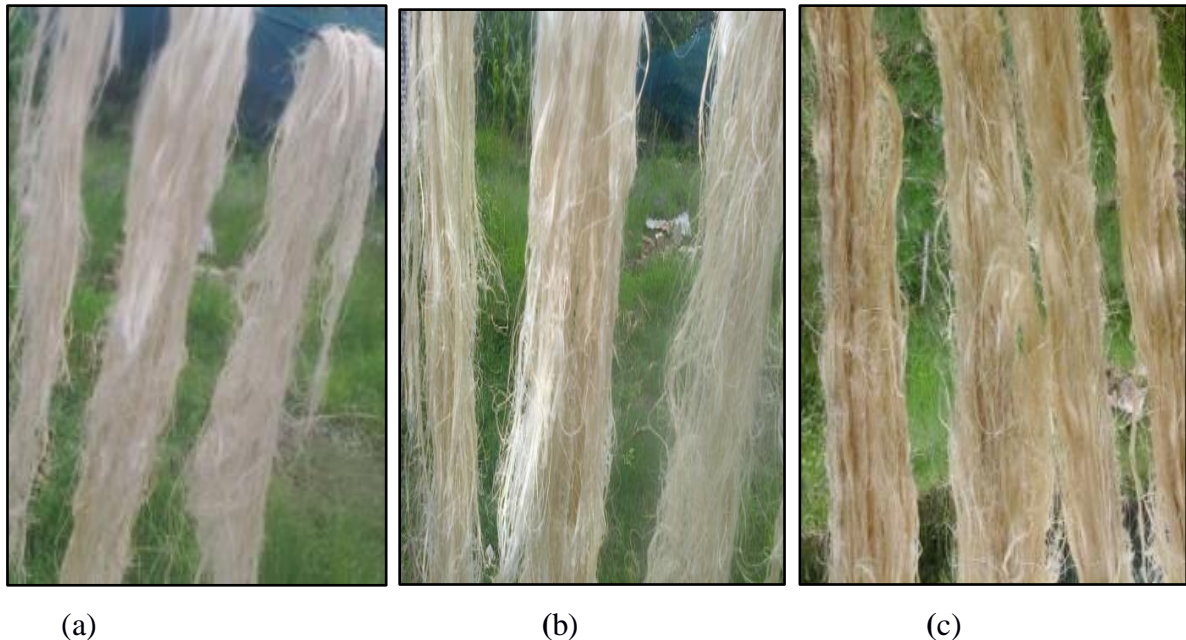
#### **4.2.3 Impact of triangulation water retting on the environment**

In traditional open, whole leaf water retting process, the huge compact *Agave americana L.* leaf undergoes decomposition very slowly and causes pollution around environment. It further imposes health hazard to fibre extractors. The triangulation water retting used in this research study naturally hastened the decomposition of the leaves by cutting and stripping the individual leaves and using insulating drums with tight fitting lids. It controlled the fermentation odour and environmental pollution. Health hazard for fibre extractors and researcher was reduced. Triangulation water retting with closed tanks controlled the volume of water used by reducing water evaporation. It also occurred successfully under rainy weather conditions. The retting and washing water as well as the leaves' non-cellulosic biomass constituents were buried in the garden to improve soil fertility because non-cellulosic biomass constituents are biodegradable. Triangulation water retting of *Agave americana L.* in this study; reduced and transformed the three forms of pollution: land, air and water because it caused non-pollution to running water bodies, inhibited greenhouse gases emissions and produced potential renewable manure and energy producing by-products that can provide employment and livelihood. It was observed that the triangulation water retting of *Agave americana L.* leaves loosened and allowed easy separation of fibre bundles from leaf biomass through removal of various cementing tissue components and partial degradation of adhesive constituents between the fibrils in the fibre bundles.

#### **4.2.4 Fibre quality in terms of colour**

The triangulation water retted *Agave americana L.* fibre produced in this study was generally more lustrous and brighter in colour than fibre obtained through conventional open retting (Mafaesa, 2006:91). The degree of colour lightness decreases proportionally with the increasing age of the leaves, which are indicated by the size and position of the leaves. Fibre obtained from the younger leaves; at the top of the plant was whiter than fibre obtained from the middle leaves which was also brighter than fibre obtained from the lower leaves which are older than the rest (figure 4.3). The older leaves produced fibre faint green coloration when wet, due to the large amounts of chlorophyll developed on the cuticle as plant leaves grow older. The faint green turns

to light brown upon drying (figure 4.3 (c)) due to oxidation. The leaves that floated above water level during retting process produced dark brown discoloured fibre. Figure 4.3 displays the *Agave americana L.* fibre extracted from leaves of different age and position on the *Agave americana L.* plant.



**Figure 4.3: Retted *Agave americana L.* fibre extracted from the (a) younger (b) middle and (c) older leaves of *Agave americana L.* plant**

#### **4.2.5 Fibre quality in terms of texture**

Based on preliminary visual and hand evaluation results, the *Agave americana L.* fibre produced in this study looked and felt softer and finer than conventional water retted fibre as reported in literature and previous research (Mafaesa, 2006:113). The fibre obtained from the older leaves was coarser, than that from the younger leaves because of larger amounts of lignin developed as plant leaves grow older. At some areas the *Agave americana L.* fibre was still held together by undissolved and under-composed gummy substances and caused sticky fibres which were not properly cleaned after fibre extraction. These were areas which experienced imperfect retting which resulted in gummy fibre. The gummy fibre was glued together by undissolved adhesive matters and produced low quality fibre with rough and hard top ends. These conditions diminished the value and quantity of useful fibre. It is difficult to determine the exact extraction time as longer extraction might have improved these but over-ret some of them.

#### **4.2.6 Fibre variability**

The great intra-and-inter plant fibre variability was observed. *Agave americana L.* fibre was not equal in length, strength, thickness, colour and texture within the same plant at different levels, in the same leaf levels and even in the same leaf. The fibre variability within different plant leaves was also obvious. The growth development of natural fibres are normally affected by numerous and varying factors such as the plant maturity, harvesting time, difference plant pattern formation of cellulose unit, availability of plant nutrients and plant metabolic rate, weather conditions and many more factors influencing the natural rate of plant cell division (Kalia *et al.*, 2013:98). The retting efficiency and the production of quality *Agave americana L.* fibre depends on several factors but two were observable: age size/thickness and depth of retting water.

Natural fibres suffer size and growth variability which is normally brought about by plant maturity during harvest time. Proper harvesting stage yields good quality fibres. The diversity in the fibre properties of different plants might be due to the variance in the pattern of formation of cellulose unit in all the plants. The cellulose is a basic unit of natural plants, but the amount of cellulose formed and the location of cellulose in the plant vary from one species to another. The formation of cellulose may be the result of discrepancies in growth rate, instigated by metabolic reactions, quality of nutrition provided, weather and growing conditions or other essential factors for cell division and development in natural fibres, thus, the growth of natural fibre are responsible for their properties (Bunsell, 2009: 83; Msahli *et al.*, 2015:2).

#### **4.3 THE AGAVE AMERICANA L. FIBRE DESIGNATIONS AND THEIR MEANINGS**

The *Agave americana L.* fibre designations used in this research study are operational and must be explained in order to reduce ambiguity and increases clarity. They are high-quality communication signs that simplify the meanings employed, and thus can become safety, quality, productivity and dissemination factors on their own right. The *Agave americana L.* fibre designation meanings indicate the fibre concepts and methodologies of fibre of interest for high-quality, effective understanding demonstration and communication. Table 4.3 provides the *Agave americana L.* fibre designations used in this research and their meanings.



**Table 4.1: The *Agave americana* L. fibre designations and their meanings**

<b>Fibre designation</b>	<b>Meaning</b>
P 1 Y	fibre extracted from top; younger leaves of plant 1
P 1 M	fibre extracted from middle leaves of plant 1
P 1 L	fibre extracted from the lower; older leaves of plant 1
P 2 Y	fibre extracted from top; younger leaves of plant 2
P 2 M	fibre extracted from middle leaves of plant 2
P 2 L	fibre extracted from the lower; older leaves of plant 2
P 3 Y	fibre extracted from top; younger leaves of plant 3
P 3 M	fibre extracted from middle leaves of plant 3
P 3 L	fibre extracted from the lower; older leaves of plant 3
P 1 YR	Raw fibre extracted from top leaves of plant 1
P 2 MR	Raw fibre obtained from middle leaves of plant 2
P 2 MRT	Raw fibre obtained from tip part of middle leaves of plant 2
P 2 MRM	Raw fibre extracted from middle part of middle leaves of plant 2
P 2 MRB	Raw fibre extracted from base part of middle leaves of plant 2
P 3 LR	Raw fibre extracted from lower leaves of plant 3
P 3 MRT	Raw fibre obtained from tip part of middle leaves of plant 3
P 3 MRM	Raw fibre extracted from middle part of middle leaves of plant 3
P 3 MRB	Raw fibre extracted from base part of middle leaves of plant 3
P 1 MB	water boiled fibre extracted from middle leaves of plant 1
P 2 MB	water boiled fibre extracted from middle leaves of plant 2
P 3 MB	water boiled fibre extracted from middle leaves of plant 3
P 2 M MnP	Manganese peroxidase delignified fibre from middle leaves of plant 2
P 1 LP	Pectinase bioscoured fibre extracted from lower leaves of plant 1
P 3 MC	Cellulase biopolished fibre extracted from middle leaves of plant 3
P 1 YX	Xylanase biobleached fibre extracted from top leaves of plant 1
P 2 YX	Xylanase biobleached fibre extracted from top leaves of plant 2
P 3 YX	Xylanase biobleached fibre extracted from top leaves of plant 3
P 1 YTE	Sequential pectinase, xylanase and cellulase biosoftened fibre extracted from top (young) leaves of plant 1
P 2 YTE	Sequential pectinase, xylanase and cellulase biosoftened fibre from extracted top (young) leaves of plant 2
P 3 YTE	Sequential pectinase, xylanase and cellulase biosoftened fibre extracted from top (young) leaves of plant 3



<b>Fibre designation</b>	<b>Meaning</b>
P 1 MFE	Sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre extracted from middle leaves of plant 1
P 2 MFE	Sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre extracted from middle leaves of plant 2
P 3 MFE	Sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre extracted from middle leaves of plant 3

#### **4.4 BIOSOFTENING OF AGAVE AMERICANA L. FIBRE**

To avoid adverse effects when using enzymes to biosoften the *Agave americana L.* fibre proper selection of lignocellulolytic enzymes, enzyme concentration, treatment time, temperature pH and mechanical agitation was done to convey a good soft hand. Enzymatic biosoftening is an eco-friendly, socially and economically sustainable biotechnology to soften the *Agave americana L.* fibre by the selective removal of fibre components that include lignin and other non-cellulosic ones, using specific commercial lignocellulolytic enzymes to yielded a good quality fibre. It is an attractive and safe alternative to chemical technology which is harmful to the environment and workers. Enzymatic biosoftening has improved the *Agave americana L.* fibre handle and visual properties. It has been proved that it imparts satisfactory physico-mechanical properties to the fibres within a short processing time. The results generally indicate that manganese peroxidase (MnP), pectinase, xylanase and cellulase have positive impact on biosoftening of *Agave americana L.* fibre.

##### **4.4.1 Weight loss percentage**

###### **4.4.1.1 Preliminary test weight loss percentage**

The *Agave americana L.* fibre lost an incredible amount of non-cellulosic biomass that include lignin, pectin, fats, oils and waxes and to some extent the cellulose component as well as other impurities during biosoftening. This fibre weight loss was used to evaluate the biosoftening and water boiling effects. The efficiency of the enzymatic biosoftening process on *Agave americana L.* fibre, is indicated by weight loss percentage. Weight loss percentage has been used to indicate the amount of non-cellulosic materials which have been removed during enzymatic biosoftening of *Agave americana L.* fibre (Suganya, 2018:129). The weight loss of manganese peroxidase (MnP), pectinase, xylanase and cellulase enzyme treated and water boiled (control) *Agave americana L.* fibre for the preliminary experiment is shown in table 4.2.

**Table 4.2: Weight loss percentages of the preliminary enzymatic biosoftening of *Agave americana L.* fibre**

<b>Fibre designation</b>	<b>Biosoftening process</b>	<b>Enzyme or agent used</b>	<b>Initial weight (gm.)</b>	<b>Time in hours.</b>	<b>Final weight (gm.)</b>	<b>percentage Weight loss (%)</b>
P 2 M	biodelignification	MnP	3	72	2.62	12.67
P 3 M	bioscouring	pectinase	3	1	2.62	12.67
P 1 M	biobleaching	Xylanase	3	8	2.59	13.67
P 3 M	biopolishing	cellulase	3	2	2.76	8
P 2 M	All bioprocesses	All enzymes	3	8	2.55	15
P 1 M	boiling	water	5	1/2	4.82	3.6
P 3 M	boiling	water	5	1/2	4.69	6.2

From Table 4.2, it is observed that weight loss percentage obtained from the individual enzyme treatment ranges from 8% to 13.67%. The sequential MnP, pectinase, xylanase and cellulase treated fibre instigated 15% weight loss percentage. The weight loss percentage difference is probable; since the enzymes function on specific substrates. For example, MnP hydrolyses lignin, pectinase removes pectin, xylanase hydrolyses mainly xylan hemicellulose and cellulase hydrolyses cellulose, leaving other substances unaffected. This could also have been affected by fibre composition. It is also observed that sequential enzymatic weight loss is 1.33% higher than the highest individual weight loss. When considering a number of treatment processes, variety of enzyme and time taken to treat the fibre; the weight loss percentage is not exceedingly high. This too; could have been due to enzyme-substrate specificity.

The enzymatic biosoftening efficacy on *Agave americana L.* fibre obtained from different leaves of different levels of maturity and different plants can be explained in part by non-cellulosic impurities amount expressed by weight loss percentage, the degree of polymerization, processing time, treatment temperature, biosoftening processes, type of enzyme, fibre structure degree and type of fibre structure crystallization (Kalia *et al.*, 2013:99 & 105). Fibre weight loss occurred due to enzymes catalysis that cleave fibre surface. The age of the fibre, the type and concentration of enzyme and the biosoftening processes used are also likely to have highly influenced the fibre weight loss (Suganya, 2018:127). The results indicate that there is a strong correlation between treatment time and weight loss, as well as treatment enzyme concentration and weight loss. Eventually biosoftening of *Agave americana L.* fibre improved its textile performance quality standards and appearance.

#### **4.4.2 Experimental weight loss percentage**

Weight loss represents the quantity of non-cellulosic impurities removed from the *Agave americana L.* fibre during boiling pre-treatment and enzymatic biosoftening (Ali *et al.*, 2015:1485). The aim of this section is to determine the weight loss percentage after enzymatic biosoftening of *Agave americana L.* fibre with increased enzyme concentrations and the use of oven instead of water bath to maintain the required temperature. The experimental weight loss percentages of manganese peroxidase (MnP), pectinase, xylanase and cellulase enzyme treated *Agave americana L.* fibre are shown in figures 4.4-4.10.

##### ***4.4.2.1 Individual experimental weight loss percentage***

###### **(a) Experimental weight loss percentage of MnP biodelignified fibre**

The noncellulosic structural impurities present in the raw *Agave americana L.* fibre are hemicellulose, pectin, lignin, fats and waxes (Hulle *et al.*, 2015c:72). These have specific activity towards various constituents of the fibre. Consequently, an individual enzyme cannot impart complete fibre cleanliness even if it provides an adequate weight loss in the requisite range. This is because a single enzyme cannot remove all non-cellulosic components evenly from the raw fibre, because enzymes are specific to one or two substrates (Shroff *et al.*, 2016:524). To investigate the enzymatic biodelignification of *Agave americana L.*, fibre; the commercial MnP, was used individually. The weight loss of the fibre sample was measured because it represents the amount of the noncellulosic components removed from the fibre. Delignification is the process through which the lignin from the natural lignocellulosic fibre is removed either with industrial chemical or natural enzymatic processes (Rafak & Benerjee, 2015:75281). Figure 4.4 shows fibre weight loss percentage of MnP biodelignified *Agave americana L.* fibre.

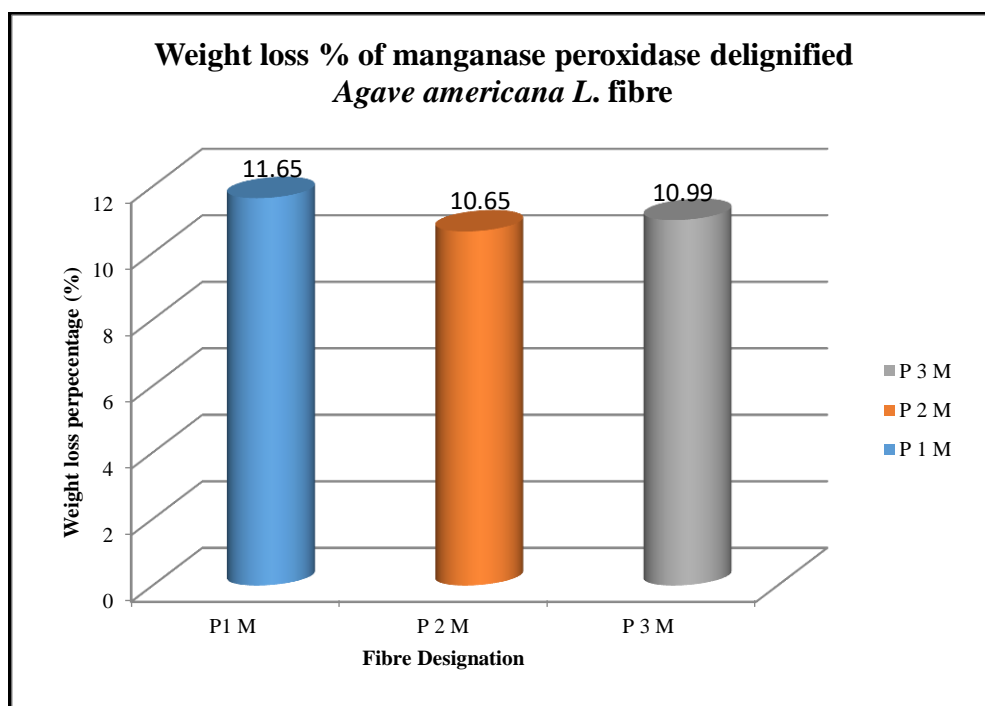


Figure 4.4: Weight loss percentage for MnP delignification of fibre obtained from middle leaves of plant 1, 2 and 3

The weight loss experienced with manganese peroxidase delignification varied for the three different fibre samples extracted from three different plants. It ranged between 10.65% and 11.65%. However, the difference is not too large to worry about. *Agave americana L.* fibre is a lingo-cellulosic fibre that contains 5-17 % lignin which covers the fibre surface (Hulle *et al.*, 2015c:72); that leads to inherent and undesirable fibre stiffness, roughness, dullness and darkness. These undesirable features contribute to fibre limited applications in textiles (Yang *et al.*, 2011:377). The lignin must therefore be partly removed to impart fibre flexibility; smoothness and lustre (Hulle *et al.*, 2015c:68). But it should be partly removed because it also contributes positively in the fibre tensile properties.

(b) Weight loss percentage of pectinase bioscoured fibre

The *Agave americana L.* fibre was bioscoured with pectinase which removed the insoluble deposit of microfibrillar debris consists mainly of pectin to soften the fibre. Pectin is extremely complicated and plentiful cell wall heteropolysaccharide found in the middle lamella that regulates intercellular linkage. Its degradation and removal was essential for biosoftening and upgrading the *Agave americana L.* fibre (Akin, 2013:7). Figure 4.5 shows weight loss percentage of pectinase bioscoured *Agave americana L.* fibre.

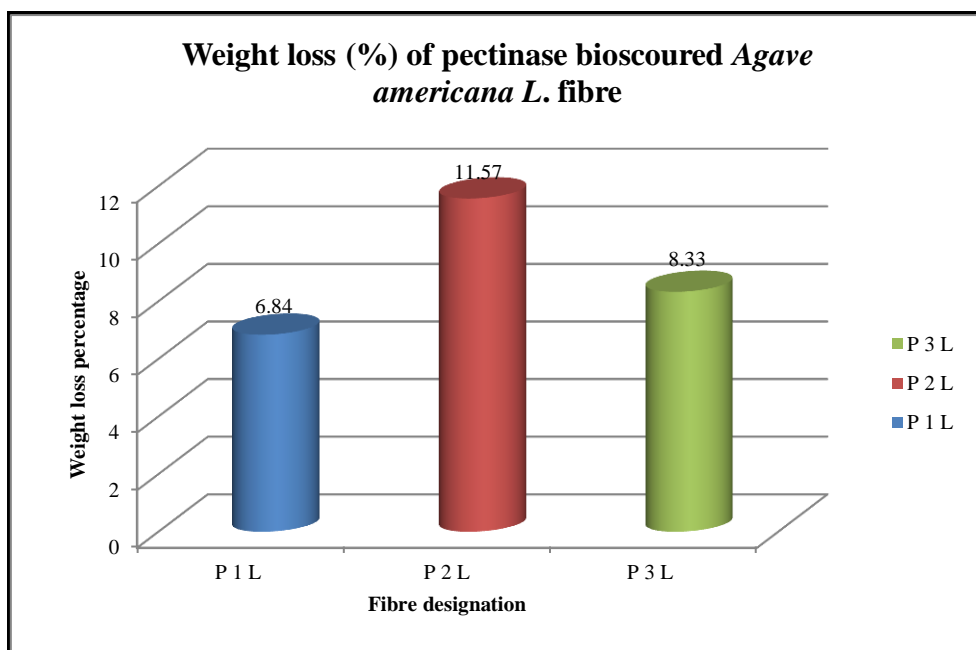


Figure 4.5: Weight loss percentage for pectinase scoured fibre obtained from the older leaves of plant 1, 2 and 3

The scouring of *Agave americana L.* fibre was aimed at removing dirt and impurities as a means of softening the fibre. When examining the pectinase bioscoured *Agave americana L.* fibre; the weight loss percentage ranged from 6.84 % to 11.57 %. There was a substantial weight loss percentage difference between P 2 L, P 1 L and P 3 L fibre that have a difference, but not as great as it is with the former fibre also treated with pectinase. This can probably be caused by the different plant age, growing conditions, genetic makeup of fibre plant, retting and extraction processes (Akin, 2013:7-8). Pectinase bioscouring of *Agave americana L.* fibre facilitated partial removal of the cuticle components by catalysing pectin which led to lowered fibre weight but to the extent that is not harmful to the fibre and environment.

After biosoftening with enzymes the fibre surface becomes ‘cleaner’ and smoother than when controlled. This could have been due to removal of the natural impurities that include pectin, fats, waxes, and the surface impurities. The pectinolytic bioscouring of the fibre removed the non-cellulosic components that are in this case fibre impurities and purified the fibre surface and increased its softness and fibre flexibility. The fibre pectin is largely generated in primary cell walls and middle lamella of the plant cell (Ochoa-Villarreal *et al.*, 2012:67). This means the removal of pectin does not negatively affect the fibre secondary cell wall structure and its physico-mechanical properties beyond limits.

Xylan is the form of hemicellulose that is widely distributed in the cell walls of the plant cell. The hemicellulose is the second most plentiful renewable polysaccharide in the biosphere. Its presence

in the plant fibre structure contributes to fibre decolouration. Xylanase enzyme is mostly used to hydrolyse hemicelluloses mostly xylan in the lignocellulosic fibre bundles to modify its structure and bleach it to improve its whiteness (De Prez *et al.*, 2018:4). Figure 4.6 presents weight loss percentage of xylanase bleached *Agave americana L.* fibre.

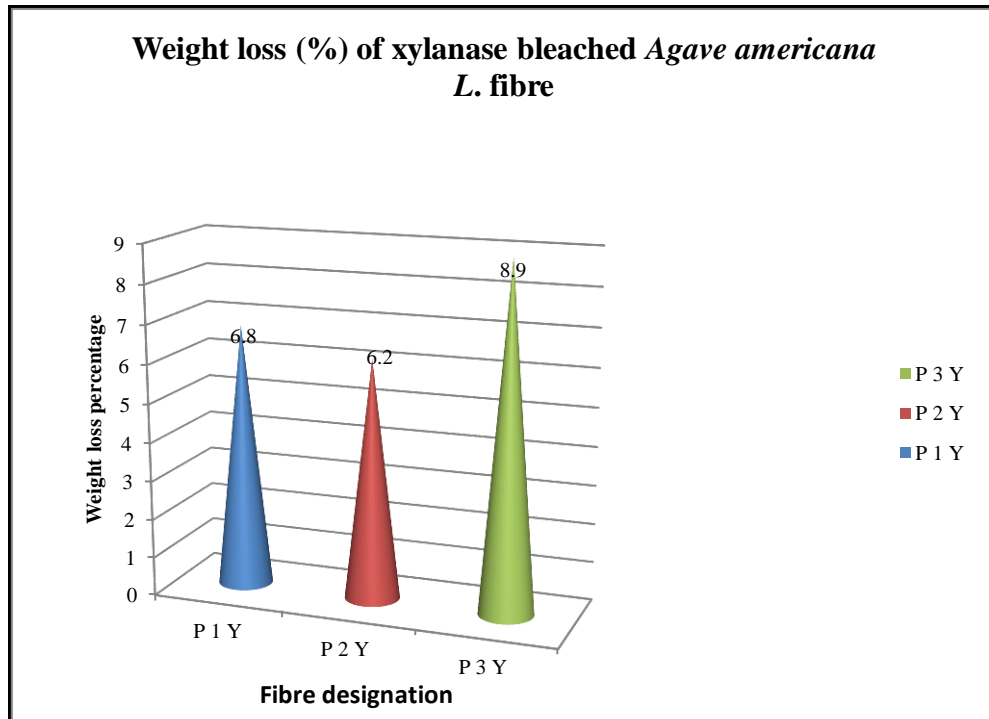


Figure 4.6: Weight loss percentage for xylanase bleached fibre obtained from the top leaves of *Agave americana L.* plant 1, 2 and 3

The weight loss percentage of the young *Agave americana L.* fibre extracted from different plants softened by xylanase ranged from 6.2% to 8.9%. Weight loss as the result of the xylanase bleaching process was lower than with the other enzymes. This can also be explained by enzyme-substrate specificity towards fibre chemical composition. Hemicellulose which is a substrate for xylanase (Narkpiban *et al.*, 2019:101) is the lowest constituent after cellulose and lignin in the *Agave americana L.* fibre: cellulose 68-80%, lignin 5-17% and hemicellulose 15% (Hulle *et al.*, 2015c:72). The other fibre constituents such as cellulose, lignin and pectin were not hydrolysed by the xylanase enzyme, hence a low weight loss percentage was observed. The efficiency of impurity elimination is usually analysed by weight loss and improved whiteness.

(c) Weight loss percentage of cellulase biopolished *Agave americana L.* fibre

Cellulase bio-polishing of lignocellulosic fibre confers calmer and softer feel and brighter luminous colour. The commercial cellulase biopolishing of *Agave americana L.* fibre was

conducted to remove loose surface fibrils and rendered the fibre clean and smooth. Figure 4.7 displays cellulase biopolished *Agave americana L.* fibre weight loss percentage.

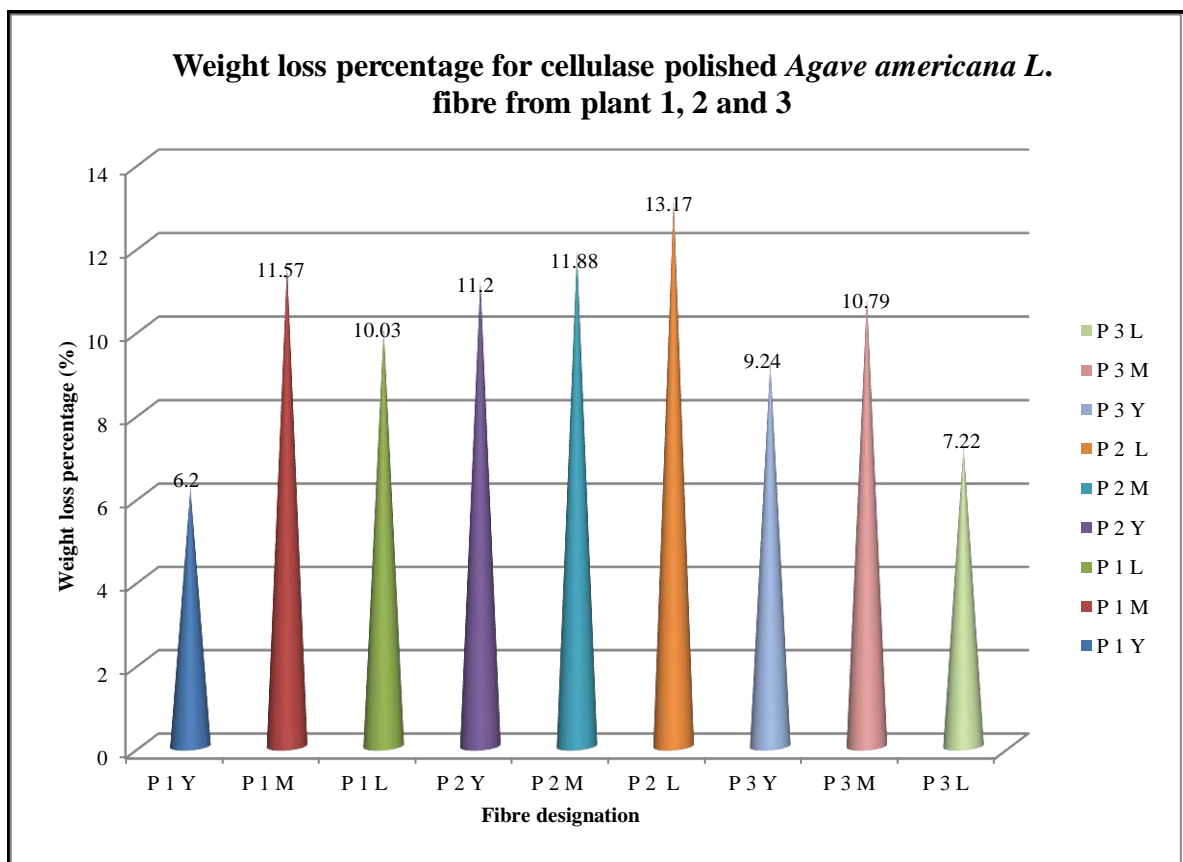


Figure 4.7: Weight loss (%) of cellulase polished *Agave americana L.* fibre obtained from younger, middle and older leaves of plant 1, 2 and 3

The weight loss as a result of the cellulase treatment varies widely between 6.2 % and 13.17 %. More often above 10 % than below which represents a high weight loss. The cellulase did remove more than expected, probably; because it was applied with intention to biopolish the fibre not to hydrolyse it because it would affect fibre qualities negatively. Low weight loss observed in individual enzymatic biosoftening was probable since the enzymes are substrate specific. For example, manganese peroxidase hydrolyses lignin only; pectinase removes pectin and cellulase functions on cellulose and leave the rest unaffected. When comparing weight loss percentages for individual enzyme biosoftening effects from figures 4.6 to 4.7; it is observed that maximum weight loss was obtained from cellulase treated fibre, followed by manganese peroxidase, the pectinase, and the xylanase respectively.

- (d) Weight loss percentage of sequential enzymatic biosoftening processes of *Agave americana L.* fibre



Sequential enzymatic biosoftening processes of *Agave americana L.* fibre were also conducted to maximize the efficiency of enzyme-substrate specificity to the desired fibre product.

#### 4.4.2.2 Sequential experimental weight loss percentage

(a) Sequential pectinase and xylanase biosoftened *Agave americana L.* fibre

Figure 4.7 shows the weight loss percentage of sequential pectinase bioscouring and xylanase biobleaching of *Agave americana L.* fibre conducted respectively.

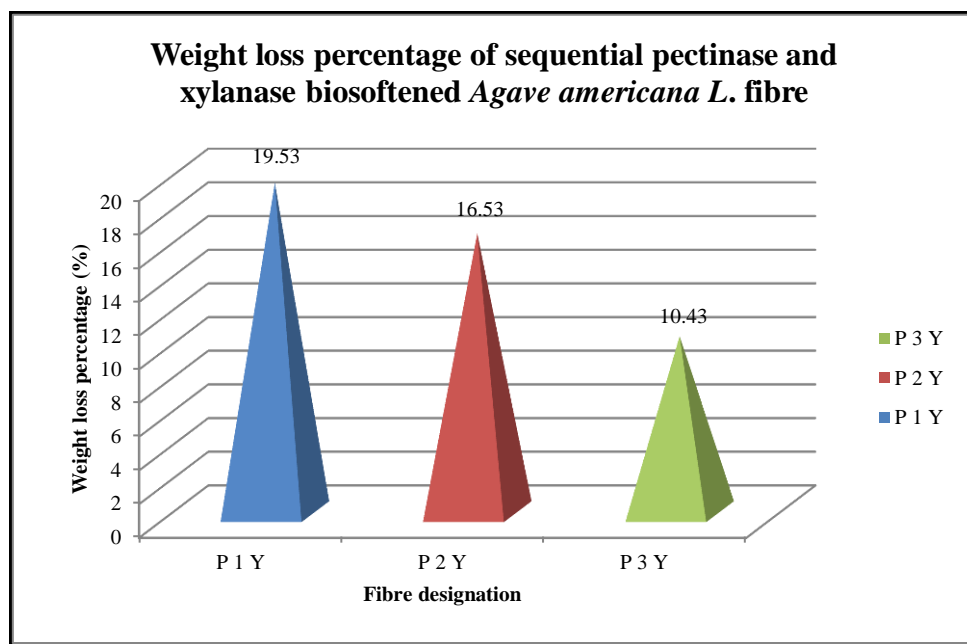


Figure 4.8: Weight loss percentage for sequential biosoftening of young fibre extracted from plant 1, 2 and 3 using pectinase and xylanase respectively

The weight loss between the different plants here is very big. It is possible that the younger leaves of different plants could be of different age even though it is young for the specific plant. It is obvious that the sequential effect is less than the individual effects added up to which indicate to me that at least partly the different enzymes remove the same substances but the sub-sequential effect will eventually be the most successful.

(b) Sequential pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre

Enzyme technology advances in textiles have eased the possibilities of exploring the potential effects of using enzymes various ways (Mojsov, 2011:233-234). Pectinase, xylanase and cellulase enzymes have been used individually but in respective sequence to biosoftened *Agave americana*

*L. fibre*. Figure 4.9 shows the weight loss percentage of sequential pectinase bioscouring, xylanase biobleaching and cellulase biopolishing of *Agave americana L.* fibre conducted respectively.

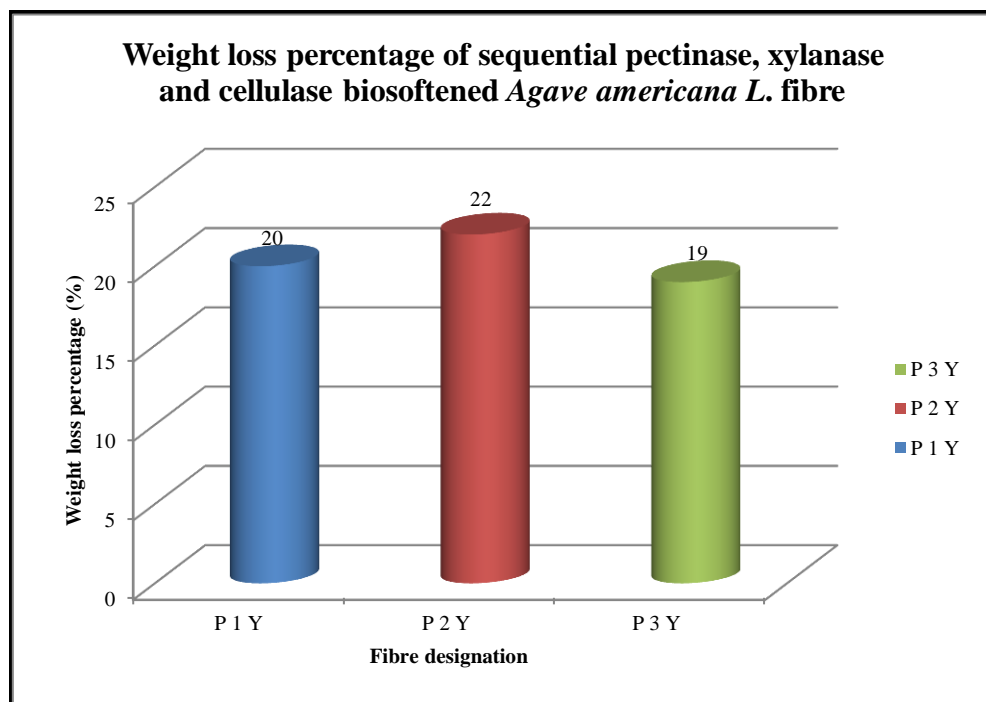
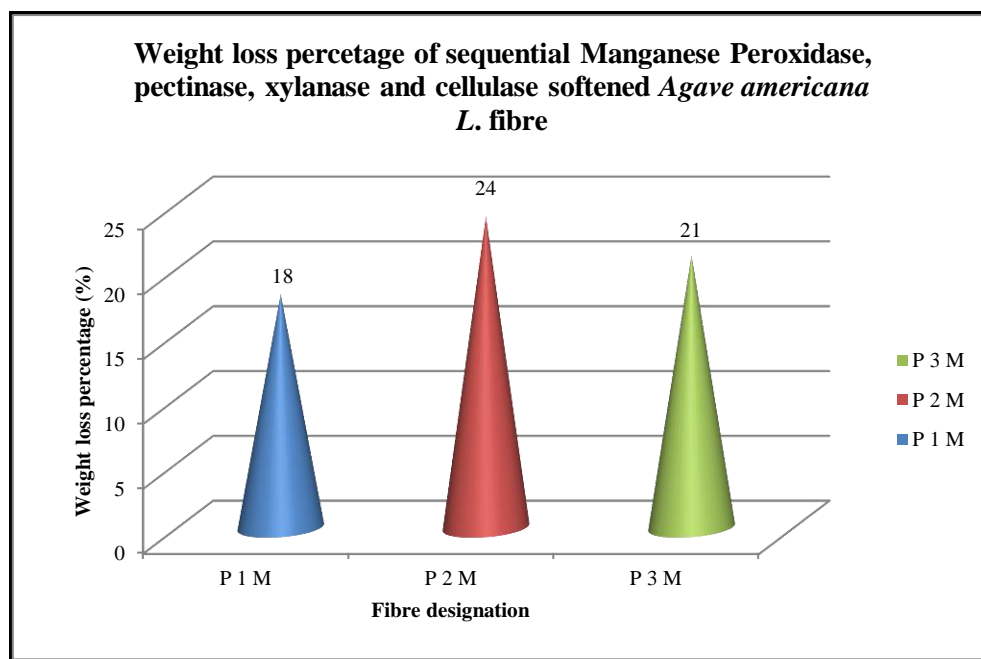


Figure 4.9: Weight loss percentage of sequential pectinase, xylanase and cellulase respectively

The fibre weight loss percentage observed shows that the enzymes used biosoftened the fibre through removing the significant amount of non-fibrous constituents in form of pectin, waxes, and other contaminations.

(c) Sequential MnP, xylanase, pectinase and cellulase biosoftened *Agave americana L.* fibre

The *Agave americana L.* fibre is a tough lignocellulosic fibre (Hulle *et al.*, 2015c:72) that was biosoftened and upgraded through the use of lignocellulolytic enzymes. The following lignocellulases: manganese peroxidase, pectinase, xylanase and cellulase were explored in respective sequence to biosoften *Agave americana L.* fibre. Figure 4.10 illustrates the weight-loss percentage of the sequential MnP, xylanase, pectinase and cellulase biosoftened *Agave americana L.* fibre.



**Figure 4.10: Weight loss percentage for sequential biosoftening of different fibre using manganese peroxidase, pectinase, xylanase and cellulase respectively**

The weight loss due to sequential biosoftening of *Agave americana L* fibre ranged from 18 to 24%. Maximum weight loss percentage of 24% was observed from the fibre obtained from plant 2 and then followed by 21% of the fibre harvested from plant 3 and the least 18 % of the fibre from plant 1. The sequential enzyme treatments have led to the highest weight loss percentage of 24 %. This weight loss percentage shows that enzymes used biosoftened substantial quantities of *Agave americana L*. fibre components that include lignin, hemicelluloses, pectin, waxes and other impurities. The weight loss percentage results show a strong correlation between treatment time and weight loss, as well as treatment intensity (due to MnP, delignification, pectinase scouring, xylanase bleaching and cellulase polishing respectively) and weight loss. They indicate that there was a significant enzymatic biosoftening effect on *Agave americana L*. fibre instigated by the selected lignocellulolytic enzymes.

When analysing the weight loss percentage values; the sequential enzymatic biosoftening of *Agave americana L*. fibre seems to be excessive but that may not be the case. According to Uddin (2010:18), the weight loss of 3-6% is commercially acceptable. Fontana *et al.*, (2014:14) suggested that the weight loss percentage of cotton fibre should not exceed 3-5%. Cotton fibre is cellulosic because it is of nearly 90% cellulose, it is the purest natural plant fibre consists; with fewer contents of impurities such as pectinous substances, fats and waxes, proteinous matter, ash and minutes others (Hsieh, 2007:3).

While *Agave americana L.* fibre is the lignocellulosic fibre; consist of 68-80% cellulose (Hulle *et al.*, 2015c:72). This implies that there are more impurities to remove from *Agave americana L.* fibre to render the fibre soft enough to provide best results. It is reasonable to think than the *Agave americana L.* fibre weight loss percentage should be more than 3-6% because *Agave americana L.* fibre has higher impurity content. Otherwise it is reasonable to think that the fibre was not clean enough after retting and decortication. From the previous preparation steps traces of surface impurities remained and increased the initial weight of the fibre measured. Weight loss percentage increased with increase in processing time because for manganese peroxidase delignification ran for 72 hours, pectinase bioscouring one hour, xylanase biobleaching 8 hours and biopolishing took 2 hours. The sequential increased weight loss percentage can also be explained as time and enzyme factors.

In general; the weight loss percentage of *Agave americana L.* fibre increased proportionately with the increase in numbers of biosoftening processes, which increased the enzymes used on individual fibre and processing time as shown by trend from preliminary tests to experimental tests. In Table 4.2 and figures 4.4-4.10; respectively for example weight loss values which indicated both the positive effects of enzymatic biosoftening and possibility of major fibre damage if occur; treatment increases to extremes.

#### **4.5 WEIGHT LOSS PERCENTAGE OF WATER BOILED AGAVE AMERICANA L. FIBRE**

The aim of this section was to assess the weight loss percentage after pure water boil biosoftening of *Agave americana L.* fibre. Boiling water is the most common pre-treatment technology that does not use harmful chemicals but uses liquid water at the boiling temperature. In boiling water pre-treatment, water functions as a solvent and a chemical agent complemented with organic acids that are released from the fibre to disturb the cell wall matrix (Li *et al.*, 2017:2). Boiling water was used as a control; since pure water is easily available, safe, cost-effective and efficient for sustainable pre-treatment technology.

##### **4.5.1 The *Agave americana L.* fibre boiled for 30 minutes**

Water boiling pre-treatment was mainly intended to improve the softening property of *Agave americana L.* fibre where water functioned as a wetting agent and also as a control experimental procedure. The boiling water treatment further improved the appearance feel and performance

properties of the fibre. Weight loss percentage for boiled water treatment ranges within the acceptable and recommended weight loss percentage of 3-8% for caustic scouring ads stated by Hoque & Azim (2016:168). Figure 4.11 shows the weight loss percentage of older *Agave americana L.* fibre; boiled for 30 minutes.

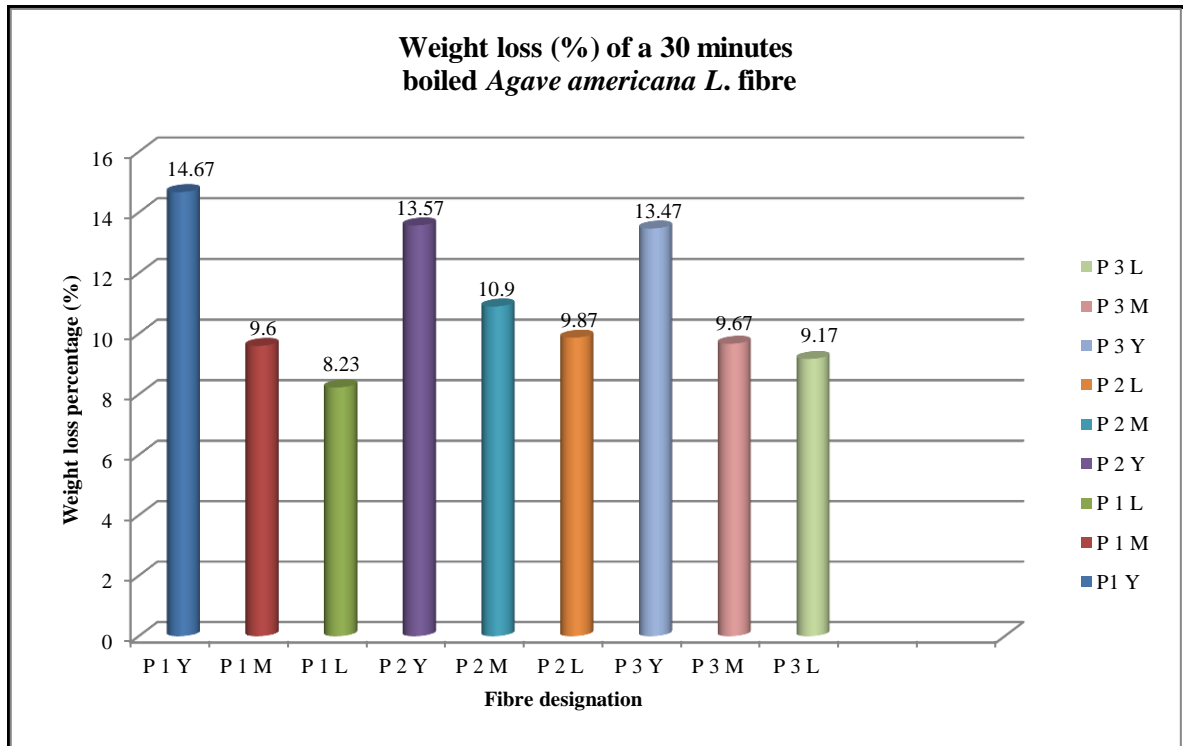


Figure 4.11: Weight loss percentage for 30 minutes water boiled fibre extracted from the younger, middle and older fibres extracted from plant 1, 2 and 3

Weight loss percentage of 30 minutes' water boiled *Agave americana L.* fibre ranges from 8.23%; the older fibre of plant 1 to 14.67% of the same plant but obtained from younger leaves. From figure 4.12; it was observed that more weight loss was achieved from younger fibre of the three used plants. The weight loss decreased with fibre age increase. This can be clarified by the point that older fibres have high biomass recalcitrance towards water boiling treatment for fibre softening.

In boiling the fibre, some impurities which include hemicellulose and lignin were hydrolysed. As a result, dissolved impurities are transferred into hot water, while cellulose remains as a solid. Agitation during several washings improved the removal of impurities. Thus weight loss was used as the response to indicate the activity of boiling water on *Agave americana L.* fibre.

The degradation efficiency of lignin showed a slight increase with a prolonged boiling process. However, a significant amount of impurities stayed as deposits in and around cellulose structure

after the boiling water pre-treatment. Increased weight loss (percentage) depicts that the water boiling pre-treatment and enzymatic biosoftening have effectively and efficiently catalysed substantial quantities of non-cellulosic *Agave americana L.* fibre components that include lignin, hemicelluloses, pectin, waxes and structural impurities. This is associated with fibre cleanliness as it is observed from figures 4.26-4.28.

#### 4.5.2 The *Agave americana L.* fibre boiled for one hour

Boiling water pre-treatment was also used because it is an expedient procedure which is non-corrosive and removes water soluble non-fibrous materials including hemicellulose with ease. But it does not remove the lignin with ease without chemical added to facilitate the process. The lignin is recalcitrant. But its disturbance can relocate it and facilitates an improved access for enzymes to soften cellulose fibre bundles (Syazwanee *et al.*, 2018:2). Figure 4.12 presents the weight loss percentage of older *Agave americana L.* fibre; boiled for one hour.

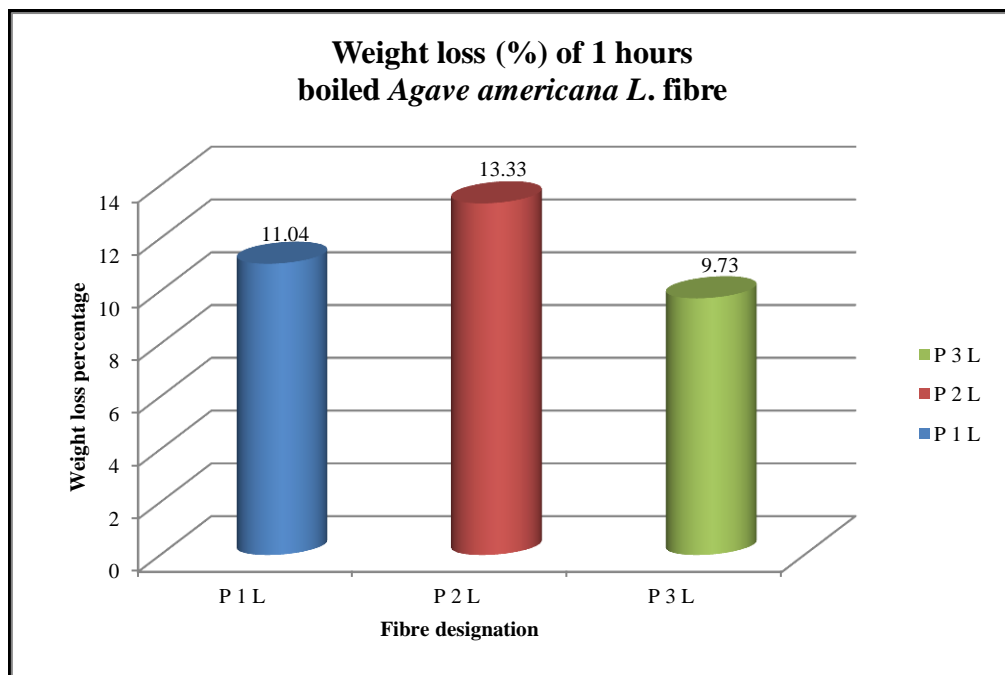


Figure 4.12: Weight loss percentage for fibre obtained from lower leaves of plant 1, 2 and 3 water boiled for 1 hour

Weight loss ranged from 9.73 to 13.33% may be due to divergences in fibre age and the growing conditions. Boiling water pre-treatment is claimed to be less destructive to the fibre surface, but removes non-cellulosic components that are water soluble even at low temperature. Likewise, it does not produce toxic products which can be unsafe to the environment (Santos *et al.*, 2018:1). The water boiled *Agave americana L.* fibre caused weight loss that ranged from 9.73-13.33 %, which is comparable to single enzyme treatments but more than that of xylanase.

## **4.6 TENSILE PROPERTIES OF AGAVE AMERICANA L. FIBRE**

The fibre tensile properties are essential because they influence the performance characteristics and end uses of the textile fibre (Zannen *et al.*, 2014:211). The mechanical properties include the tensile properties that deal with applied different loads in tension load form (Shesan *et al.*, 2019:2). The textile tensile test results should provide reliable data that enable the researcher to characterise the physico-mechanical properties of the textile fibres. The tensile test was conducted to determine the mechanical properties of *Agave americana L.* fibre. The significant mechanical properties determined in this study were the maximum load (N), displacement at maximum load, Young's modulus and tensile stress-strain.

The *Agave americana L.* fibre is a natural plant fibre; therefore, its mechanical properties are expected to vary considerably due to its individual chemical composition, age, physical structure like size of the fibre, number of defects in them, the existence of non-cellulosic components, mostly lignin, and the sizes of the cells in the tissues and the growing, experimental and testing conditions (Da Paixao, 2017:27, Fortea-Verdejo *et al.*, 2017:444-445). It chemically consists primarily of cellulose, hemi-cellulose and lignin; which affects its tensile properties. The age of the plant and /or the leaves from which the fibre was extracted also influences the tensile properties of the fibre (Shesan *et al.*, 2019:4). Older *Agave americana L.* fibre are expected to dispose higher tensile strength than the younger fibre from younger plants and/or leaves. However, the fibre properties can differ from the expectations and literature data because of these internal and external factors.

### **4.6.1 Mean values of tensile maximum Load (N), displacement at maximum load (mm) and initial Young's modulus of *Agave americana L.* fibre**

Conversely, it is challenging to evaluate the tensile properties of *Agave americana L.* fibre, since it is a natural lignocellulose fibre and as such it has great variability due to growth irregularities (Hulle *et al.*, 2015c:71, Hulle *et al.*, 2015b:4). The study of physico-mechanical properties of raw, water boiled and enzyme biosoftened *Agave americana L.* fibre was conducted with the aim to determine how much the innate tensile properties of raw fibre, can be affected by boiling water pre-treatment and enzymatic biosoftening technology.

The following tensile properties: the maximum breaking load; which is the tension requisite to breakdown the fibre sample, displacement at maximum load; which is the actual length extension;



the fibre sample experiences before breaking due to load application and initial Young's modulus which expresses the stiffness of the fibre (Da Paixão, 2017:29) were measured in this study. Table 4.3 presents the mean tensile maximum load in newtons (N), displacement at maximum load in millimetres (mm) and initial Young's modulus in megapascals (MPa) of raw (untreated), water boiled and enzyme treated *Agave americana L.* fibre. Starting from the top of the table the first nine fibre samples are of untreated (raw); subsequent three; are boiled samples and the last twelve are enzyme treated fibres.

**Table 4.3: The mean tensile maximum load (N), displacement at maximum load (mm) and initial Young's modulus (MPa) of raw (untreated), water boiled and enzyme treated *Agave americana L.* fibre**

<b>Fibre designation</b>	<b>Maximum Load (N)</b>	<b>Displacement at maximum load (mm)</b>	<b>Initial Young's modulus (MPa)</b>
P 1 YR	22.34	37.82	543.84
P 2 MR	14.42	45.83	496.68
P 2 MRT	4.71	28.27	278.71
P 2 MRM	6.27	34.57	215.90
P 2 MRB	9.15	39.54	436.44
P 3 LR	11.76	29.55	555.92
P 3 MRT	22.61	30.92	584.36
P 3 MRM	10.09	33.73	467.97
P 3 MRB	15.21	46.65	598.13
P 1 MB	4.82	22.24	346.11
P 2 MB	11.43	50.40	826.33
P 3 MB	8.44	39.47	354.26
P 2 M MnP	9.03	39.17	366.90
P 1 LP	12.8	35.41	411.39
P 3 MC	12.29	26.27	264.20
P 1 YX	18.85	32.11	369.31
P 2 YX	18.62	34.36	0
P 3 YX	9.54	42.73	405.76
P 1 YTE	6.76	29.01	322.79
P 2 YTE	8.15	39.31	309.17
P 3 YTE	4.64	24.13	530.78
P 1 MFE	16.09	20.12	1101.54
P 2 MFE	11.80	42.41	315.10

<b>Fibre designation</b>	<b>Maximum Load (N)</b>	<b>Displacement at maximum load (mm)</b>	<b>Initial Young's modulus (MPa)</b>
P 3 MFE	8.50	34.06	468.54

The *Agave americana L.* fibre strength was indicated with tensile maximum load. The *Agave americana L.* fibre with lower strength was indicated by low maximum load and was significantly deformed and has high displacement value while fibre with higher strength bear more load at maximum load because it is harder and less deformed and has lower displacement values. These results show expected trend because displacement at maximum load increases divergent to the fibre strength or vice versa. Based on the values provided in figure 4.3, the raw fibre was the strongest with the mean average value of 12.95 N, followed by enzyme biosoftened fibre with 11.42 N and water boiled for 1 hour had 8.23 N. The raw fibre further exhibited highest Young's modulus mean of 598.13 MPa; followed by boiled fibre with 508.90 MPa and finally the enzyme treated fibre with 409.46 MPa. Boiled fibre on the other hand exhibited the highest mean value of displacement at maximum load of 37.37 mm then followed by the raw fibre with 36.65 mm while the enzyme biosoftened fibre became last with 33.26 mm. These fibre mechanical properties differences can be attributed to the cell wall thickness ratio to fibre diameter, fibre morphology and structure, fibre polymers' composition and their removal, enzyme-substrate specificity, experimental procedure and cleaning agitation rate (Bourmaud *et al.*, 2018:348).

The results shown in figure 4.3 indicate that *Agave americana L.* fibre is a strong fibre. Its high tensile properties can be due to its many years of growth which results in high cellulose polymerisation and crystallisation (Hulle *et al.*, 2015b:4). The *Agave americana L.* fibre is a natural multicellular filament fibre consists of overlapping ultimate fibres glued together by waxy film. The high breaking strain of *Agave americana L.* fibre shown in table 4.4 can also be explained by the tensile modelling behaviour of ultimate fibres. These ultimate fibres have helical structures that disentangle when the force is applied (Thamae *et al.*, 2009:92). The *Agave americana L.* fibre tensile properties indicate that it is strong enough to become a textile fibre. It has reasonably high tenacity and elongation at break properties which are comparable to those of other lignocellulosic fibres like jute, flax and sisal as studied by Azanaw *et al.*, (2018:50). *Agave americana L.* fibre tensile results showed that the removal of non-cellulosic fibre components through enzymatic biosoftening and water boiling did not cause any significant fibre strength loss that can disqualify the fibre to become a textile fibre.

It is observed from table 4.3 that the tensile properties of *Agave americana L.* fibre obtained from the same leaf fibre differ greatly. This variability is typical of natural plant fibres. This variability may be due to different genetic makeup, growing, harvesting, extraction and testing conditions and procedures, as well as chemical and structural compositions of individual *Agave americana L.* fibre, which influence chemical composition, luminal porosity and microfibrillar angle (Fortea-Verdejoa *et al.*, 2017:444). The tensile properties of *Agave americana L.* fibre vary between fibres harvested from the same leaf at different positions such as the base, middle and tip and from the single fibre at different positions (base, middle and tip). The *Agave americana L.* fibre is a lignocellulosic multi-cellular filament fibre of a diameter ranging from 100-150  $\mu\text{m}$ ; mainly of cellulose, hemicellulose and lignin (Mafaesa, 2006:96, Hulle *et al.*, 2015c:69 & 72). The fibre is in the form of crystalline network of lengthy chains of cellulose molecules, termed microfibrils have size ranging from 10-30  $\mu\text{m}$  (Kolte & Dabera, 2012:8). The number and the size of the ultimate fibres have great influence on the tensile strength of the fibre. The complicated natural lignocellulosic fibre structure affects its properties (Shah D.U. *et al.*, 2016:1487).

It is also observed from table 4.3 that displacement at maximum load is inversely proportional to, the tensile strength that is represented as maximum load and the initial Young's modulus of the *Agave americana L.* fibre. That is, greater the tensile maximum load and initial Young's modulus, lesser values of displacement at maximum load are under stress. These results are more or less the same with those obtained by Ravichandran *et al.* (2019:66). The *Agave americana L.* fibre tensile maximum load, displacement at maximum load and Initial Young's modulus properties can be correlated with some structural parameters that vary from fibre to fibre and within the same fibre. As a plant fibre; *Agave americana L.* fibre should have tissues that have mechanical support.

The *Agave americana L.* is a lignocellulosic fibre that has physical and mechanical characteristics that are influenced partly by its chemical composition to make it qualify to become a textile fibre. it is composed of 68-80% cellulose, 5-17% lignin, 15% Hemicelluloses, 0.26% wax and 8% moisture (Hulle *et al.*, 2015b:4). Cellulose which is the main framework element that provides stiffness, strength and structural stability to the fibre (Eder and Burgert, 2010:27). The tensile behaviour of *Agave americana L.* fibre can also be explained by the cellulose unique structural arrangement of glucose molecules and the degree of polymerisation that varies but not with less than 500 molecules form parallel and mainly crystalline microfibrils that are bound together by hydrogen bonds (Eder and Burgert, 2010:27).

A number of micro fibrils are bundled up to form macrofibrils. The cellulose hydrogen bonds induce extreme toughness and inflexibility to cellulose microfibrils (Asim *et al.*, 2015). Thus cellulose structural arrangement influences the physico-mechanical properties of the *Agave americana L.* An *Agave americana L.* fibre is a natural complex composite material comprising of cellulose fibrils entrenched in an unstructured matrix of lignin, hemicelluloses and pectin which bind together the cellulose framework structure of the fibre. It is also comprised of minute amounts of organic and inorganic components namely extractives and ash respectively. Organic extractives provide resistance to colour, smell and decay while the inorganic substances improve the natural fibre abrasiveness. All the fibre components influence its physico-mechanical properties (Msahli *et al.*, 2007:3952).

#### **4.6.2 Variability evaluation of tensile properties of *Agave americana L.* fibre**

The amount of dispersion in a dataset is normally represented by measures of variability (Bunsell, 2009:62). The rate at which data generally spreads about an average is referred to as data variation. There are several measures of dispersion from which researches can use to present data variation (Javaid, 2018:15). The mean, standard deviations (SD), coefficient of variation (CV) and median values of maximum load (N), displacement at maximum load (mm) and initial Young's modulus (MPa) were used as measures for *Agave americana L.* fibre tensile variability. Like other lignocellulosic fibres; *Agave americana L.* fibre showed great variability in tensile properties which could have been due to plant growing conditions and test parameters (Aseer *et al.*, 2013:202). Because the *Agave americana L.* fibre' properties depend, on its morphological constructions and chemical components the maximum load at break, the elongation at maximum load and the initial Young's modulus show a high coefficient deviation. This variance can be due to the natural growing condition of the fibre studied, the definite structural arrangements, the processing method and conditions (Zannen *et al.*, 2014:212).

##### ***4.6.2.1 Mean maximum load (N), standard deviations (SD), coefficient of variation (CV) and median values of tensile properties***

The maximum load mean (N), standard deviations (SD), coefficient of variation (CV) and median values of *Agave americana L.* fibre are presented in figure 4.5 and are very important because they can be used to model the chances of the failure of fibre properties in more or less the same systems, define design allowable and assurance levels in component design and compare and contrast the variability of tensile strength of *Agave americana L.* fibre to that of other natural fibres.

*Table 4.4: Mean maximum load (N), standard deviations (SD), coefficient of variation and median values of tensile properties of Agave americana L. fibre*

<b>Fibre Sample designation</b>	<b>Mean Maximum load (N)</b>	<b>Standard Deviation</b>	<b>Mean - 2.00 Standard Deviation</b>	<b>Mean + 2.00 Standard Deviation</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Coefficient of Variation</b>	<b>Median</b>
P 1 YR	22.34	4.11	14.12	30.56	15.89	28.18	18.40	22.92
P 2 MR	14.42	4.26	5.90	22.95	6.49	20.88	20.88	15.49
P 2 MRT	4.71	0.95	2.81	6.61	3.22	6.49	20.21	4.64
P 2 MRM	6.27	1.03	4.20	8.33	4.61	8.8	16.47	6.15
P 2 MRB	11.43	2.36	6.70	16.16	6.71	14.33	20.68	11.51
P3LR	11.76	4.04	3.67	19.84	4.02	17.39	34.39	10.74
P 3 MRT	22.61	3.66	15.29	29.92	16	30.06	16.18	22.63
P 3 MRM	10.09	2.71	4.68	15.50	5.42	14.87	26.80	10.89
P 3 MRB	15.21	4.12	6.98	23.44	10.36	21.85	27.06	15.59
P 1 MB	4.82	2.83	0.84	10.48	1.44	10.89	58.72	4.35
P 2 MB	11.43	2.36	6.70	16.16	6.71	14.33	20.68	11.51
P 3 MB	8.44	3.00	2.43	14.45	2.36	11.97	35.60	8.86
P1LP	12.8	5.00	2.79	22.81	5.42	19.27	39.09	14.06
P2MMNP	9.03	3.27	2.48	15.57	4.99	14.92	36.25	8.72
P 3 MC	12.29	3.74	4.82	19.76	6.12	15.94	30.39	12.83
P 1 YX	18.85	3.12	12.61	25.09	14.76	25.02	16.56	17.31
P 2 YX	18.62	3.21	12.20	25.05	12.88	23.89	17.25	19.35
P 3 YX	9.54	2.76	4.03	15.05	5.95	14.44	28.88	9.23

P 1 YTE	6.76	3.50	0.24	13.75	1.93	11.54	51.77	6.22
P 2 YTE	8.15	3.43	1.28	15.02	3.43	15.3	42.15	7.09
P 3 YTE	4.64	1.83	0.98	8.30	1.71	8.00	39.48	4.32
P1MFE	16.09	1.54	13.00	19.17	13.04	19.22	9.56	16.16
P2MFE	11.80	3.62	4.55	19.04	4.83	15.73	30.70	12.54
P3MFE	8.50	3.02	2.46	14.53	3.22	13.42	35.51	8.42

For a fibre to become a textile fibre, it should possess fibre strength as one of the basic characteristics (Sinclair, 2015:14). A distribution manifestation in tensile maximum load that ranges from 4.64 N to 22.61 N is a proportion of standard deviation that ranges from 0.95 to 5.00 to mean value  $\mu$  referred to as coefficient of variation that varies from 16.18 to 58.72. It is observed that the standard deviation for the mean value was more than 1 ( $\sigma > 1$ ) except for the raw fibre obtained from tip part of middle leaves of plant 2 (P 2 MRT). This shows that there is a large amount of variability in the tensile mean maximum load properties of *Agave americana L.* fibre has been studied. It can be due to several different factors like the specific plant and its growth conditions or the level of development of the specific fibre and the fibre size as result of it (Ravichandran *et al.*, 2019:66).

The results of the relevant statistics portrayed in table 4.4 clearly show the variation in the performance of the individual fibres with the big differences between the minimum and maximum values with each group and the standard deviation, although the mean maximum loads carried before breaking and the median values are very similar, indicating that variation in the results are largely caused by outliers. The variation in the size of the fibre was prominent and the most likely cause for the variation performance.

Table 4.4 presents variability distribution of tensile maximum load of *Agave americana L.* fibre through statistical approach and to identify hiccups and search ways of minimising the gaps in between the fibre tensile properties and exploration and/or utilisation of *Agave americana L.* fibre as a textile fibre.

#### ***4.6.2.2 Displacement at maximum load mean (mm), standard deviation (SD), coefficient of variation and median values of Agave americana L. fibre***

Displacement at maximum load (mm) is known as elongation. It is normally inversely proportional to fibre stiffness, tensile strength and initial Young's modulus. This means that, greater the stiffness, tensile strength, and initial Young's modulus the lesser; the displacement at maximum load under stress and it needs more force to stretch a stiff fibre, which is consuming high tensile strength (Ravichandran *et al.*, 2019:66). Table 4.5 presents the mean displacement at maximum load (mm), standard deviations (SD), coefficient of variation and median values of tensile properties of *Agave americana L.* fibre.



*Table 4.5: Displacement at maximum load mean (mm), standard deviation (DS), coefficient of variation and median values of the tensile properties of Agave americana L. fibre*

<b>Fibre Sample designation</b>	<b>Mean tensile Displacement at maximum load (mm)</b>	<b>Standard Deviation</b>	<b>Mean - 2.00 Standard Deviation</b>	<b>Mean + 2.00 Standard Deviation</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Coefficient of Variation</b>	<b>Median</b>
P 1 YR	37.82	16.09	5.63	70.00	9.41	53.6	42.552	47.46
P 2 MR	45.83	11.96	21.90	69.75	21.11	55.74	26.11	50.68
P 2 MRT	28.27	6.82	14.63	41.92	14.59	37.23	24.13	28.35
P 2 MRM	34.57	3.74	27.09	42.05	29.85	42.37	10.81	33.56
P 2 MRB	39.54	9.55	20.45	58.63	24.1	54.09	24.14	39.37
P 3 LR	29.55	9.68	10.19	48.91	9.82	41.45	32.76	33.18
P 3 MRT	30.92	11.42	8.08	53.75	13.05	48.41	36.93	27.76
P 3 MRM	33.73	7.27	19.20	48.26	23.43	53.05	21.55	32.10
P 3 MRB	46.65	5.54	35.58	57.73	37.16	53.38	11.87	47.76
P 1 MB	22.24	14.12	-6.01	50.48	2.9	49.88	63.51	23.44
P 2 MB	50.40	4.81	40.79	60.02	41.26	61.02	9.54	49.76
P 3 MB	39.47	12.11	15.25	63.69	11.71	52.38	49.76	43.215
P 2 M MnP	39.17	9.90	19.37	58.97	16.53	51.78	25.28	43.05
P 1 LP	35.41	8.59	18.24	52.59	17.22	48.64	24.25	35.98
P 3 MC	26.27	12.20	1.87	50.68	6.26	44.55	46.44	23.45
P 1 YX	32.11	13.18	5.75	58.47	11.54	50.75	41.04	34.31

P 2 YX	34.36	13.79	6.78	61.94	8.35	56.74	40.13	35.51
P 3 YX	42.73	8.15	26.42	59.04	22.13	56	19.08	44.34
P 1 YTE	29.01	14.27	0.47	57.55	8.12	47.2	49.19	29.77
P 2 YTE	39.31	13.03	13.25	65.37	23.92	61.92	33.14	36.95
P 3 YTE	24.13	10.03	4.08	44.19	4.56	43.24	41.56	26.24
P 1 MFE	20.12	9.55	1.03	39.21	0.47	34.73	47.44	21.18
P 2 MFE	42.41	10.17	22.08	62.75	21.8	52.48	23.97	48.1
P 3 MFE	34.06	12.71	8.63	59.49	10.54	49.26	37.33	35.36

The standard deviation is greater than 1. This indicates great variation. The results of the tensile displacement as portrayed in table 4.5 show the variation in the displacement performance of the different fibre samples in each group. The mean and median values are in most cases very close as expected, but the minimum and maximum values vary widely and so do the standard deviation values. These are most likely because fibre morphologies differ due to a number of factors: Within a species, fibre tensile displacement diverge due to climatic and seasonal conditions, soil type and fertility rate, the different positions of a plant on which fibres grow can have different tensile displacement (Sorieul, 2016:18; Ayele *et al.*, 2018:47-48). In the case of P 1 YR group the influence of a specific outlier with a value of 9.41 evident in the substantial difference between the mean; 37.82 and the median; 47.66.

#### ***4.6.2.3 Initial Young's Modulus mean (MPa), standard deviation (SD), coefficient of variation and median values of *Agave americana L.* fibre***

Initial Young's modulus, is also referred to as an elastic or tensile modulus. It is a measure of the stiffness of a fibre (Ravichandran *et al.*, 2019:66). Table 4.6 presents the mean initial Young's modulus (MPa), standard deviations (DS), coefficient of variations and median values of the tensile properties of *Agave americana L.* fibre.

*Table 4.6: The initial Young's modulus mean (MPa), standard deviation (DS), coefficient of variations and median values of the tensile properties of Agave americana L. fibre*

<b>Sample ID</b>	<b>Mean</b>	<b>Standard Deviation</b>	<b>Mean - 2.00 Standard Deviation</b>	<b>Mean + 2.00 Standard Deviation</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Coefficient of Variation</b>	<b>Median</b>
P 1 YR	543.84	182.82	-178.19	909.48	267.77	840	33.62	534.12
P 2 MR	496.68	104.22	288.24	705.11	297.27	665.30	20.98	500.12
P 2 MRT	278.71	73.04	132.62	424.79	149.50	384.05	26.21	288.36
P 2 MRM	215.90	215.90	107.54	324.26	128.30	306.62	25.10	221.20
P 2 MRB	436.44	95.45	245.54	627.34	192	581.80	21.87	428.69
P 3 LR	555.93	108.43	339.07	772.78	445.04	790.40	19.50	537.30
P 3 MRT	584.36	215.09	154.17	1014.55	257.94	1059.53	36.81	546
P 3 MRM	467.97	258.65	-49.32	985.27	279.09	1254.08	55.27	394.69
P 3 MRB	598.13	223.66	150.80	1045.46	368.24	1234.18	37.39	535.42
P 1 MB	346.11	55.20	235.72	456.50	252	444	15.95	352.33
P 2 MB	826.33	723.16	-619.99	2272.64	172.71	1854.64	87.52	278.09
P 3 MB	354.26	93.182	167.90	540.63	184.59	459.759	26.3032	387.90
P 2 M MnP	366.90	115.35	136.19	597.60	166.65	520.10	31.44	394.62
P 1 LP	411.39	214.96	18.53	841.30	12	748.21	52.25	483.22
P 3 MC	264.19	259.56	254.93	783.31	0	578.82	98.25	373.57
P 1 YX	369.31	87.79	193.73	544.89	204.36	524.58	23.77	364.08
P 2 YX	0	0	0	0	0	0	0	0

P 3 YX	405.76	67.05	271.66	539.86	311.67	488.85	16.53	411.18
P 1 YTE	322.79	146.76	29.27	616.31	177.17	702.86	45.47	317.09
P 2 YTE	309.17	127.06	55.049	563.29	161.43	634.97	41.10	297.12
P 3 YTE	530.78	750.36	-969.93	2031.49	204.00	2905.76	141.37	334.43
P 1 MFE	1101.54	3805.35	-6509.16	8712.24	89.91	17704.2	345.46	314.17
P 2 MFE	315.10	106.53	102.03	528.16	152.87	489.55	33.81	306.97
P 3 MFE	468.54	98.96	270.63	666.45	275.31	647.55	21.12	443.65

A distribution manifestation in tensile initial Young's modulus that ranges from 0 N to 1101.45. N is a proportion of standard deviation that ranges from 0.00 to 3805.35. The mean value  $\mu$ , is referred to as the coefficient of variation varies from 0.00 to 345.46. It is observed from table 4.6 that; the standard deviation for the mean value was more than 1. The variations in the tensile initial Young's modulus behaviour can be explained by the chemical and structural heterogeneity between the different parts of a fibre, fibres extracted from different leaves at different levels from the same plant and from different plants and their layers that include variation in cellulose content against lignin and hemicellulose proportions and microfibril angle along the cell axis (Shesan *et al.*, 2019:4-6). It is observed that *Agave americana L.* fibre exhibited the tensile properties that are highly variable. The tensile fibre variability was evident, even among the same batches from the same fibre or the leaves from the same plants obtained from the same levels. This can be due to varied structural arrangements like lumens, wall thickness and diameter which are of different sizes which are distinctive natural fibre characteristics (Ayele et al. 2018:48).

**4.6.2.4 The tensile properties of the raw *Agave americana L.* fibre from different positions of the plant leaves**

**Table 4.7: The Comparison of the tensile properties of raw fibre obtained from different positions of plant 2 and 3 leaves**

<b>Position of leaf</b>	<b>Fibre sample designation</b>	<b>Maximum breaking load (N)</b>	<b>Displacement at maximum load (mm)</b>	<b>Initial Young's Modulus (GPa)</b>
Top	P 2 MRT	4.71	28.27	278.71
Middle	P 2 MRM	6.27	34.57	215.90
Bottom	P 2 MRB	9.15	39.54	436.44
Top	P 3 MRT	22.61	30.92	584.36
Middle	P 3 MRM	10.09	33.73	467.97
Bottom	P 3 MRB	15.21	46.65	598.13

Tensile properties of fibre bundles extracted from three different positions in the leaf: bottom, middle and top, were evaluated and the results are shown in table 4.7. For plant 2 medium leaf fibres, the top endured the lowest load then followed by the middle and the bottom /base fibre tolerated the highest load before breaking. But for plant 3 medium, the fibre from top part of the leaf, withstood the highest load before breaking followed by the fibre from the base and

finally the middle. But in general, the *Agave americana L.* fibre tensile properties tested showed the same trend. The fibre from top part exhibits the highest average mean tensile properties; then followed by the fibre obtained from the base and lastly the fibre from the middle part showed lowest the tensile properties. However, the differences between middle and bottom parts were not much different.

These results differ with those of Surajarusarn *et al.* (2019:148) where the pineapple leaf fibre bundles obtained from the middle part exhibited the highest tensile strengths and those from the top position were the weakest. These results confirm the theory that the plant fibre tensile properties are substantially divergent between different plants, between fibres harvested from the same plant at different levels and within an individual fibre at different positions. This variability is effected by several factors like; the intrinsic distribution of the fibre composition properties, procedures followed to extract the fibre and to explore the tensile properties of individual fibres, the plant fibre structural variations among themselves, affecting fibre diameter, crystallinity, microfibrillar angle, physical and chemical composition and luminal porosity due to different growth conditions (Celino *et al.*, 2014:3; Ahmad *et al.* 2019:2).

Under normal circumstances; the Young's modulus and tensile strength of lignocellulosic fibres increases with increase in cellulose content (Feigel *et al.*, 2019:8). Fibre attained from the base of the leaf is older with higher cellulose content than the younger fibre from the middle and top of the leaf. The same trend is observed in table 4.8; with exception of P 3 MRT which shows the highest maximum load and higher Young's modulus (N) values. This can be explained by the orientation of cellulosic fibre ultimates (microfibrils) towards fibre axis, which controls fibre rigidity. The cellulosic fibres are unyielding and can bear high tensile load when their constituent microfibrils are parallel orientation to the fibre axis. Otherwise, they are more yielding if they possess a spiral orientation towards fibre axis (Bourmaud *et al.*, 2018:348). It is therefore reasonable to conclude that P 3 MRT might have had a natural spiral orientation towards its axis and therefore became more pliable when loaded.

From Tables 4.4, 4.5 4.6 and 4.7 it is observed that the tensile properties of *Agave americana L.* fibre varies substantively, even in the same fibre. This variability is caused by the natural distribution of the fibre properties and investigational procedures used to test the tensile properties of each individual fibre. It is observed from the results, that the *Agave americana L.* fibre tensile properties diverge between fibres extracted from the leaves of the same *Agave americana L.* plant. This is influenced by the fibre and maturity levels and structural deviations



that affect the fibre physical and chemical composition, microfibrillar angle, crystallinity and luminal porosity due to different growth conditions (Bourmaud *et al.*, 2018:348).

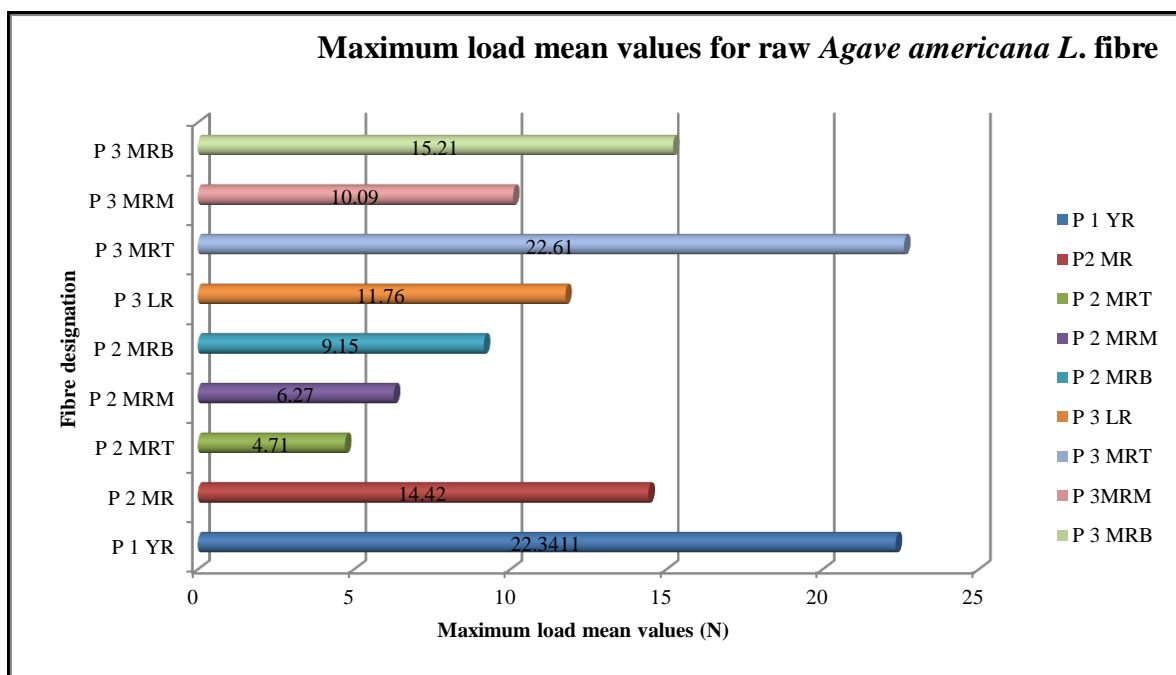
The variability of the tensile properties of *Agave americana L.* fibre can also be impacted mainly by variability in fibre diameter that is influenced by the composition of an individual fibre bundle. An individual fibre bundle is composed of a number of ultimate fibres (as illustrated in figures 4.25, 4.26 & 4.27). The fibre diameter variability posts a problem when determining the transverse-sectional area of the fibre. This is explained by a substantial scatter observed in the measured tensile strength and initial moduli of the *Agave americana L.* fibre. The evaluated tensile modulus decreases with increasing fibre diameter that shows the significance of the identification of the fibre width. The mechanical actions of fibre extraction applied to separate the fibre bundles from the leaf biomass (figure 4.1), washing, drying, straightening and cleaning fibre debris on fibre surface could have induced fibre defects. Fortea-Verdejo *et al.* (2017:445), claimed that the defects induced by fibre extraction are probable to influence the tensile properties of the fibre and increase probabilities of its failure during textile manufacturing, causing the fibre length to be shorter than its own acute length.

The difference among *Agave americana L.* fibre cells is influenced by factors that include the thickness of the secondary cell-walls which are affected by the number of fibre cells, the cell-wall size, the fibre bundle cross-section area and the real cross-section which is equal to the total area minus the lumen area, differences in fibre morphology can be associated with the tensile strength properties (Bourmaud *et al.*, 2018:348). This implies that the fibre mechanical properties are correlated to its particular morphological characteristics which are also related to its tensile strength properties. The plant fibre selection for specific textile performance is highly influenced by fibre strength properties (Shesan *et al.*, 2019:4).

### **4.6.3 Representation of the tensile properties of *Agave americana L.* fibre**

#### ***4.6.3.1 Tensile maximum load mean values (N) of raw *Agave americana L.* fibre***

The maximum load mean values of the raw *Agave americana L.* fibre are presented in figure 4.13.



**Figure 4.13: Tensile maximum load values (N) of raw *Agave americana L.* fibre extracted from the leaves harvested from three plant levels and from three different parts of the leaves: base, middle & tip**

The lowest maximum load value of 4.71 N was obtained from the tip fibre extracted from the middle leaves of plant 2. The highest maximum load value of 22.61 N was obtained from the tip fibre extracted from the middle leaves of plant 3, followed by fibre obtained from the young leaves of plant 1 with 22.34 N. This can be explicated by the fact that they were from different plants which had the same ecological niche in which they adapted to it differently. The *Agave americana L.* fibre is a lignocellulosic fibre comprises of chemical cellulose, lignin, hemicelluloses, pectin, and waxes. Its power stations are cellulose microfibrils; which are protected by hemicelluloses and lignin. When a force is applied, the microfibrils align themselves with fibre axis (Ahmad *et al.*, 2019:2).

When matrix hemicelluloses and lignin lose their bonding power with cellulose microfibrils; the cellulose hydrogen bonds within the cellulose microfibrils also break and fibre failure occurs. That is why; the fibre with higher cellulose content has the higher tensile strength and vice versa. It can also be explained from a chemical composition viewpoint that an increase of hemicelluloses content; results in a decreased tensile strength because of the amorphous nature of hemicelluloses that ends up with the heterogeneous properties. Lignin is another essential chemical component of the cell wall that protects and strengthens the fibre. The middle lamella also strengths the lignocellulosic fibre when joining the neighbouring cell walls of the fibre. The highly lignified sclerenchyma fibres may also be found in the fibre and add extra strength

to the fibre. This difference in tensile properties of the fibre extracted from the same position of the leaves and the fibre could be due to retting process (Fortea-Verdejo *et al.*, 2017:442). The P 2 MRT might have been over-retted and P 3 MRT under-retted. However, these *Agave americana L.* fibre tensile tests results indicated that the triangulation water retting followed by extractive removal of pectin from *Agave americana L.* fibre leaves did not change the fibre tensile strength adversely.

#### 4.6.3.2 Displacement values at maximum load mean (mm) of raw *Agave americana L.* fibre

The displacement mean value at maximum load is also referred to as the elongation at break or fracture strain. It defines the average relationship between ultimate length and initial length of the test specimen; following the fracture caused by load applied (Da Paixão, 2017:29). Figure 4.14 presents the displacement mean value at maximum load of raw *Agave americana L.* fibre.

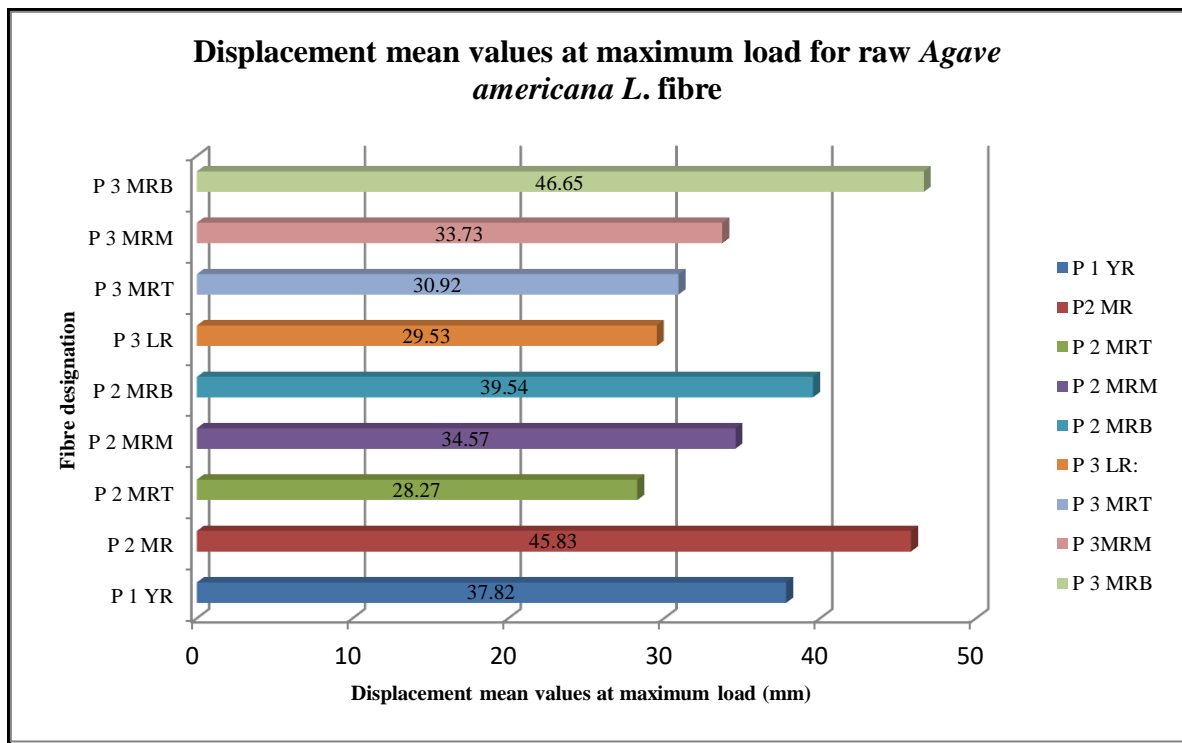


Figure 4.14: Tensile displacement at maximum load mean (mm) of raw *Agave americana L.* fibre extracted from the leaves harvested from three plant levels and from three different parts of the leaves: base, middle & tip

Figure 4.14 shows that at maximum load, the maximum displacement value of 46.65 mm was moved by fibre extracted from the base of middle leaves (P 3 MRB). It was followed by fibre extracted from middle leaves of plant 2 (P 2 MR) with 45.83 mm; which was also followed by fibre extracted from the base of middle leaves (P 2 MRB) with 39.54 mm. the minimum

displacement at maximum load; of 28.27 mm was displayed by fibre extracted from the tip of the middle leaves of plant 2 (P 2 MRT), followed in an ascending order by fibre extracted from the lower leaves of plant 3 (P 3 LR) with 29.53 mm. These results can be explicated by a number of hypotheses: the plant fibre molecular structure of cell wall polymers, their architectural structure, age and diameter of the fibre. It is, therefore, ideal for one to consider fibre extracted from middle leaves to have relatively high elongation to failure, which is mostly attributed to stress relaxation, that occurs when fibre is loaded for residual stress that exists in the fibre microfibrils and cause them to self-align during deformation (Msahli *et al.*, 2007:3953-3955; Kolte & Daberao, 2012:6 & 7).

The fibre with maximum displacement is from the base of the leaf. It is therefore ideal to think that it was older and thicker in diameter than the fibre from the middle and tip of the leaf. Diameter variation could affect the displacement at maximum load for the fibre. According to these results; as the fibre diameter increased the mechanical behaviour (in terms of displacement at maximum load) increased substantially. The used plants were wild with unknown age; one could conclude that plant 2 was older than plant 1 and 3 from which the fibre was extracted. There is difference in displacement between the different parts of the leaves. The fibres extracted from the base stretch further before they break in the middle part less but least at the tip of the leaves.

#### ***4.6.3.3 Initial Young's modulus mean values for raw *Agave americana* L. fibre***

The initial Young's modulus is a measurement of fibre flexibility or stiffness. It is the diagonal slope of the linear section on the stress-strain curve. Thus, it is longitudinal stress divided by vertical strain, the fibre experience. Normally; the stiffer fibre exhibits higher initial modulus (Ravichandran *et al.*, 2019:66). Figure 4.15 shows a tensile Young's modulus (MPa) of raw (untreated *Agave americana* L. fibre.

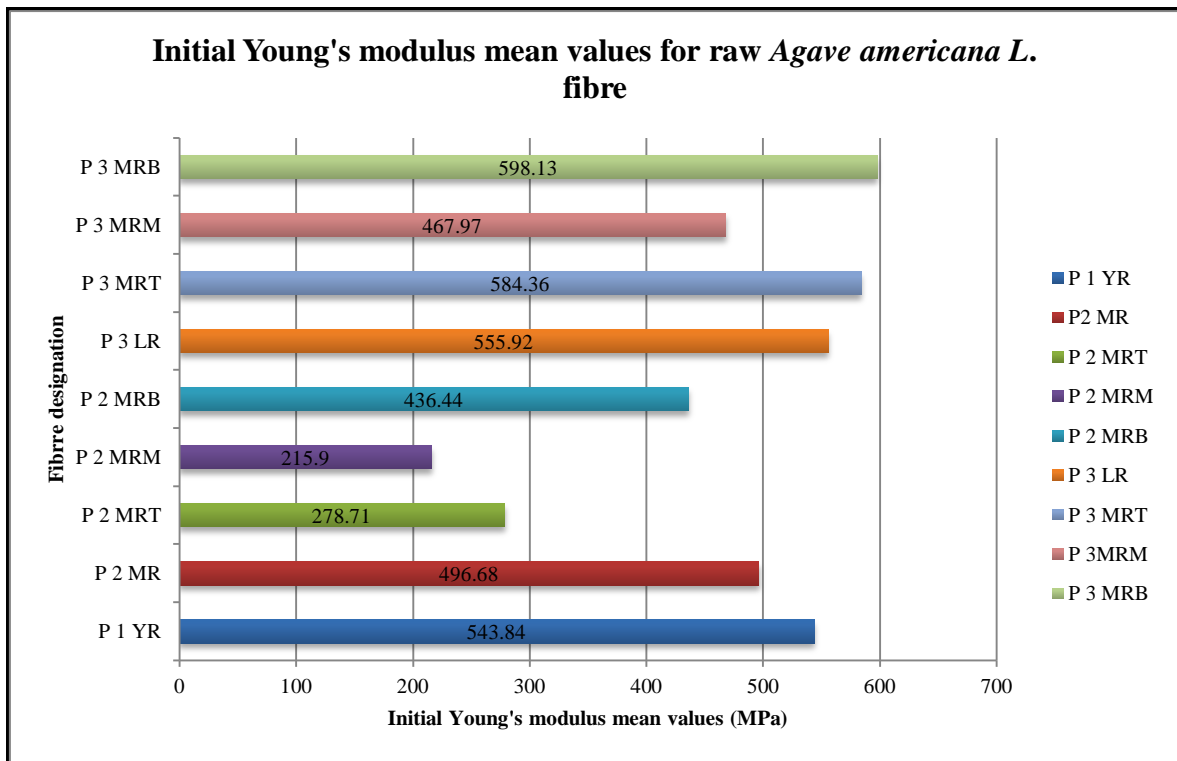


Figure 4.15: Tensile Initial Young's modulus mean values (MPa) of raw *Agave americana L.* fibre extracted from the leaves harvested from three plant levels and from three different parts of the leaves: base, middle & tip

Figure 4.15 shows that the initial young's modulus mean of raw *Agave americana L.* fibres ranged from 278.71 MPa obtained from the middle fibre of the medium leaves of plant 2 (P 2 MRM), to 598.13 MPa obtained from base fibre of the medium leaves of plant 3. Initial Young's modulus load was exhibited by the fibre at the base of the medium leaves of plant 3. These results show no statistical relationship with the diameter variation and position of the leaf from where the fibre was extracted. The highest mean value was attained from the base fibre and the second highest initial Young's modulus value was from the tip fibre can also be explained by the same hypotheses as those shown in figure 4.15. It is therefore ideal to think that it was thicker in diameter than the fibre from the middle and tip of the leaf. Fortea-Verdejo *et al.* (2017:444), claimed that fibre diameter variation could affect the displacement at maximum load for the fibre. The result of the initial modulus is measured in MPa. The problems with the individual natural fibres are the differences in size of the fibre which make the results less comparable. The facts that the mean and median values are closely related make the results more comparable.

#### 4.6.3.4 Tensile Maximum load mean values (N) of boiled *Agave americana L.* fibre

Boiling water treatment was applied on *Agave americana L.* fibre and removed water soluble extractives from the fibre. This then softened fibre to some extent. Maximum load mean values of water boiled for *Agave americana L.* fibre is presented in figure 4.16

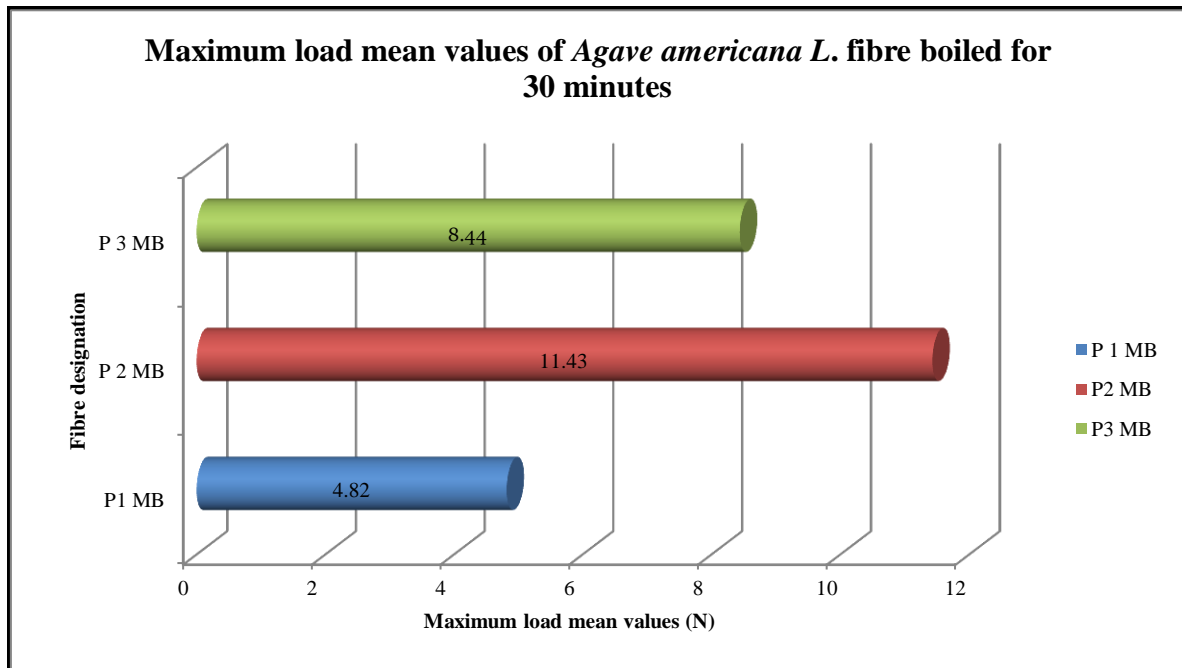
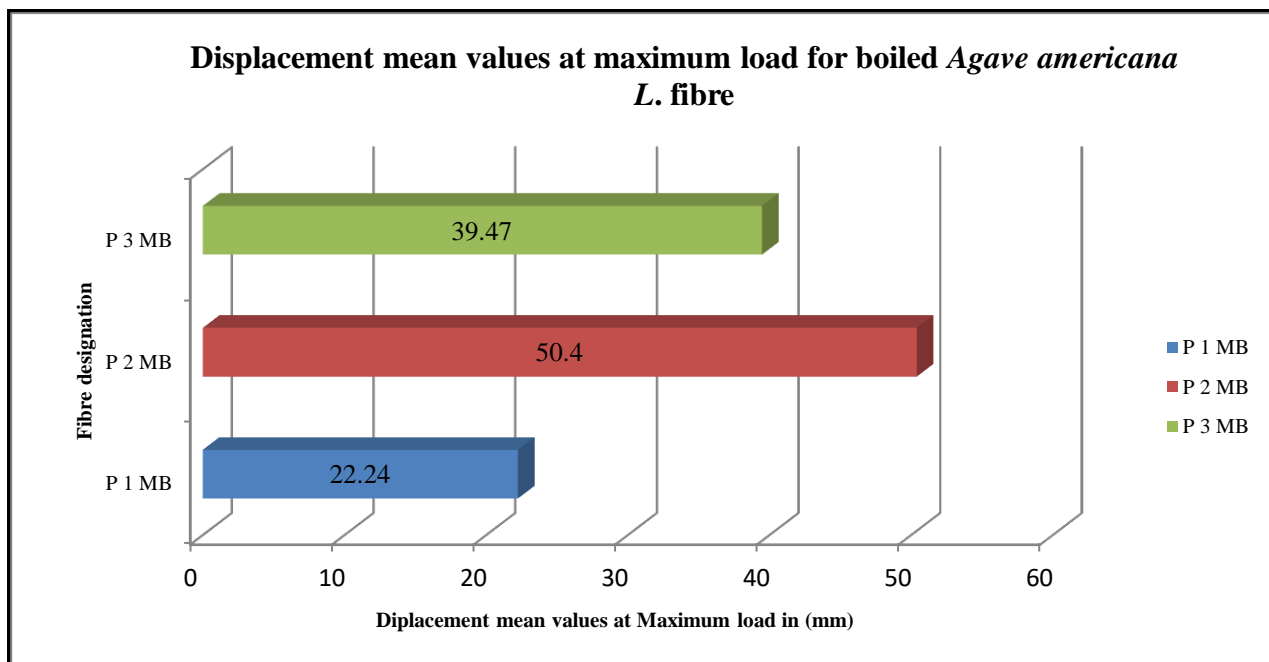


Figure 4.16: Tensile maximum load mean values (N) for boiled *Agave americana L.* fibre obtained from plants 1, 2 and 3

The tensile maximum load mean values of *Agave americana L.* fibre ranged from 4.82 N to 11.43 N. This implies that water boiled fibre extracted from middle leaves of plant was the least strong while the water boiled fibre extracted from middle leaves of plant 2 was the strongest. These values are comparable to those of raw fibre. One other important observation is that the load carried before breaking of the boiled fibre was lower than for the raw fibre, expected as the boiling process would break the bigger fibre bundles down in smaller fibre bundles or individual fibres.

#### 4.6.3.5 Displacement mean values at maximum load for boiled *Agave americana L.* fibre

The displacement at maximum load of the fibre is measured to indicate the ductility; which indicates the amount of load the fibre can withstand before fracture. The displacement means values at maximum load (mm) for 1-hour water boiled *Agave americana L.* fibre, which is the distance the sample can expand before breaking, was determined and presented in figure 4.17.



**Figure 4.17: Tensile displacement mean values at maximum load (mm) for 1 hour water boiled *Agave americana* *L.* fibre obtained from plants 1, 2 and 3**

The boiling water treatment removed dirt and surface attached fragmental tissues and debris. Thus rendered the fibre surface smoother and cleaner and contribute a softer feeling than the raw fibre. Boiling the *Agave americana* *L.* fibre in water for 1 hour rendered fibre extracted from the middle leaves of plant 2 to become the most ductile of the three samples; because it exhibited the highest displacement mean value of 50.4 mm; followed by fibre from plant 3 with 39.47 mm and fibre from plant 1 exhibited the lowest mean value; 22.24 mm of fracture strain, thus; it has least ductile properties. Nonetheless, it is difficult to comprehend a correlation between the water boiling treatment effect and the difference in tensile displacement values and the fibre designation.

The known effect of boiling water on cellulosic fibre is that it increases the fibre fibrillation and outer surface to improve fibre swelling, flexibility and intra fibre bonding interaction (Motamedian *et al.*, 2019:4099). The Tensile displacement at the boiled fibre does not differ observably much from the displacement of the raw fibre. Indicating that the boiling process did not harm the fibre characteristics.



#### 4.6.3.6 Initial Young's modulus mean values for boiled *Agave americana L.* fibre

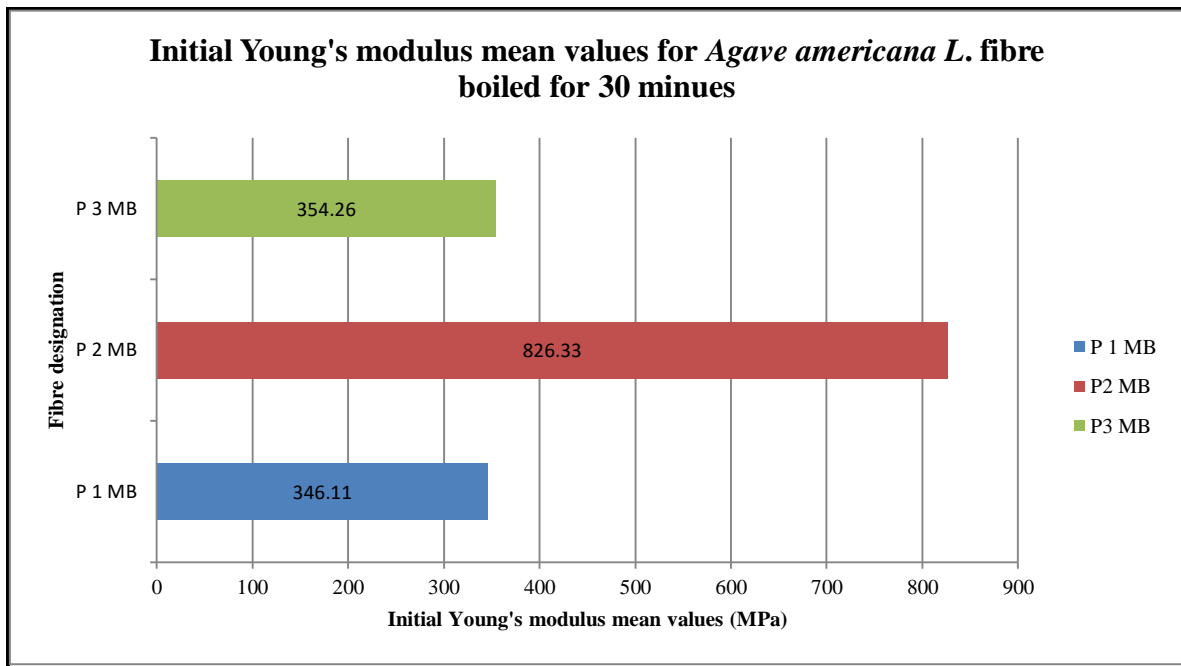
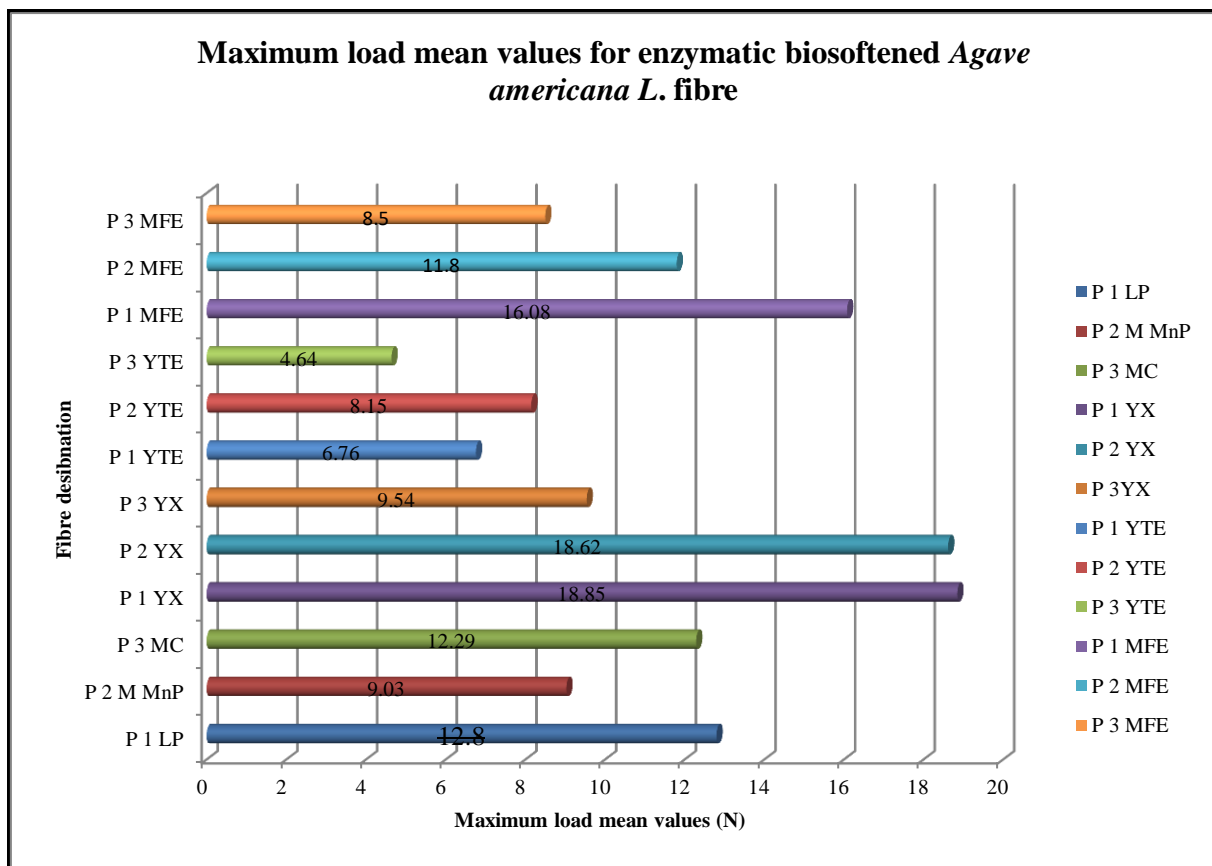


Figure 4.18: Tensile initial Young's modulus mean values (MPa) for boiled *Agave americana L.* fibre obtained from plants 1, 2 and 3

The initial Young's modulus mean value for the boiled fibre extracted from the middle leaves of plant 2; (826.33 MPa) was double; higher than the boiled fibre extracted from middle leaves of plant 1 and 3; (354.26 MPa) and (346.11 MPa) respectively. This means that fibre obtained from plant 2 was the stiffest; its shape exchanged slightly under elastic loads; when compared to plant 1 and 3 fibres which were more flexible/elastic and changed its shape considerably under elastic loads. This is not much different from that of the raw fibre. The initial Young's module for the boiled fibre varies widely from 345.11 MPa and 826.33 MPa. Thus not indicate that the boiling process did any damage to the original initial young's modulus values at the *Agave americana L.* fibre.

#### 4.6.3.7 Maximum load mean values of enzyme biosoftened *Agave americana L.* fibre

Figure 4.19 displays the maximum load mean values of enzyme biosoftened *Agave americana L.* fibre.



**Figure 4.19: Maximum load mean values (N) for single and sequential enzymatic biosoftened *Agave americana* L. fibre**

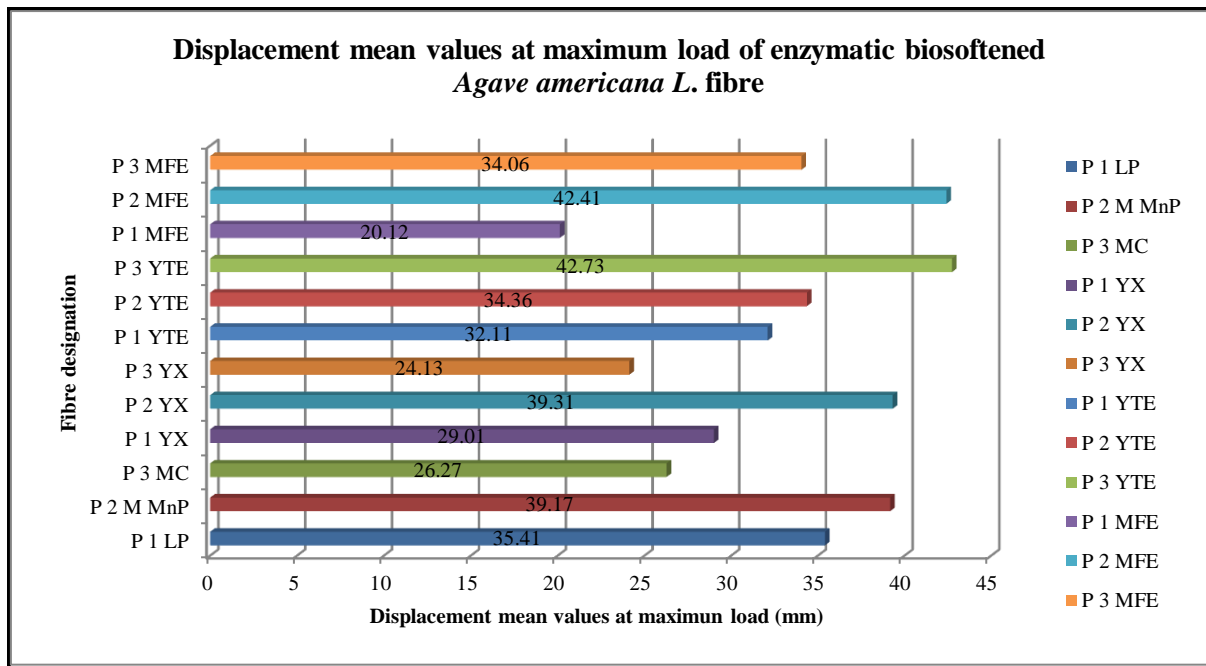
Results from figure 4.19 show that the enzyme biosoftened *Agave americana* L. fibre can bear maximum load that ranged from 4.64–18.85 N. The lowest value denotes the lowest tensile strength and was obtained from sequential pectinase, xylanase and cellulase biosoftened fibre extracted from top (young) leaves of plant 3 (P 3 YTE). The optimal value designates the maximum tensile strength and was obtained from xylanase biobleached fibre extracted from top (Younger) leaves of plant 1 (P 1 YX). There are certain theories to expound on the possible reasons for these results;

- (i) Enzymes are highly substrate selective and specific. Therefore, xylanase was likely to have hydrolysed hemicellulose only and left other components in touch. The sequential pectinase, xylanase and cellulase biosoftening led each enzyme to hydrolyse its component. However, this may not be substantive, because sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre exhibited stronger properties than the sequential pectinase, xylanase and cellulase biosoftened fibre.

- (ii) The chemical composition of the *Agave americana L.* fibre of which literature explained as mainly; 68-80% cellulose, 15% hemicellulose and 5-17% lignin might be another cause of difference.
- (iii) Furthermore, hemicellulose which is xylanase substrate is amorphous, cellulose microfibrils' supporting matrix; it is weaker and less abundant than crystalline cellulose in the *Agave americana L.* fibre (Hulle *et al.*, 2015c:71-72).
- (iv) It may be due to the fact that the *Agave americana L.* fibre is a natural lignocellulosic fibre. Intra-inter-fibre variability tensile strength due to growth irregularities is a typical feature for natural plant fibres,
- (v) The *Agave americana L.* fibre variability might be due to the position from which the fibre is extracted can further explain the different results observed for example fibre obtained from the outermost layer of the leaf sheath which is stronger than the fibre extracted from the inner leaf sheath (Hulle *et al.*, 2015c:71). In general, the load carried by the enzymatic biosoftened *Agave americana L.* fibre before breaking is lower than that of the raw *Agave americana L.* fibre. The *Agave americana L.* fibre tensile results showed that the removal of non-cellulosic fibre components through enzymatic biosoftening caused fibre strength loss. The same result was manifested in an experiment conducted by Osorio *et al.* (2012:86).

#### ***4.6.3.8 Displacement mean values at maximum load of enzyme biosoftened Agave americana L. fibre***

Figure 4.20 displays the displacement mean values at maximum load of enzyme biosoftened *Agave americana L.* fibre.



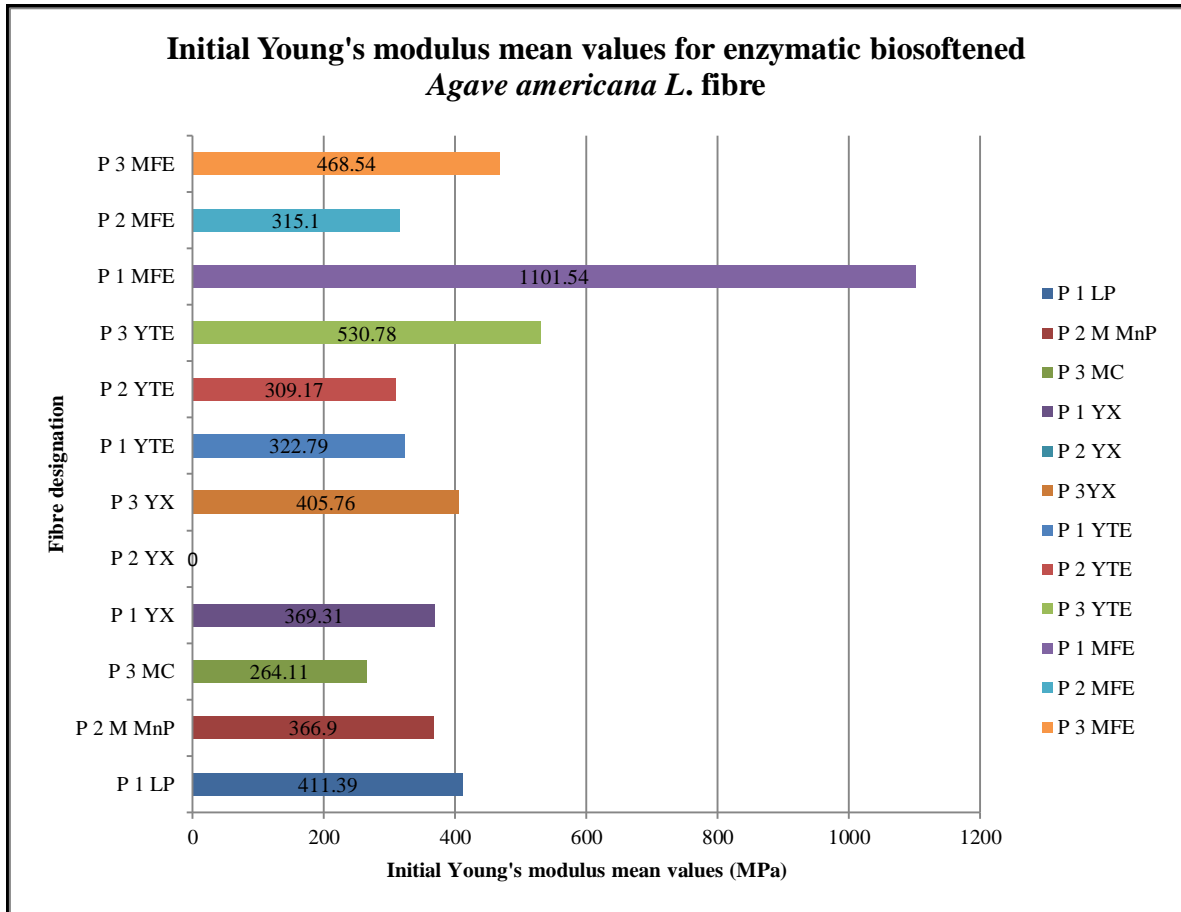
**Figure 4.20: Displacement mean values at maximum load (mm) for enzymatic biosoftening of *Agave americana* L. fibre**

The results from figure 4.20 shown more or less the same trend as other tensile properties results displayed on table and figures above. The fibre extracted from plant 2 treated with different enzymes, generally exhibited significantly higher displacement mean values, when compare to fibres obtained from plant 1 and 3. It is also observed that P 3 YTE exhibited the highest displacement mean values at maximum load of 42.73 mm, followed by P 2 MFE with 42.41 mm and P 2 YX; 39.31mm respectively. P 1 MFE exhibited the lowest displacement mean value of 20.12 mm. Displacement value at maximum load is inversely proportional to tensile maximum load at break and modulus.

When fibre tensile maximum load and initial Young’s modulus are higher, the fibre displacement value at maximum load; stretch under stress. Displacement at maximum load is a ductility degree of which the fibre experience before failure because they experience irreversible plastic deformation. A more ductile fibre exhibits a higher displacement at failure whereas a more brittle fibre exhibits extremely low displacement at failure; it exhibits lower displacement value at break, which is mostly elastic and reversible. It is therefore reasonable to think that P 3 YTE was the most ductile fibre while P 1 MFE was the most brittle of all enzyme biosoftened *Agave americana* L. fibre.

**4.6.3.9 Initial Young's modulus mean values (MPa) for enzymatic biosoftening of *Agave americana L.* fibre**

Figure 4.21 displays the initial Young's modulus mean values of enzyme biosoftened *Agave americana L.* fibre.



**Figure 4.21: Initial Young's modulus mean values (MPa) for enzymatic biosoftening of *Agave americana L.* fibre**

The impact of enzymatic biosoftening on *Agave americana L.* fibre Young's modulus is shown in figure 4.21. The maximum modulus of raw fibre was 598.13 MPa. However, the modulus for enzyme treated fibre fluctuated between 264.11 MPa for the sequential pectinase, xylanase and cellulase biosoftened fibre extracted from younger leaves of plant 3 and 1101.54 MPa for the sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened fibre extracted from middle leaves of plant 1. The highest modulus value might have been due to an effective increase in cellulose fibre orientation that might have occurred because of the elimination of the hemicellulose and lignin constituents that cause the fibres become comparatively ductile with cellulose chains increased self-realignment and stiffness.

However the general trend observed was that the initial Young' modulus decreased slightly with enzymatic treatment intensification with sequential manganese peroxidase, pectinase, xylanase and cellulase. The decrease in stiffness; resistance to deformation observed with treatment intensity might have been initiated by the elimination of lignin; the rigid component on the fibre and other non-cellulosic fibre components to the extent that it damaged the fibres inter-laminar bonding. It is also observed that P 2 XY showed zero or no initial Young's modulus. This implies that the sample was highly ductile and lacked resistance to stretching and strength (Kamarudin *et al.*, 2018:9). The initial Young's modulus of enzymatic biosoftened *Agave americana L.* fibre was slightly lower than that of a raw *Agave americana L.* fibre with the exception of P 2 MFE indicating that the biosoftening technology did not affect the fibre tensile properties negatively.

The tensile tests result, evidently designated that the tensile strength of *Agave americana L.* fibre decreased proportionately with the intensity of enzymatic biosoftening technology applied when compared to that of raw fibre (tables 4.4 and 4.5 as well as figures 4.7-4.8). The decrease in strength is mainly caused by structural changes which take place during enzymatic biosoftening. As shown by the SEM analysis, non-cellulosic surface components of the fibre were catalysed by the action of enzymes. The primary wall of the fibre consists mainly of pectin; one of the most complex non-cellulosic components. Its main function in a fibre cell is to glue together all other fibre components. Thus; its hydrolysis and elimination can destabilize other constituents and the original fibre structure as well as its tensile properties. This happened because the pectin lamellae have been disintegrated.

The enzymatic biodelignification hydrolyses lignin another important structural component of the lignocellulosic fibre cell wall which should be partially removed in order to soften the fibre. Xylanase bioleaching technology also hydrolyses hemicellulose; xylan which is one of the three main components: cellulose, hemicellulose and lignin; of the complex structural network of lignocellulosic fibres; that contributes to the decrease of fibre tensile properties. Cellulase biopolishing hydrolysed the readily accessible surface cellulose fibrils so as to biosoften the fibre with minimum tensile strength losses. Increasing enzymatic number of sequential treatments which increase effect and time also increases the breaking tenacity and initial modulus of the treated *Agave americana L.* fibre. The high values of *Agave americana L.* fibre tensile properties are a good indication that it is a potential textile fibre.

#### 4.6.4 Tensile stress-strain curves *Agave americana L.* fibre.

The *Agave americana L.* fibre mechanical properties are undoubtedly the most substantial properties to study because, they characterize the mechanical performance of the fibres during processing and use of the end products (Msahli *et al.*, 2007:3953). The stress-strain behaviour under tension is normally determined by tensile testing. The specimens' response to tensile testing force (stress) is measured during application samples are subjected to controlled tension until failure occurs. Tensile test determines how strong a material is and how long it can be stretched. The tensile test carried out in this research study provided the following important information yield maximum load in N, displacement at maximum load in mm and initial Young's modulus in GPa.

The typical strain stress curve consists of three regions: At region 1 where there is elastic behaviour, with a small deformation, the lignin bears the stress without passing it to the fibre ultimates. The first part of a stress strain curve slope is a straight line and is considered the initial modulus (Hulle *et al.*, 2015c:71). If the load was relieved at this stage the fibre would return to its original dimension as it is still elastic. It is therefore reasonable for one to conclude that the physico-mechanical properties of a composite fibre bundle are related to the physico-mechanical properties of non-cellulosic components of the cellulosic fibre. At region 2, there is viscoelastic deformation, fibre fibrils begin to change without being really deformed; due to the spring arrangement of the fibre fibrils which make the fibre bundle very extensible and begin to distort until they rupture (Hulle *et al.*, 2015c:72). At region 3 where plastic deformation of the fibre fibrils results in a deep slope; indicating the beginning of fibre failure until rupture (Gorjanc & Bukosek, 2008:63).

The tensile stress-strain curve for the tested *Agave americana L.* fibre samples were plotted to observe its behaviour when subjected to forces. Figures 4.21-4.23 are the representative stress strain curves for *Agave americana L.* fibre tested in this research study. These figures depict typical tensile maximum load (N) versus displacement at maximum load (mm) curves directly obtained from the machine digital recorded data, for each one of the tested samples of *Agave americana L.* fibre. In these diagrams shown below; the strains are plotted along the horizontal axis; converse to the stresses that are plotted along the perpendicular axis.



#### 4.6.4.1 Tensile stress-strain curves of raw *Agave americana* L. fibre

The stress-strain curves of *Agave americana* L. fibre were drawn automatically by instron tensile tester during fibre tensile testing process. The *Agave americana* L. fibre stress-strain curve indicates the relationship between force per unit area and the proportional deformation; which is a measure of its stiffness and is termed the initial Young's modulus (Da Paixao, 2017:28). Figures 4.22-4.26 show the typical the stress-strain curves and behaviour of the *Agave americana* L. fibre. Figure 4.22 displays the stress-strain curve of raw *Agave americana* L. fibre extracted from middle leaves of plant 2 (P 2 MR).

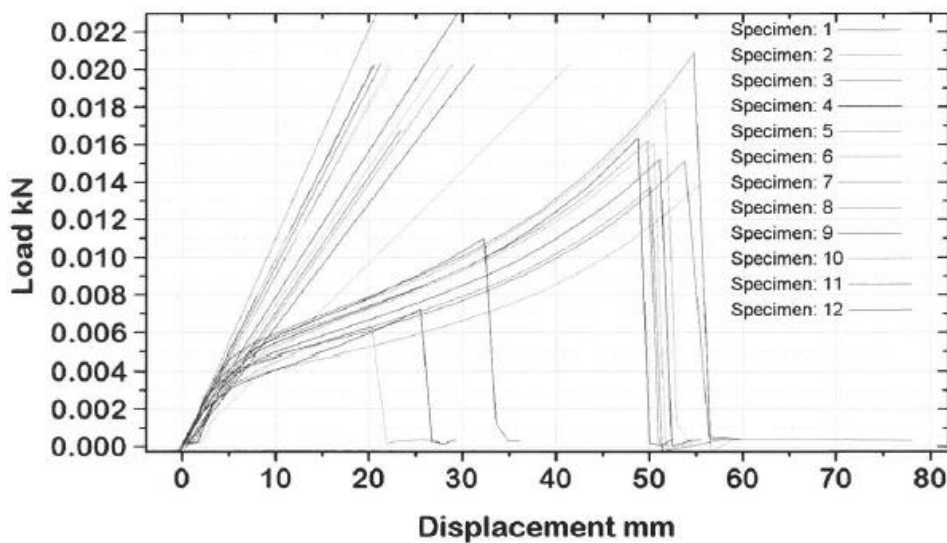


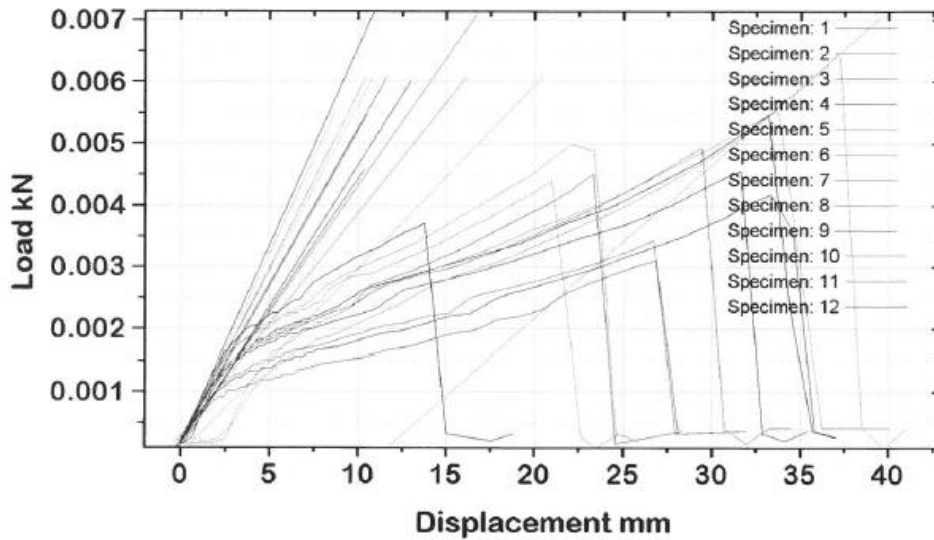
Figure 4.22: The stress-strain curve of raw *Agave americana* L. fibre extracted from middle leaves of plant 2 (P 2 MR)

The tensile stress-strain diagrams of the tested *Agave americana* L. fibre provide the maximum tensile load strengths and the ratio of stress over strain which indicates the Young's modulus within the linear region. Young's modulus is a degree of the fibre's resistance to the elastic deformation. The fibre that exhibits higher stiffness has higher Young's modulus values and has fewer propensities to the deformation along an axis when opposite forces are applied (Shah A.U. *et al.*, 2016:10661).

The fibre tensile strength and elongation influence the performance properties of the textile products manufactured from it. The tensile strength is measured when the force is employed in the form of springs or weights on the fibre and the fibre is stretched and resistant to breaking (Sinclair, 2015:15). The stress-strain curve in (figure 4.22), *Agave americana* L. fibre shows a relatively brittle nature that is characterised by skipping of the plasticity region to the elastic

limit where the fibre undergoes a fracture followed by an immediate load drop when subjected to excess stress.

Figure 4.23 exhibits the stress-strain curve of raw *Agave americana L.* fibre extracted from tip part of middle leaves of plant 2.



**Figure 4.23: The stress-strain curve of raw *Agave americana L.* fibre extracted from tip part of middle leaves of plant 2 (P 2 MRT)**

The stress-strain curve in figure 4.23 is an example of the typical viscoelastic behaviour of the natural lignocellulosic fibre. This can be expounded by the fact that the fibre is obtained from the tip area of the leaf. The tip is the growing zone of the leaf; this implies that the fibre is thinner and younger than the fibre at the base of the leaf. Hence why the displacement is short. the curve slope showed that the tensile strength of P 2 MRT was lower than that of the other raw fibres.

Figure 4.24 presents the stress-strain curve of raw *Agave americana L.* fibre extracted from the lower leaves of plant 3.

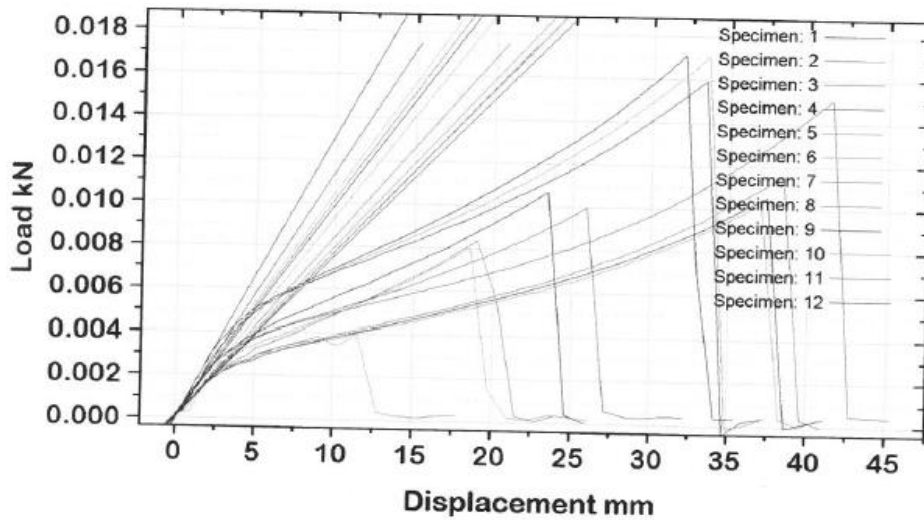


Figure 4.24: The stress-strain curve of raw *Agave americana L.* fibre extracted from lower leaves of plant 3 (P 3 LR)

With the exception of the initial; lowest strain rate, the curve indicates that the fibre mainly exhibits the brittle rupture. The curve showed strain-hardening that can be reasoned as a microfibrillar progressive orientation of internal structures that includes defects and microfibrillar angle.

Figure 4.25 presents the stress-strain curve of raw *Agave americana L.* fibre extracted from middle part of middle leaves of plant 3.

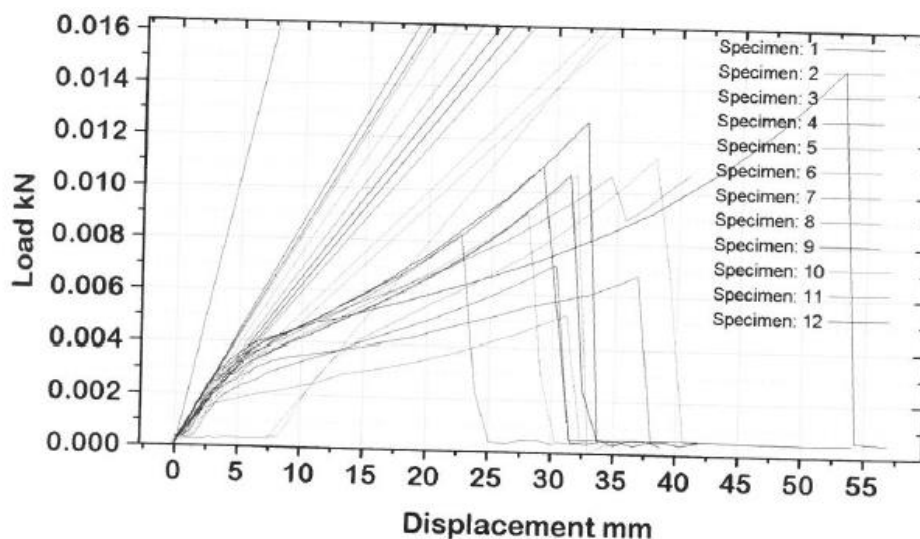
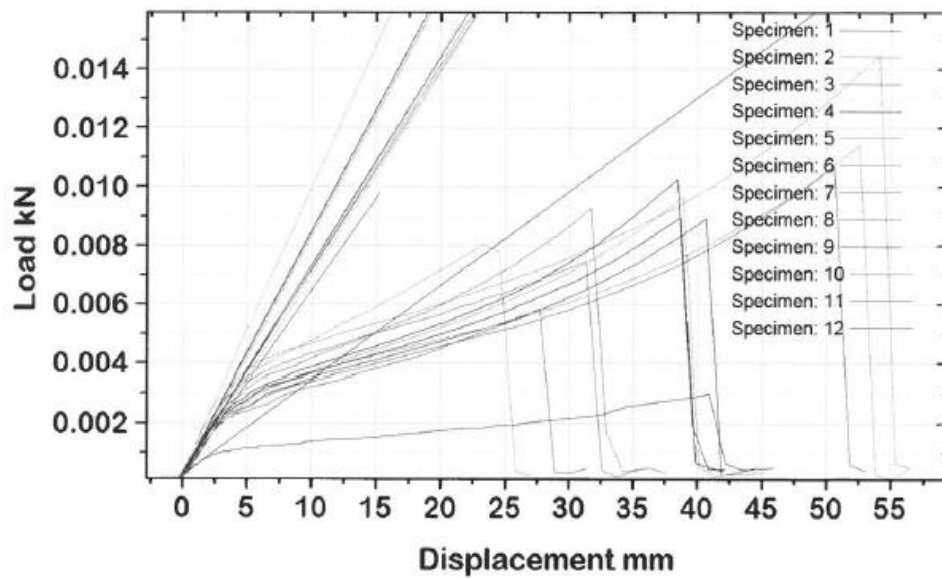


Figure 4.25: The stress-strain curve of raw *Agave americana L.* fibre extracted from middle part of middle leaves of plant 3 (P 3 MRM)

Generally, the untreated fibre shows a brittle breaking behaviour with an abrupt load fall when fibre failure occurs. The stress-strain curves of raw *Agave americana L* fibre except that of fibre extracted from tip part of middle leaves of plant 2 (P 2 MRT), indicate the fibre tensile strength and stiffness are proportionally high when compared to those of boiled and enzyme treated fibre.

Figure 4.26 illustrates the stress-strain curve of raw *Agave americana L*. fibre extracted from middle part of middle leaves of plant 3.



*Figure 4.26: The stress-strain curve of raw Agave americana L. fibre extracted from middle part of middle leaves of plant 3 (P 3 MRM)*

#### **4.6.4.2 Tensile stress-strain curves of boiled *Agave americana L*. fibre**

Figures 4.27-4.29 present the tensile stress strain curves of boiled *Agave americana L*. fibre. Figure 4.27 displays the tensile stress strain curves of boiled *Agave americana L*. fibre extracted from middle leaves of plant 1.

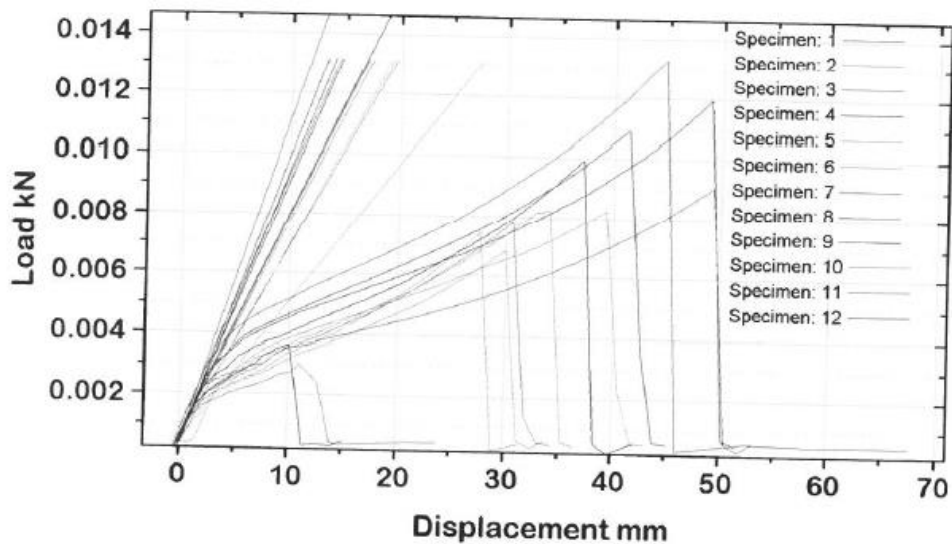


Figure 4.27: The stress-strain curve of boiled *Agave americana L.* fibre extracted from middle leaves of plant 1 (P 1 MB)

The slope of the curve in the linear region is where the evaluation of the fibre’s young modulus occurs in the stress-strain curve (Osorio *et al.*, 2012:86).

Figure 4.28 displays the tensile stress strain curves of boiled *Agave americana L.* fibre extracted from middle leaves of plant 2.

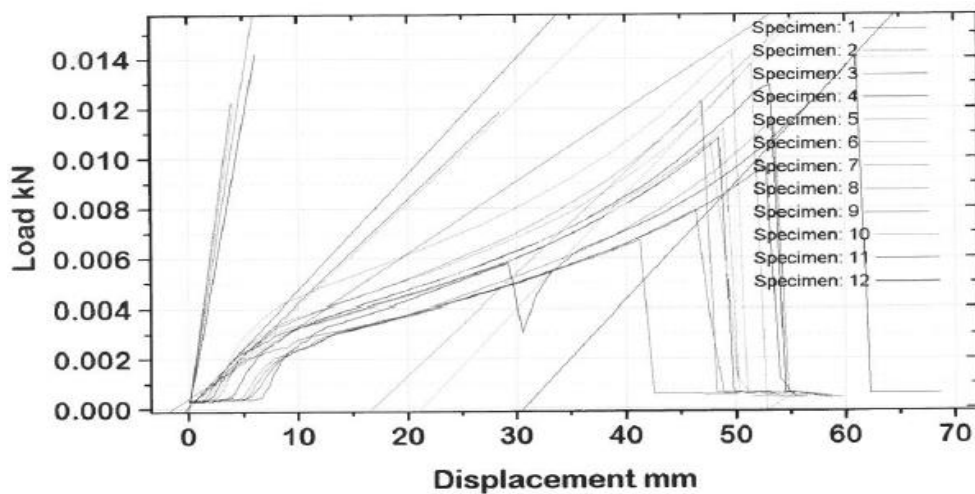
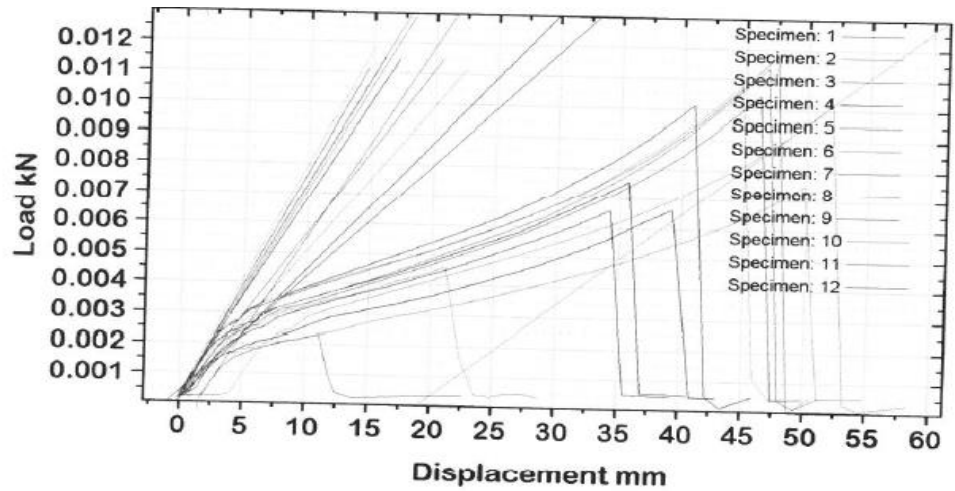


Figure 4.28: The stress-strain curve of boiled *Agave americana L.* fibre extracted from middle leaves of plant 2 (P 2 MB)

Figure 4.29 displays the tensile stress strain curves of boiled *Agave americana L.* fibre extracted from middle leaves of plant 3.



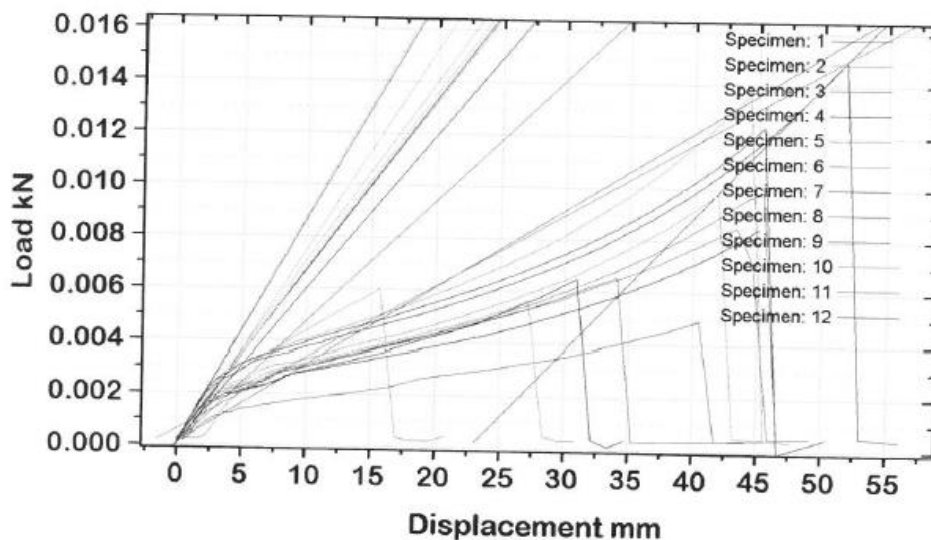


**Figure 4.29: The stress-strain curve of boiled *Agave americana L.* fibre extracted from middle leaves of plant 3 (P 3 MB)**

The Young’s modulus of the boiled fibre is lower than that of the raw fibre and this fact may be ascribed to the elimination of some fibre impurities.

**4.6.4.3 Tensile stress-strain curves of enzyme biosoftened *Agave americana L.* fibre**

Figures 4.30-4.37 present the stress–strain curves of enzyme biosoftened *Agave americana L.* fibre. Figure 4.30 displays the stress–strain curves of manganese peroxidase biodelignified *Agave americana L.* fibre extracted from middle leaves of plant 2.



**Figure 4.30: The stress-strain curve of manganese peroxidase biodelignified *Agave americana L.* fibre extracted from middle leaves of plant 2 (P 2 M MnP)**

The stress-strain curve exhibited variation in some specific areas. This can be explained as a cause of constant microfibrillar readjustments that occur in the fibre axial direction during testing (Osorio *et al.*, 2012:84).

Figure 4.31 displays the stress-strain curves of xylanase biobleached *Agave americana L.* fibre extracted from top leaves of plant 1.

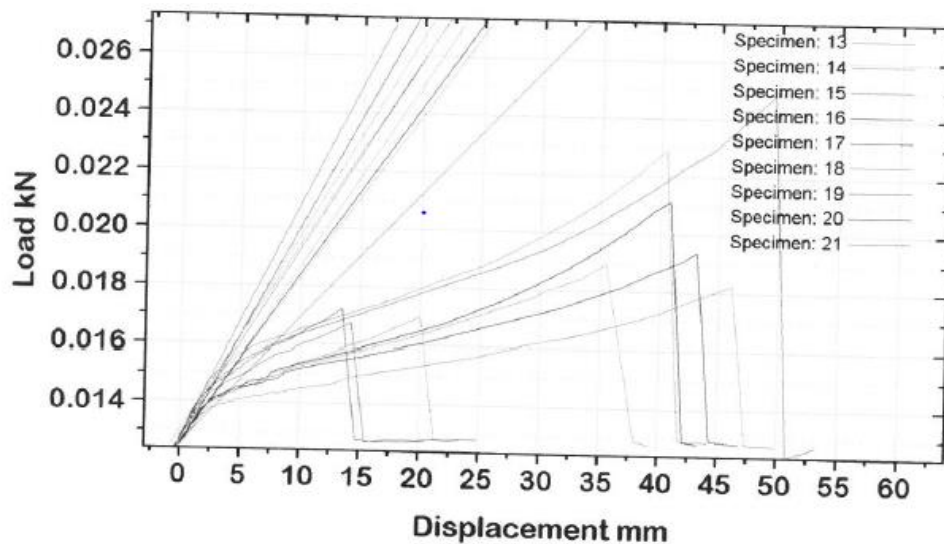


Figure 4.31: The stress-strain curve of xylanase biobleached *Agave americana L.* fibre extracted from top leaves of plant 1 (P 1 YX)

Figure 4.32 displays the stress-strain curves of xylanase biobleached *Agave americana L.* fibre extracted from top leaves of plant 2.

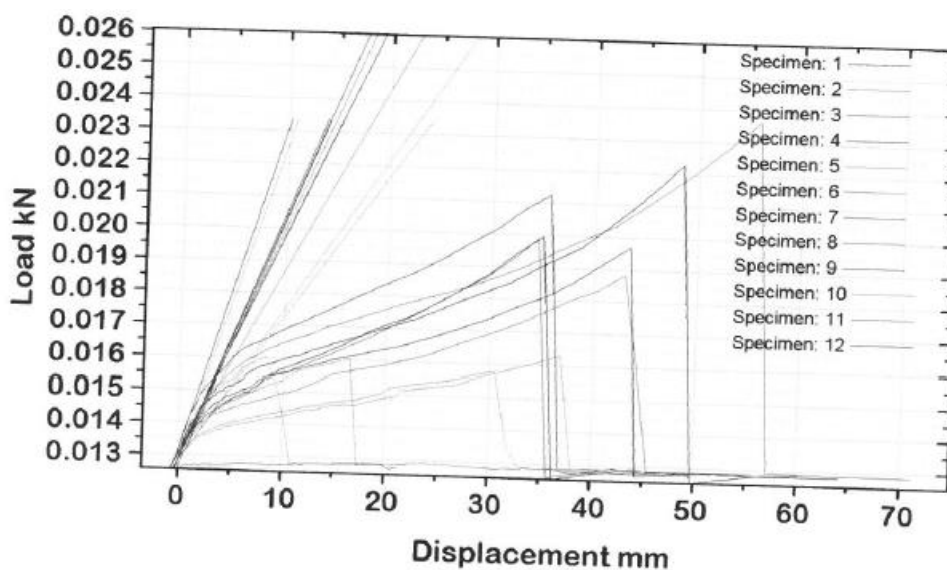


Figure 4.32: The stress-strain curve of xylanase biobleached *Agave americana L.* fibre extracted from top leaves of plant 2 (P 2 YX)



Figure 4.33 displays the stress–strain curves of xylanase biobleached *Agave americana* L. fibre extracted from top leaves of plant 3.

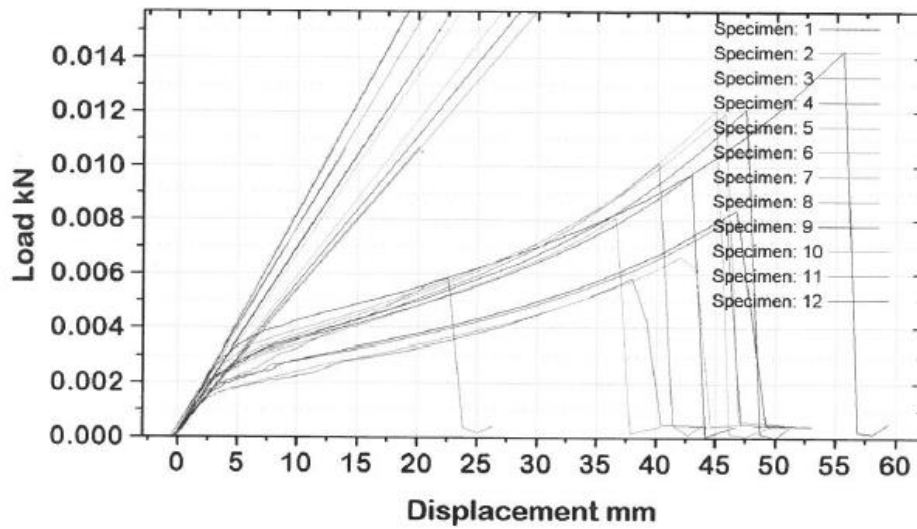


Figure 4.33: The stress-strain curve of xylanase biobleached *Agave americana* L. fibre extracted from top leaves of plant 3 (P 3 YX)

The stress-strain curve of sequential pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from the top young leaves of plant 1 is presented in figure 4.34.

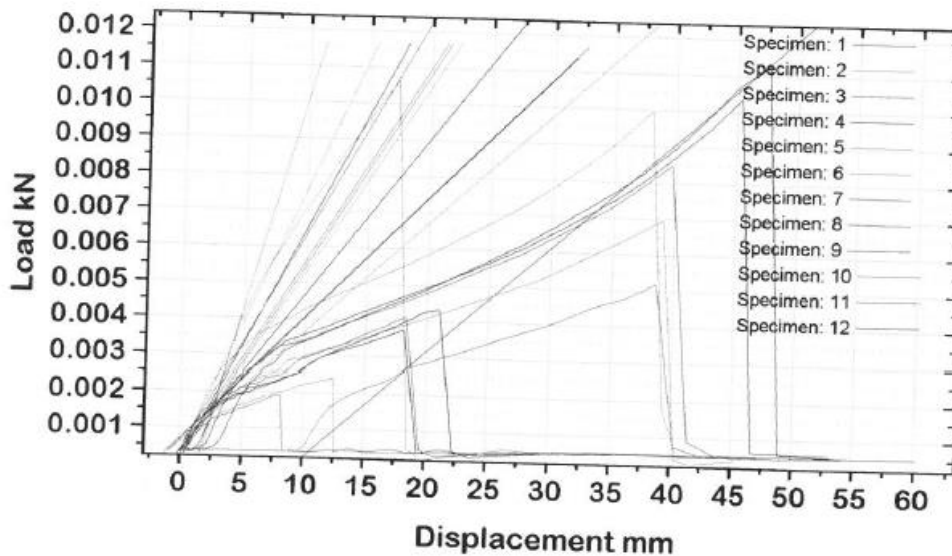
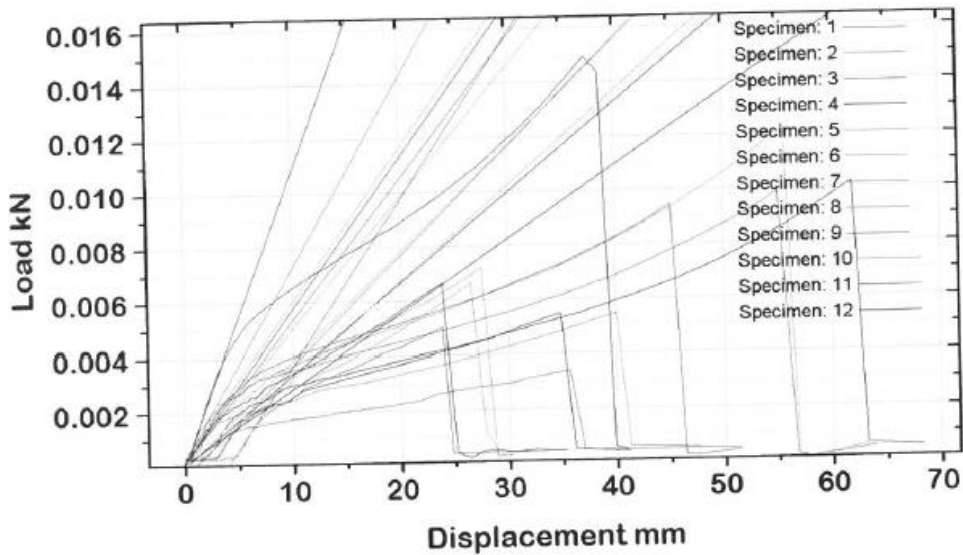


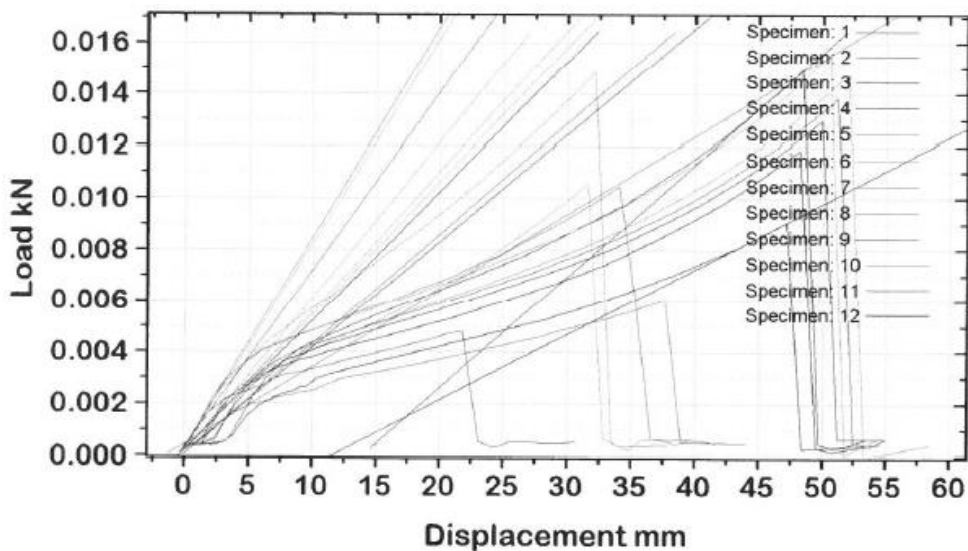
Figure 4.34: The stress-strain curve of sequential pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from top (young) leaves of plant 1 (P1 TYE)

Figure 4.35 displays the stress-strain curve of sequential pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from top (young) leaves of plant 2. In the softer fibre the stress-strain curves exhibited longer and deeper curvatures figures.



**Figure 4.35: The stress-strain curve of sequential pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from top (young) leaves of plant 2 (P 2 YTE)**

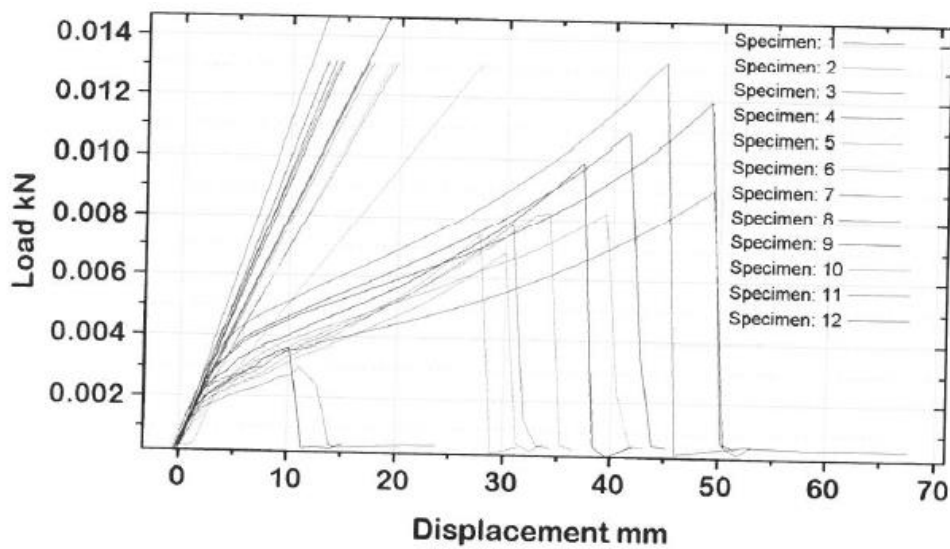
Figure 4.36 shows the stress-strain curve of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from middle leaves of plant 2. The stress-strain curve designated the fibre ruptured in a brittle mode subsequent to the yield point. The viscoelastic nature under loading of the *Agave americana* L. fibre is indicated by the stress-strain curvature as observed in figures 4.22-4.37. The viscoelastic nature of the fibre can be explained by the fact that *Agave americana* L. fibre is a natural composite, consists of helically wound crystalline cellulose microfibrils (Hulle et al., 2015b:3).



**Figure 4.36: The stress-strain curve of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre extracted from middle leaves of plant 2 (P 2 MFE)**

The MnP, pectinase, xylanase and cellulase respective sequential enzymatic biosoftening of *Agave americana L.* fibre separated the fibre from its non-cellulosic constituent; the sequential enzymatic biosoftening technique removed from the surface of the fibre; the lignin and hemicelluloses. This reduced the breaking strength of the biosoftened fibre. The same results were found by Osorio *et al.* (2012:86), using a mixture of cellulase, xylanase and pectinase.

Figure 4.37 depicts the stress-strain curve of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre extracted from middle leaves of plant 3.



**Figure 4.37:** The stress-strain curve of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre extracted from middle leaves of plant 3 (P 3 MFE)

Enzyme treated fibre exhibits a nonlinear; viscoelastic deformation behaviour when fibre failure occurs. It is observed from figures 4.30-4.37 that *Agave americana L.* fibre stress-strain curves are characterized by short, initial linear region of Young’s modulus, followed by a viscoelastic curvature before failure, indicating a variation of the strain rate produced with the variation of the stresses. The short, initial linear region of Young’s modulus is characterised by a small elongation and a full instant elastic recovery. This means the fibre stress is constantly proportional to strain as the Young’s modulus that obeys Hooke’s law to a reasonable approximation. From the proportional limit, the strain normally increases, in such a way that the fibre deviated from the linear proportionality.

The subsequent curvature observed in the curve illustrated the viscoelastic nature of the *Agave americana L.* fibre. To understand the observed viscoelastic behaviour, it is necessary to

consider that *Agave americana L.* fibre, is regarded as natural composites of cavities of spiral, multi-cellular crystalline and non-crystalline cellulose microfibrils that are entrenched in an amorphous hemicellulose-lignin matrix. As the stress was constantly applied, it was also shared between crystalline and non-crystalline fibre components. The spiral cellulose microfibrils undergo deformation, by first unwinding and then followed by matrix resilience and slippage of molecules, ending up with crystalline and non-crystalline molecule decohesion especially at weaker joints or defects. This complicated *Agave americana L.* fibre structure can influence its strain–stress behaviour (Hamad *et al.*, 2017:296). The fibre age and origin can also influence its resistance to deformation when the strain is low (Júnior *et al.*, 2016:20).

The *Agave americana L.* fibre stress strain curves were characterised by skipping the plastic behaviour and ending with sudden fibre rupture; as the applied stress increased; to a point where the fibre could not bear it any more, it broke at the maximum strain. In the plastic region the ductile fibre is believed to undergo permanent deformation where it rearranges its internal molecular structure. a mechanism for molecular movement, is required by the fibre plasticity from which dislocation of crystalline materials can arise. It is assumed that *Agave americana L.* fibre is deficient in this mobility, and has internal microstructures that restrict dislocation movement.

It exhibited the typical linear viscoelastic stress-strain curve that breaks without appreciable plastic flow because it is a brittle fibre that shows very little bend before fractures when exposed to additional stress. The resistance of *Agave americana L.* fibre fibrils in the rupture zone, is due to their size and number, dispersal and their connection to the natural matrix which explain the behaviour of fibre bundles. It is therefore reasonable for one to conclude that the mechanical behaviour of *Agave americana L.* fibre bundles is greatly correlated to its fine structure. A progressive realignment of cellulose microfibrils was observed for some fibre samples (figure 4.22b and 4.24h).

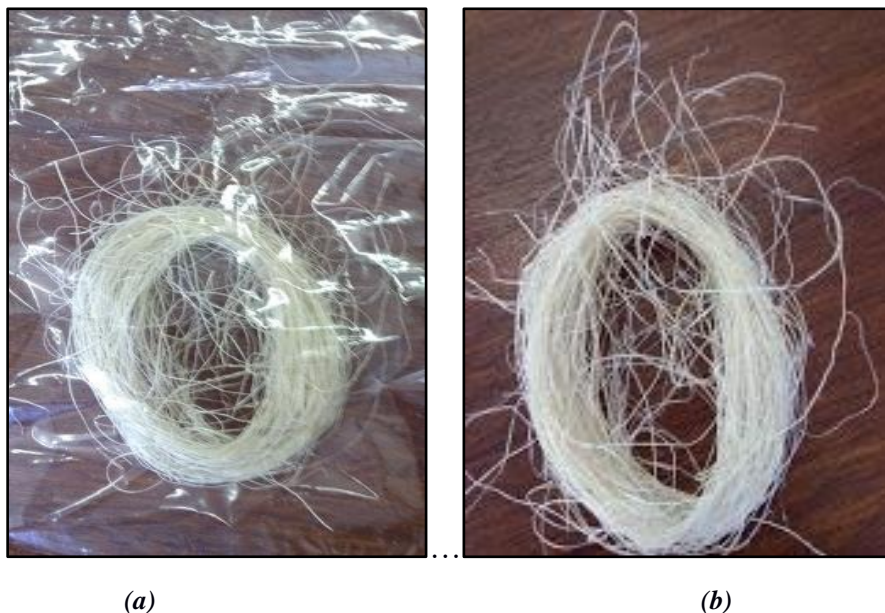
Its stress-strain curves show were comparatively the same and comparable to those of other natural lignocellulosic fibres like sisal (Ananjiwala *et al.*, 2010:189), jute (Bourmaud *et al.*, 2018:379-380) angustifolia HawAgave (Silva-Santos *et al.*, 2009:105), banana (Aseer *et al.*, 2013:202). A number of *Agave americana L.* fibre stress-strain curves relatively confirm the high fibre variability of all its mechanical properties. This scattering shows the particular structure of *Agave americana L.* fibre. The sizes of the fibre bundles fluctuate along its length and among different fibres within the same leaf from the same plant and from the different



plants due to varying numbers of fibre fibrils that comprise the fibre bundle. It is observed that *Agave americana L.* fibre presented high tensile strength and modulus values because it is made up of more crystalline regions and showed little or no plastic curvature at failure because it is a brittle fibre. These results further confirm the results obtained by El Oudiani *et al.* (2008:3) and El Oudiani *et al.* (2009:3954).

#### 4.7 THE ANALYSIS OF AGAVE AMERICANA L. FIBRE SURFACE MORPHOLOGY

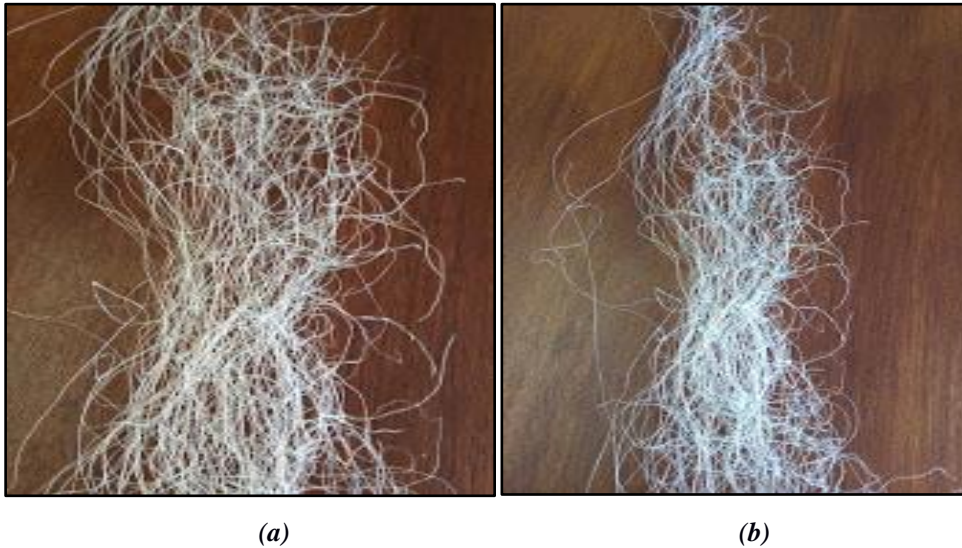
The *Agave americana L.* fibre is extracted as fibre bundles consist of minute individual microfibrils referred to as ultimates (Hulle *et al.*, 2015b:3). It is in the form of elongated cemented and approximately cylindrical fibre bundles, not the individual microfibrils *per se*. In this case, the *Agave americana L.* fibre bundles' morphology resembles the morphology of other multicellular plant fibres, such as *Agave sisalana*, *Agave tequilana*, banana leaf fibre, Doum palm fibres (Zannen *et al.*, 2014:204; Hidalgo-Reyes *et al.*, 2015:813-814). Biosoftening of *Agave americana L.* fibre with commercial lignocellulolytic MnP, pectinase, xylanase and cellulase resulted in improved physical properties of the fibre. Figure 4.38 illustrates the photographs for individual enzymatic biosoftened *Agave americana L.* fibre



**Figure 4.38: Photographs of the single enzymatic biosoftened *Agave americana L.* fibre (a) Cellulase biopolished fibre (b) Xylanase biobleached fibre.**

Figure 4.38 shows the surface modification of individual enzyme biosoftened *Agave americana* L. fibre conferring more even, softer and smoother texture with lesser surface fuzzing and brighter glossier lustre and whiter colour than the raw fibre. The cellulase biopolishing improved fibre appearance by hydrolysing apparent loosely hanging surface fibrils and microfibrils which are then easily sheared off during wet and dry cleaning (figure 4.38 (a)). Biobleaching with xylanases softens the fibre by hydrolysing and removing xylan the main hemicellulose that is associated with other non-cellulosic impurities which are air oxidised to decolourise and bring about a characteristic brown colour to fibre, which is removed to whiten the fibre(figure 4.38 (b)).

Figure 4.39 illustrated the photographs for sequential enzymatic biosoftened *Agave americana* L. fibre.



**Figure 4.39: Photographs of the sequential enzymatic biosoftened *Agave americana* L. fibre (a) Sequential pectinase, xylanase and cellulase biosoftened fibre. (b) Sequential MnP, pectinase, xylanase and cellulase biosoftened fibre.**

Enzymatic *Agave americana* L. fibre was biosoftened under eco-friendly environment and it looked and felt cleaner, with fewer or no fuzz, smoother appearance and softer texture than the raw and boiled fibres. The sequential biosoftening of *Agave americana* L. fibre hydrolysed more non-cellulosic impurities and rendered the fibre more even, softer, suppler and whiter in colour from the fibre were removed more evenly. One can conclude that the increased biosoftening time with a number of enzymes used in sequential biosoftening processes; removed more impurities whitened, and thinned the *Agave americana* L. fibre more than

individual figure 4.38 and fewer sequential enzymatic biosoftening processes as illustrated in figures 4.39 (b) fibre has more improved physical textile properties than figure in 4.39 (b).

#### **4.7.1 Scanning Electron Microscope (SEM) analysis**

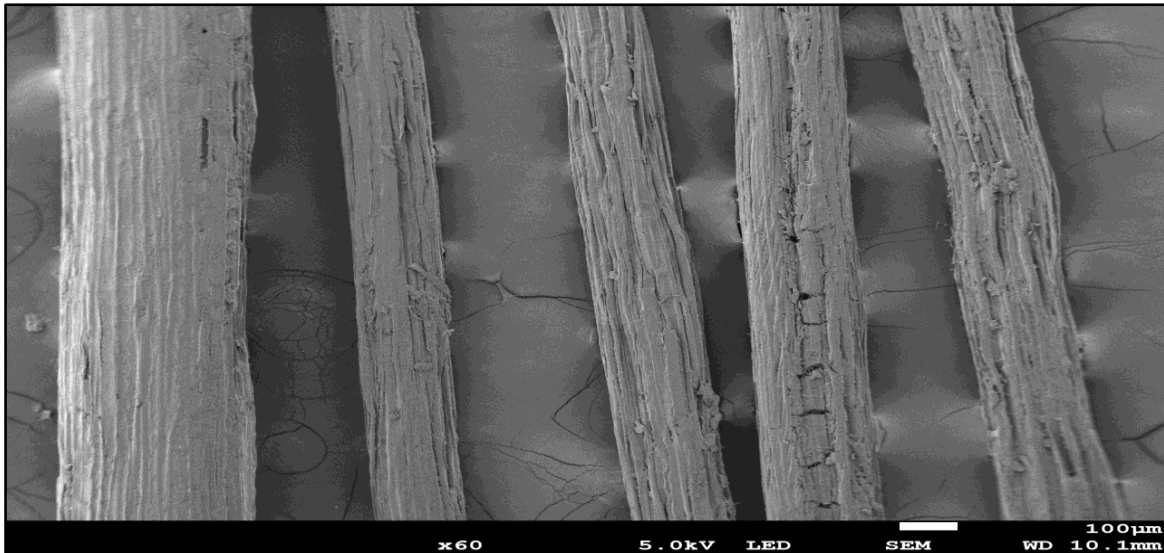
Jabasingh and Nachiyar, (2012:14) claimed that SEM images can effectively be used to validate qualitative results for research analysis. The longitudinal SEM images, of individual *Agave americana L.* fibre, were shown in figures 4.40, 4.41, 4.42, 4.43 and 4.44. These views show that *Agave americana L.* fibre is a composite vascular bundle and consists of several minute elongated sclerenchymal and parenchymal microfibrillar cells. The sclerenchyma and parenchymal cells are then embedded in the thin-walled tissues (figures 4.40-4.46). During fibre extraction, the thin cell walls are broken down to release fibre bundles.

However, there is cell wall debris that remains attached on the fibre bundles surface as observed in figures 4.40-4.57, which must be removed to improve the quality of the fibre bundles. The removal of fibre bundle debris attached on the surface was achieved through enzymatic biosoftening technologies, which also removed the cuticle; outer waxiest layer and other non-cellulosic fibre components. The structural morphology of untreated (raw) and enzyme-treated *Agave americana L.* fibre has been investigated using the scanning electron microscope (SEM) to express the structural improvements that occur in fibre during enzymatic biosoftening. The longitudinal SEM analysis of raw, boiled and enzyme-treated *Agave americana L.* fibre samples was conducted and the results are shown in figures 4.40-4.57.

##### ***4.7.1.1 The SEM analysis of the raw Agave americana L. fibre***

Figures 4.40-4.46 presents the scanning electron micrographs of raw *Agave americana L.* fibre extracted from the top and middle leaves of the plant 1 and 3. Figure 4.40 displays longitudinal images of the *Agave americana L.* fibre extracted from the top leaves of the plant 1.

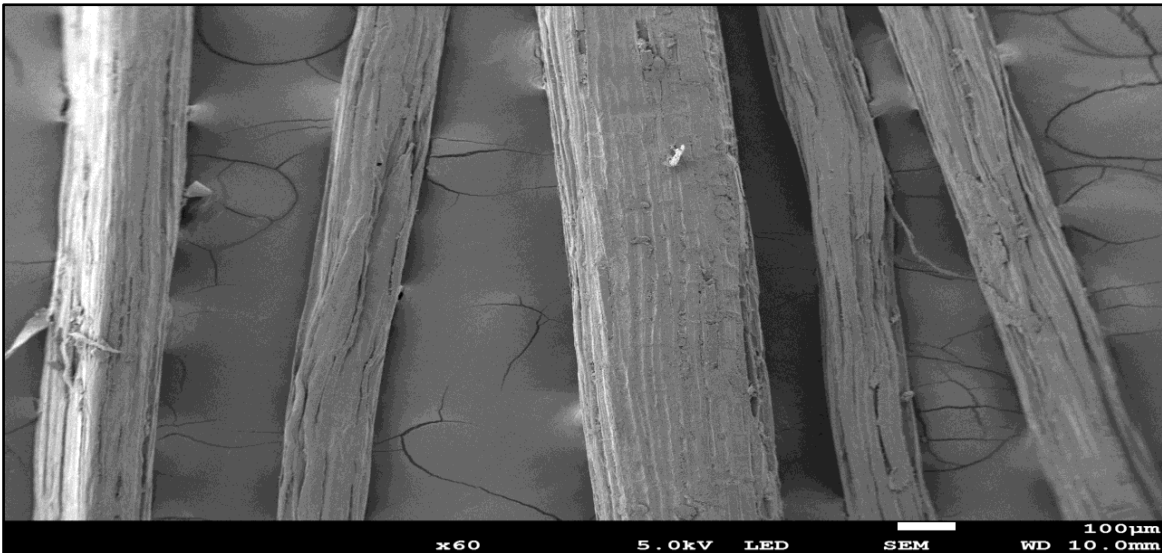




*Figure 4.40: The longitudinal SEM micrographs of raw Agave americana L. fibre harvested from the top leaves of plant 1*

Based on the SEM analysis shown in figure 4.40, raw *Agave americana L.* fibre surface is compact and rigid with elongated irregular cellular structures. It is also observed that *Agave americana L.* fibre is approximately cylindrical in shape. The surface morphology of raw *Agave americana L.* fibre displays parallel lines of cellulose microfibrils entrenched in the soft milieu of the amorphous non-fibrous fibre components; lignin and hemicelluloses as well as the non-cellulosic (Sosiati *et al.*, 2013:44) debris that remained on the fibre surface and resulted in surface roughness. The irregular cell wall topographies of raw *Agave americana L.* fibre were shown by microscopic micrograph. There are some lengthwise and crosswise cracks causing parallel ridges and splits observed. The fibre surface is also characterized by small striations and deep fissures. It is also observed that some parts of the fibre are damaged and ruptured in the raw *Agave americana L.* fibre. This might have been due to the mechanical processes employed.

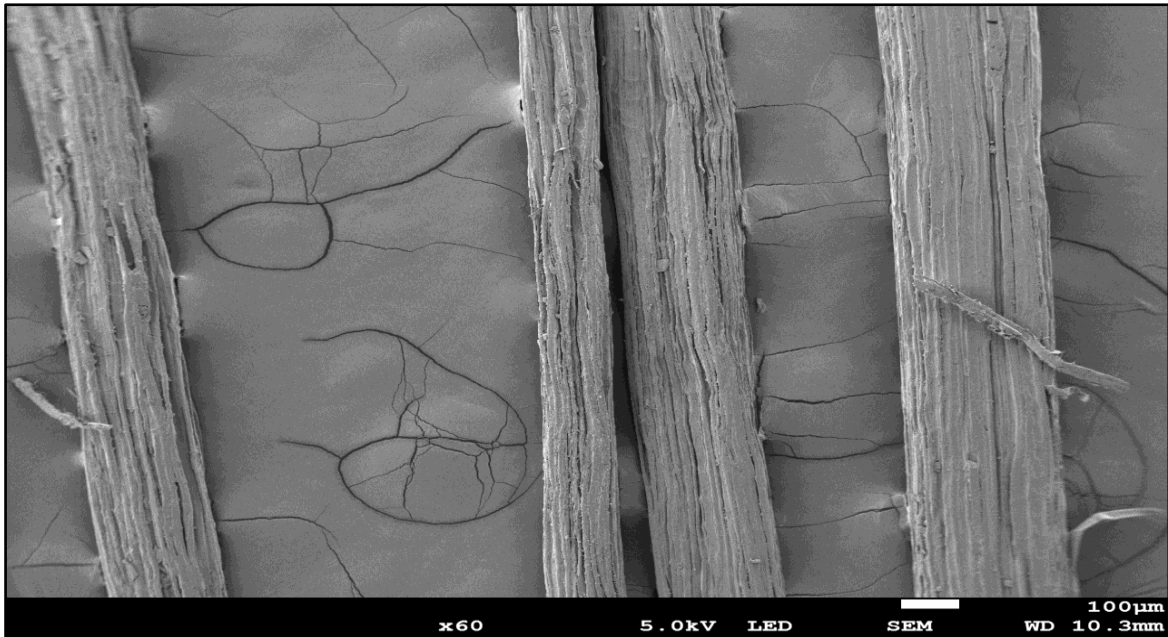
Figure 4.41 presents the raw *Agave americana L.* fibre scanning electron micrographs, extracted from the top leaves of plant 1.



*Figure 4.41: The longitudinal SEM micrographs of Raw Agave americana L. fibre harvested from the top leaves of plant 1*

One can also notice from figure 4.41 that the fibre thickness was longitudinally not uniform throughout. Fibre part from the base of the leaf consists of more ultimate fibres, which decrease gradually in number toward the tip of the leaf. This means the fibre tapers towards the tip. The SEM image reveals that the surface of the raw *Agave americana L.* fibre was covered with a primary wall structure with impurities were found embedded on the fibre surface and had parallel rough streaks and hollows in them. Voids are also observable defects in figure 4.41. The surface of raw *Agave americana L.* fibre shows more impurities compared to the treated fibre surface. The longitudinal views confirm morphological fibre variability in size and shape.

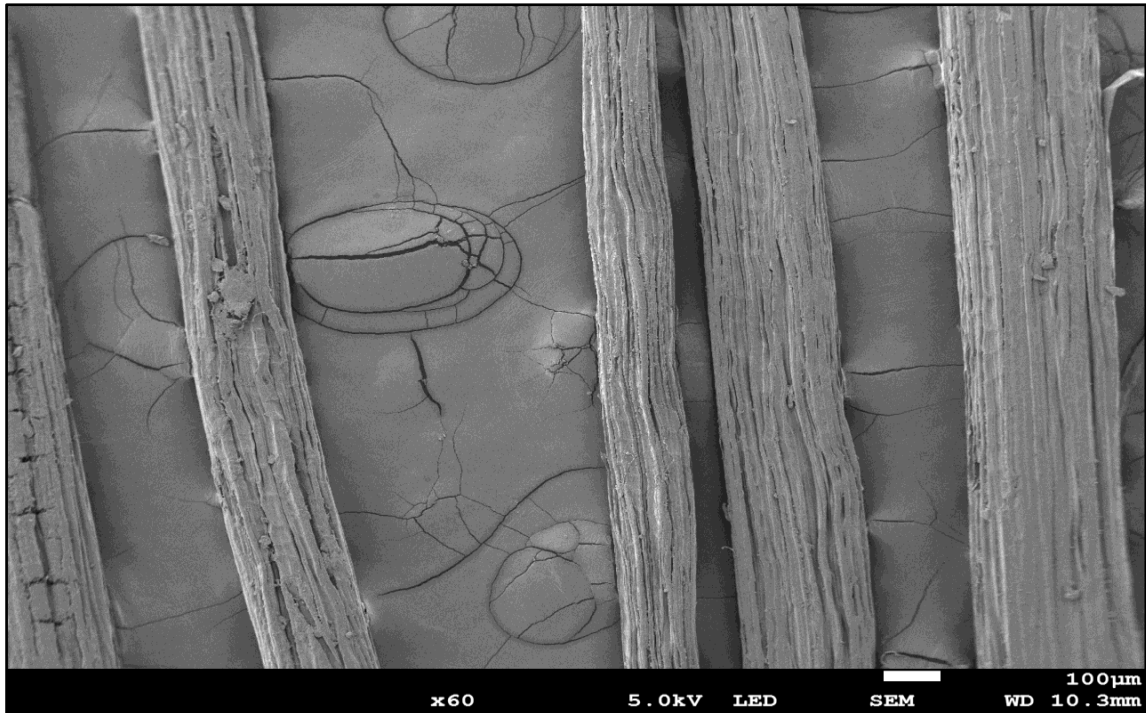
Figure 4.42 illustrates the raw *Agave americana L.* fibre scanning electron micrographs, extracted from the top leaves of plant 1.



*Figure 4.42: The longitudinal SEM micrographs of raw Agave americana L. fibre extracted from the top leaves of plant 1*

It is observed that the fibre surface is waxy with protruding microfibrils and rough. *Agave americana L.* fibre is a natural technical leaf fibre composed of ultimate fibrils embedded in the natural cementing non-cellulosic fibre constituents. The fibre surface of raw *Agave americana L.* fibre is contaminated with parenchymatous tissue remains, and the collection of non-fibrous debris. The primary cell wall structure covering the entire surface; along with ultimate cells of the technical fibre were noticeable in the raw *Agave americana L.* fibre. As a natural fibre *Agave americana L.* fibre illustrated extreme fibre bundle variability. The fibre surface looked irregular in texture, size and shape. The longitudinal surface shows multicellular over-lapping micro-fibrils that run parallel to the fibre's axis.

Figure 4.43 shows the longitudinal SEM micrographs of raw *Agave americana L.* fibre harvested from the top leaves of plant 1.

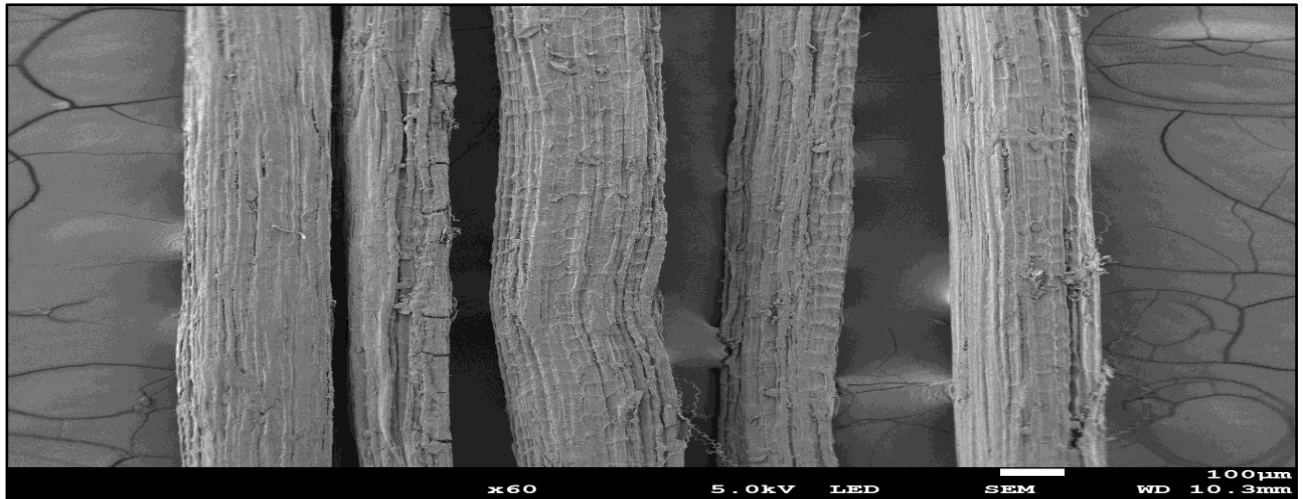


*Figure 4.43: The longitudinal SEM micrographs of raw Agave americana L. fibre harvested from the top leaves of plant 1*

The sample of raw *Agave americana L.* fibre (figure 4.43) the fibre surface is observed contaminated with parenchymatous tissue remains and the collection of non-fibrous debris. There are some lengthwise cracks causing parallel ridges with some splits observed. The primary cell wall structure covering the entire surface; along with ultimate cells of the technical fibre were noticeable in the raw *Agave americana L.* fibre. It is also observed that *Agave americana L.* fibre consists of the individual microfibrils (figure 4.43).

Figure 4.44 presents the longitudinal SEM micrographs of raw *Agave americana L.* fibre harvested from the middle leaves of plant 3.

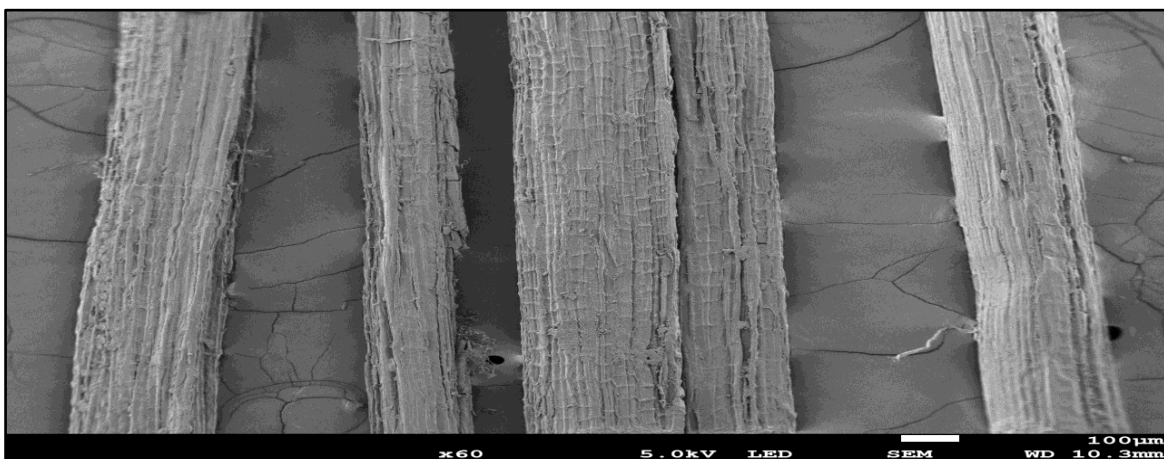




**Figure 4.44: The longitudinal SEM micrographs of raw *Agave americana L.* fibre harvested from the middle leaves of plant 3**

Figure 4.44 shows the compact technical *Agave americana L.* fibre with no or very little spaces in between the longitudinal fibre ultimates that are embedded in adhesive residual non-fibrous impurities observed on the surface; indicating that fibre retting could have been minimal (Ray *et al.*, 2015b:694). It looks stiff, harsh, coarse and hard-surfaced. The sample fibres are akin to other plant leaf fibres such as banana and sisal and other lignocellulosic fibres that include jute, flax, to mention a few (Hulle *et al.*, 2015c:71).

Figure 4.45 illustrates the longitudinal SEM micrographs of raw *Agave americana L.* fibre harvested from the middle leaves of plant 3.

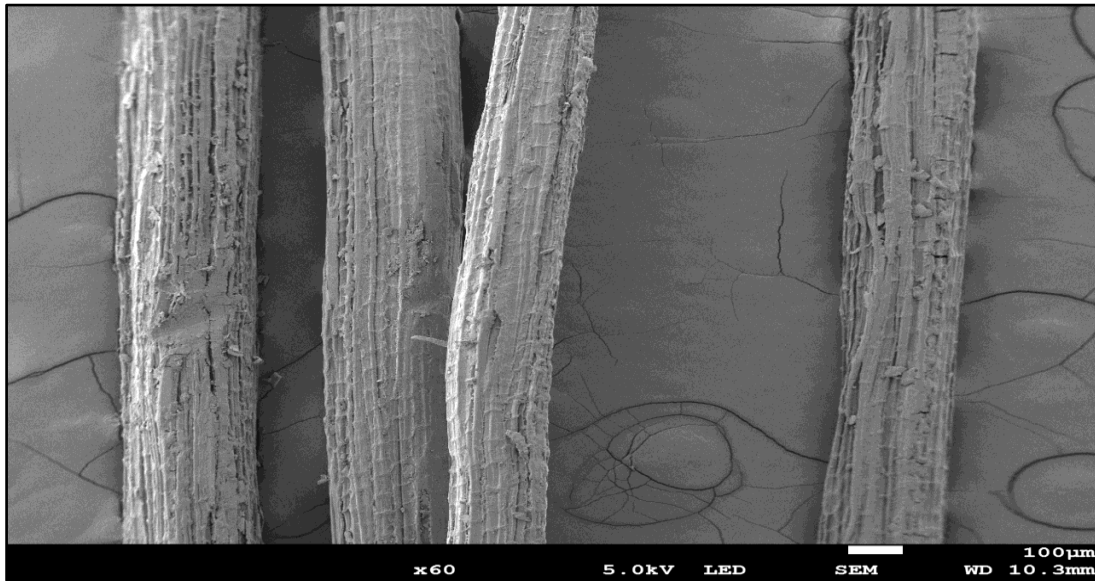


**Figure 4.45: The longitudinal SEM micrographs of raw *Agave americana L.* fibre harvested from the middle leaves of plant 3**

Figure 4.45 shows that there is substantial inconsistency in surface roughness with entrenched impurities and middle lamellae residues. This may be instigated either by insufficient retting or

fibre extraction cleaning processes, which can be influenced by a substantial lignification or improper mechanical means. The irregular cell wall characteristics and extracellular non-fibrous impurities were observed on the surface of SEM images of raw fibre which results in bumpy and uneven fibre surfaces.

Another longitudinal SEM micrograph of raw *Agave americana L.* fibre harvested from the middle leaves of plant 3 is presented in figure 4.46.



*Figure 4.46: The longitudinal SEM micrographs of raw Agave americana L. fibre harvested from the middle leaves of plant 3*

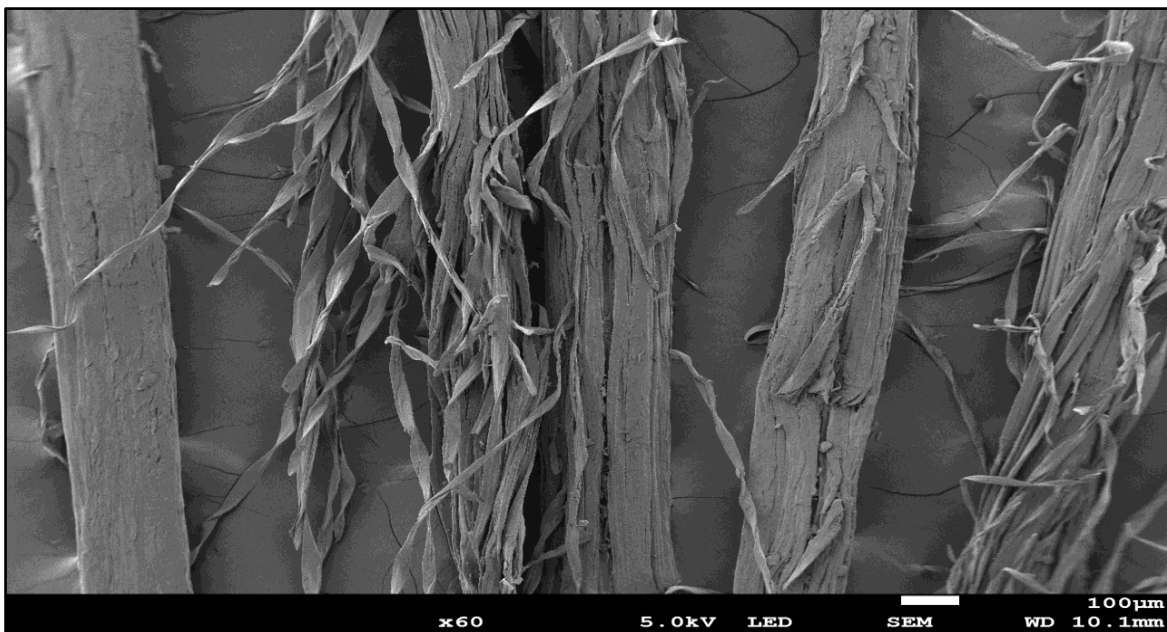
The extracellular non-fibrous impurities are observed in figure 4.46. There are also some dents caused by the hammering method of fibre extraction observed from figure 4.46. It is also observed that the surface morphology of the raw *Agave americana L.* fibre samples consist of ultimate fibres embedded and covered with non-fibrous gummy components. Hulle *et al.* (2015b:3), claimed it to be lignin, pectin, hemicelluloses, waxes and other extracellular impurities. There is no fibre defibrillation observed. Lee *et al.* (2019:2), confirmed that the role of non-fibrous gummy components of the fibre is to preserve the morphological structure and physical form of the textile fibre. A comparable observation was done by Reddy *et al.* (2013:288).

#### ***4.7.1.2 The SEM analysis of the enzyme biosoftened Agave americana L. fibre surface morphology***

The structural changes that occur during enzymatic biosoftening of *Agave americana L.* fibre surface were determined by SEM analysis. Figures 4.47-4.57 exhibited SEM micrographs of enzymatic biosoftened *Agave americana L.* fibre specimen. The enzyme(s) used have eliminated most of the extra noncellulosic fibre components and surface impurities. Fibre morphologies had partly decomposed to be individual fibres with relatively high surface roughness. The surface morphology looks smoother with less irregular cell wall features than the raw *Agave americana L.* fibre because waxy materials and cuticles have been removed. It is also observed that external fibre defibrillation has occurred on enzyme biosoftened *Agave americana L.* fibre because the cementing non-cellulosic fibre constituents have been removed from the fibre surface (figures 4.47-4.57). The separation of technical fibre bundles into smaller loose fibre is an indication that enzymatic biosoftening has been effective.

(a) The SEM images of manganese peroxidase biodelignified *Agave americana L.* fibre

Figure 4.47 shows the longitudinal SEM micrographs of manganese peroxidase biodelignified *Agave americana L.* fibre extracted from the middle leaves of plant 1.

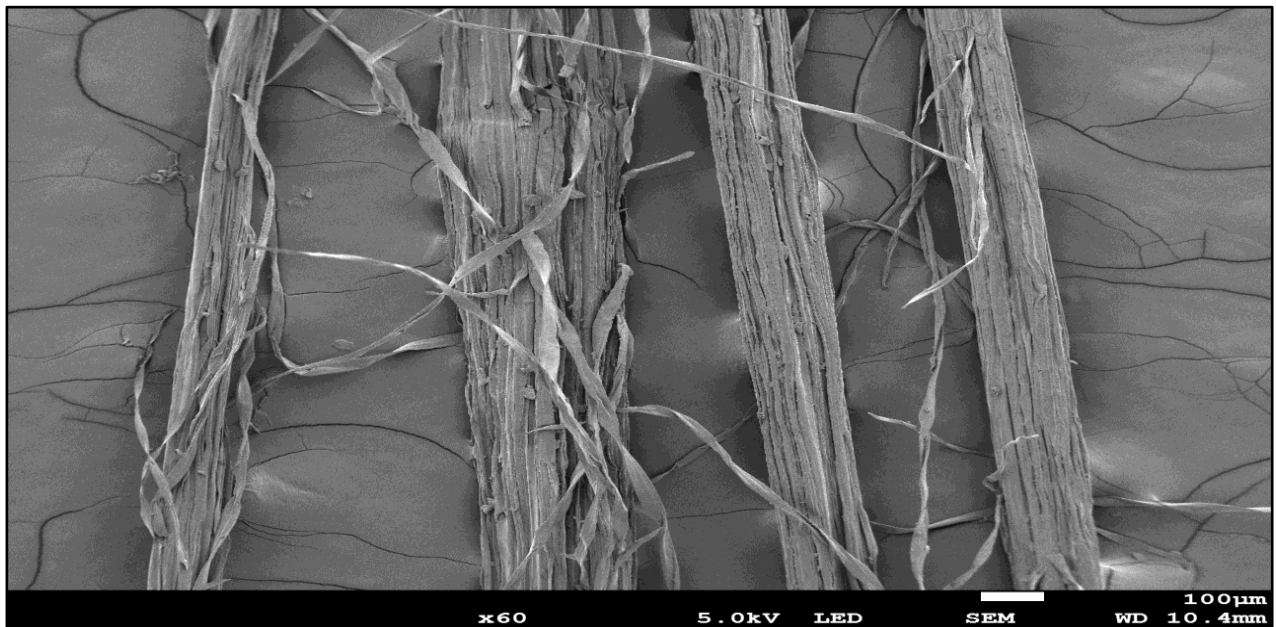


***Figure 4.47: The longitudinal SEM micrographs of manganese peroxidase biodelignified Agave americana L. fibre harvested from the middle leaves of plant 1***



Figure 4.47 illustrates that the fibre surface impurities and debris had been partly removed by the biodelignification with manganese peroxidase. It shows that fibre ultimates are loosened from the parent fibres and as such the *Agave americana L.* fibre defibrillation has as a result of effective degradation of the middle lamellar binding materials. The separated twisted ribbon-shaped cellulosic ultimate fibres adhere over the surface due to the surface tensile force that occurred during drying. It has caused an attraction between the neighbouring ultimate cells to remain attached loosely to one another. These results validate the existence of the complex lignocellulose network and that the removal of lignin leads to the degradation of bonds (Rahman & Sayed-Esfani, 1979:118).

Figure 4.48 also shows the longitudinal SEM micrographs of manganese peroxidase biodelignified *Agave americana L.* fibre extracted from the middle leaves of plant 1.

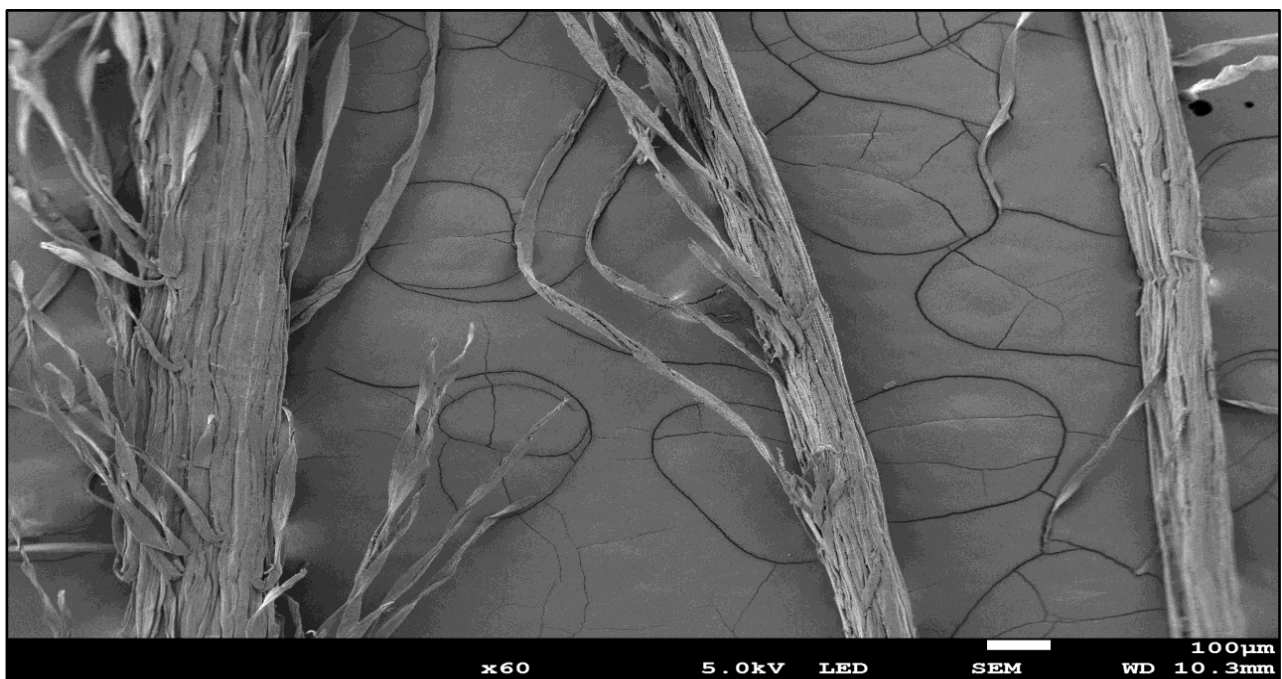


**Figure 4.48:** *The longitudinal SEM micrographs of manganese peroxidase biodelignified Agave americana L. fibre harvested from the middle leaves of plant 1*

The ligninolytic enzyme used in this research, to delignify the *Agave americana L.* fibre cell wall to soften and improve its tactile properties for textile use was Manganese peroxidase (MnP). MnP has a good reputation in plant fibre delignification (Martinez *et al.*, 2009:348). Upon defibrillation, the introduction of surface roughness was increasingly perceived (Figure 4.48). The SEM micrographs reveal that MnP delignification *Agave americana L.* fibre has effectively and partially separated its different constituents (cellulose, lignin, hemicelluloses, and minor constituents) as indicated by thinning out of the fibre when compared to raw fibres

in figures 4.40-4.46. Fibre defibrillation resulted in microstructural changes, which reduced fibre diameters along the entire fibre length. The fibre defibrillation indicated the dissociation of hemicelluloses and lignin from the cellulose microfibrils and extra softening of the fibre and increase of fibre surface unevenness might have occurred as a result of the variance between the coefficient of increase and decrease of the *Agave americana L.* fibre and non-cellulosic components (Le Moigne *et al.* 2018:39-40). There were some remaining non-cellulosic components on the surface of the microfibrils; observed with partial separation of ultimate fibrils from the fibre bundles. After enzymatic biosoftening of *Agave americana L.* fibre with MnP, the non-cellulosic fibre components had been partly removed to reveal the morphology of the microfibrils, including partial separation of microfibrils from the fibre bundles.

Figure 4.49 presents the SEM images of manganese peroxidase treated *Agave americana L.* fibre harvested from the middle leaves of plant 1.



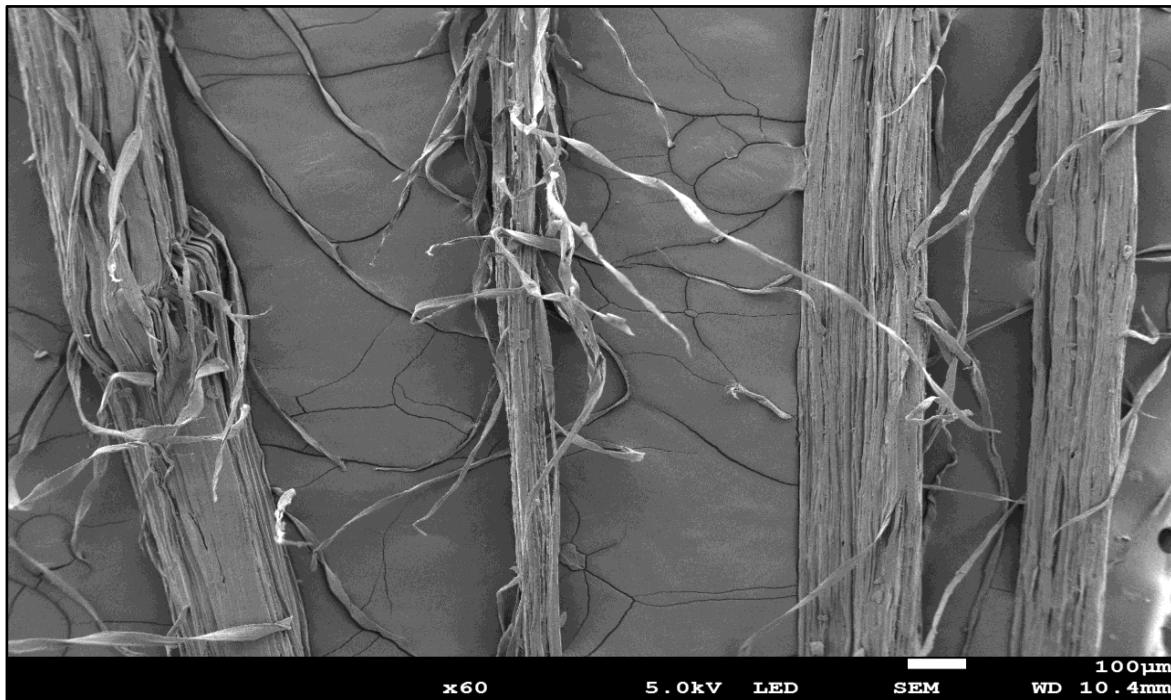
**Figure 4.49: The longitudinal SEM micrographs of manganese peroxidase biodelignified *Agave americana L.* fibre harvested from the middle leaves of plant 1**

The SEM images depict the MnP bio softened *Agave americana L.* fibre resulted in defibrillated structures (figure 4.49). After biosoftening *Agave americana L.* fibre with MnP, the fibre surface becomes cleaner and smoother than the raw fibre shown in figures 4.47-4.49. This is due to the removal of natural fats, waxes, and other impurities especially lignin from the fibre surfaces. It is also observed that the fibre diameter varies a lot and decreased when compared to the topographic characteristics of raw fibre (figures 4.40-4.46). It is also observed

from figure 4.49 that fibre diameter decreased due to microfibrillar defibrillation and individualization when compared to raw *Agave americana L.* fibre topographic characteristics (figures 4.49).

Figure 4.50 portrays the longitudinal SEM images of *Agave americana L.* fibre harvested from the top leaves of plant 1 treated in sequence with pectinase, xylanase and cellulase respectively.

#### 4.7.1.3 The SEM analysis of the sequential enzyme-treated *Agave americana L.* fibre

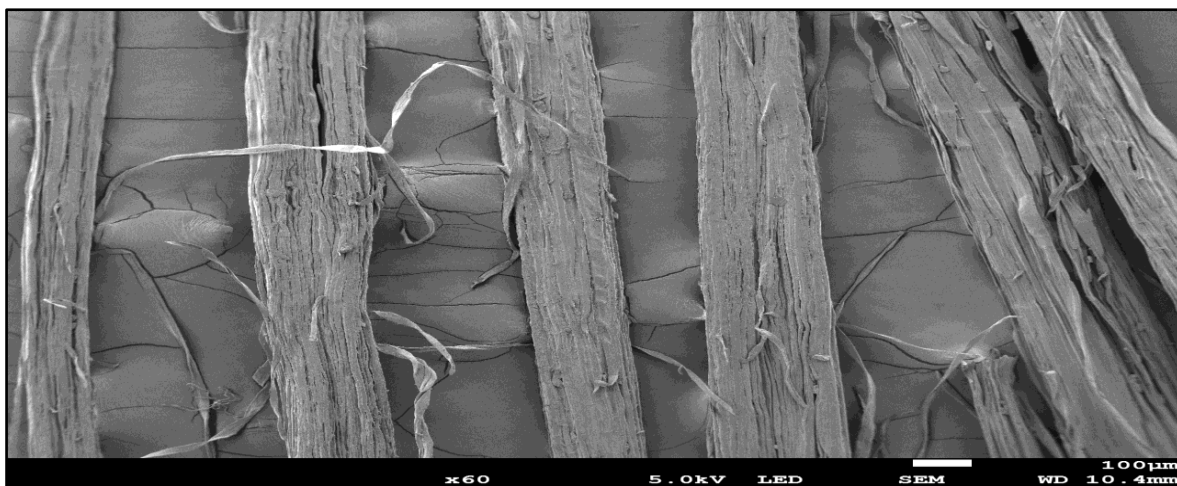


*Figure 4.50: The longitudinal SEM micrographs of sequential pectinase, xylanase and cellulase biosoftened Agave americana L. fibre harvested from the top leaves of plant 1*

Figure 4.50 shows the twisted ribbon-shaped cellulosic microfibrils that are separated from the technical fibre bundles but adhered over the parent fibre surface. The fibre surface looks cleaner with almost no impurities present. The white areas created on the twisted ribbon-shaped cellulosic may be instigated by the light beam used to take the fibre micrograph (Sorieu *et al.*, 2016:5).

Figure 4.51 also reveals the longitudinal SEM micrographs of *Agave americana L.* fibre harvested from the top leaves of plant 1 biosoftened in sequence with pectinase, xylanase and cellulase respectively.

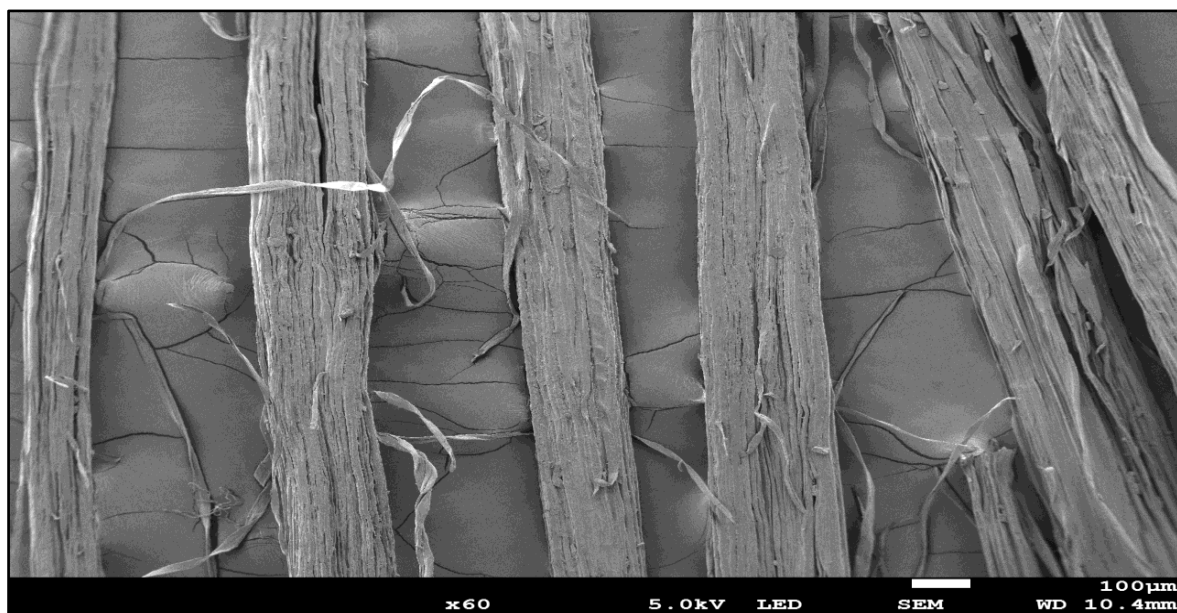




**Figure 4.51: The longitudinal SEM micrographs of sequential pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the top leaves of plant 1**

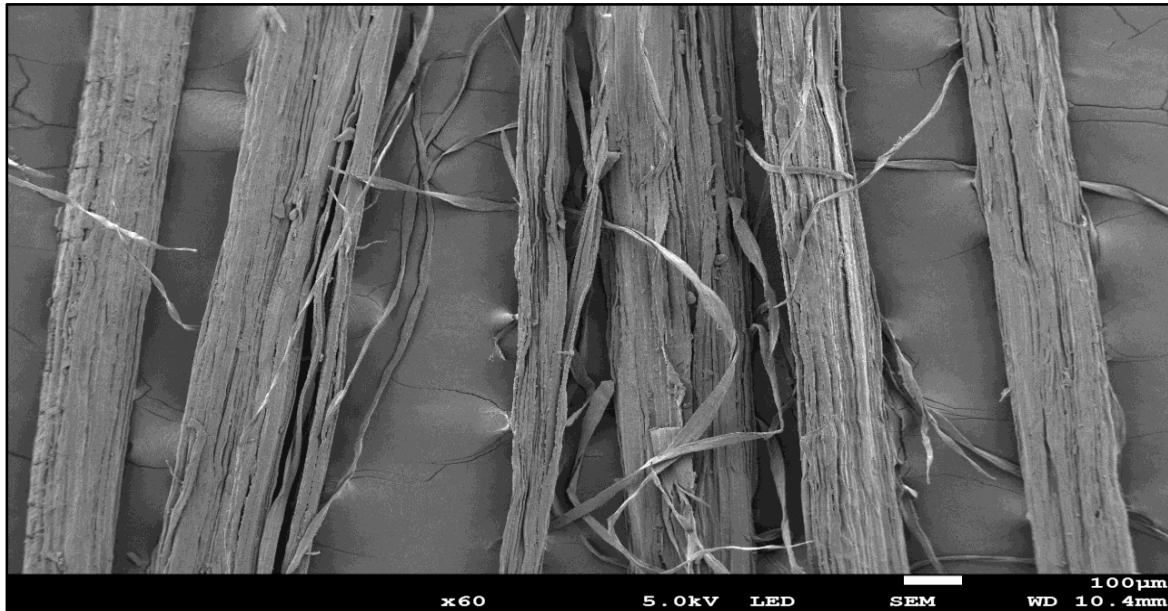
Sequential enzymatic biosoftening of *Agave americana L.* fibre that included cellulase biopolishing; imparted defibrillation and reduce the fuzz formation to bring about increased softness and upgrading on handle, surface structure and fibre appearance and conveys cooler feel and improve colour brightness.

Figure 4.52 also illustrates the longitudinal SEM micrographs of *Agave americana L.* fibre harvested from the top leaves of plant 1 biosoftened in sequence with pectinase, xylanase and cellulase respectively.



**Figure 4.52: The longitudinal SEM micrographs of sequential pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the top leaves of plant 1**

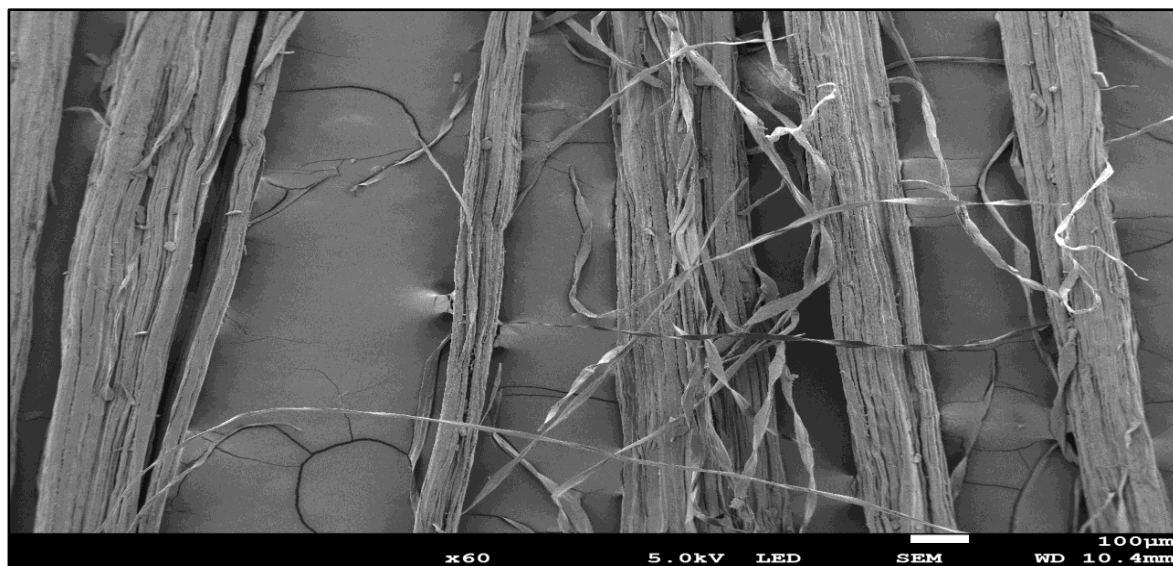
Figure 4.53 also shows the longitudinal SEM micrographs of *Agave americana* L. fibre harvested from the top leaves of plant 1 biosoftened in sequence with pectinase, xylanase and cellulase respectively.



**Figure 4.53: The longitudinal SEM micrographs of sequential pectinase, xylanase and cellulase biosoftened *Agave americana* L. fibre harvested from the top leaves of plant 1**

Enzyme biosoftened fibres exhibited some surface folds or swellings of which it can be reasonably inferred that they are mostly caused by the elimination of the non-fibrous constituents and chemical bonds between the crystalline fibrils and the cementing non-fibrous fibre components. Literature states that the occurrence of the primary cell wall components and the outer layer (s1) of the secondary cell wall exhibited the fibre surface that instigated swelling damage (Singh *et al.*, 2009:685). SEM images show the initiation of fibre separation into fibre ultimates that are flat ribbon-shaped like cotton fibre. The fibre ultimates were twisting due to the disintegration of the lumens and the removal of the protective layer of non-cellulosic heterogeneous fibre components which mark the initiation of defibrillation of *Agave americana* L. fibre (Rahman & Sayed-Esfahani, 1979:116).

Figure 4.54 demonstrates the longitudinal SEM micrographs of *Agave americana* L. fibre harvested from the top leaves of plant 1 biosoftened in sequence with manganese peroxidase, pectinase, xylanase and cellulase respectively.

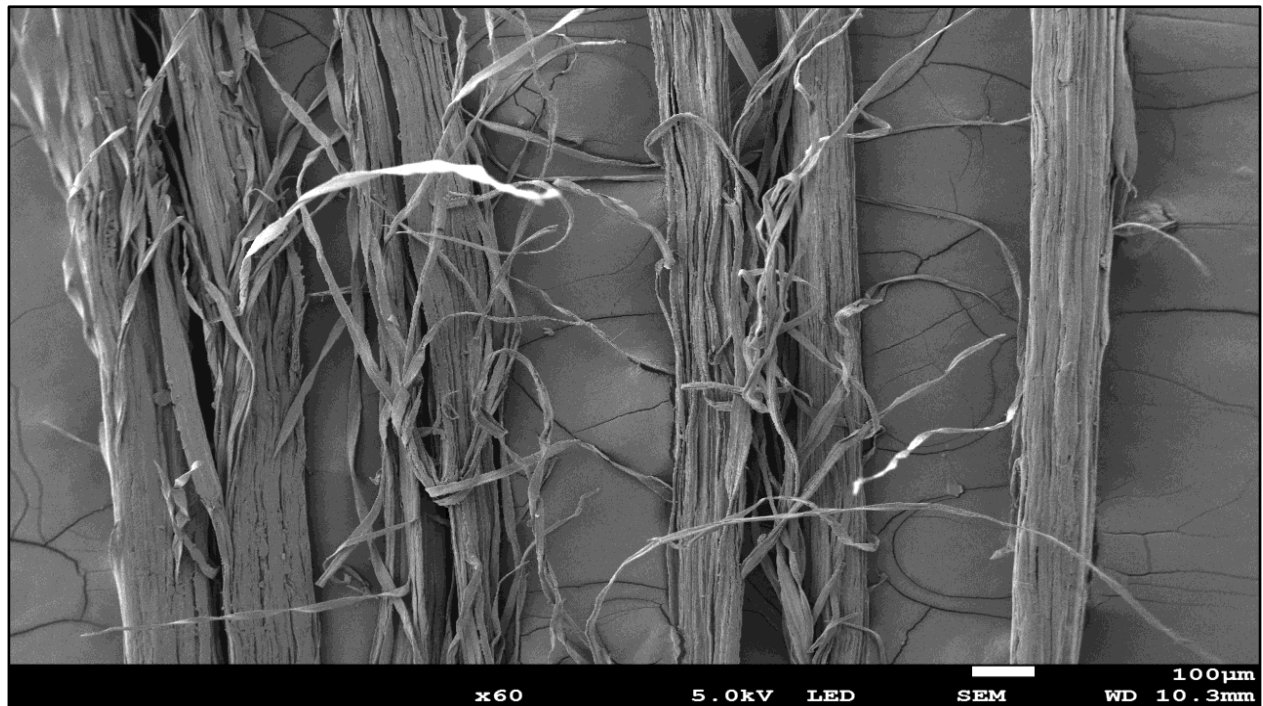


**Figure 4.54: The longitudinal SEM micrographs of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the middle leaves of plant 1**

The highest degree of loosening of fibre ultimates was observed on *Agave americana L.* fibre biosoftened with sequential MnP, pectinase, xylanase and cellulase followed by sequential pectinase, xylanase and cellulose enzymes. The enzymatic biosoftening had a substantially positive influence on the textile advancement of *Agave americana L.* fibre.

The SEM images of sequential manganese peroxidase, pectinase, xylanase and cellulase respectively; biosoftened *Agave americana L.* fibre harvested from the middle leaves of plant 1; is shown in figure 4.55.



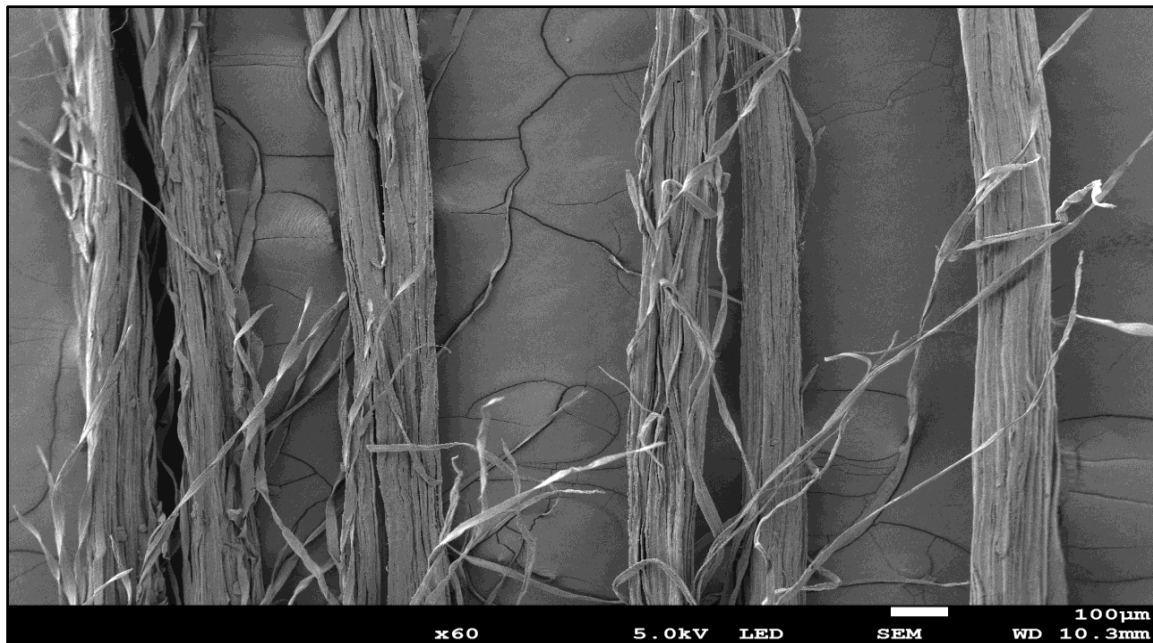


**Figure 4.55: The longitudinal SEM micrographs of sequential Manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the middle leaves of plant 1**

The SEM images of enzymatic biosoftened *Agave americana L.* fibre (Figures 4.47-4.57) reveal that there is an internal fibrillation; that breaks the crosslinks between microfibrils and leads to the separation of microfibrils from the fibre (Wang *et al.*, 2007:381). These separated flat, ribbon-shaped fibrils tend to twist like cotton fibre because of the disintegrated lumens and the surface tension relaxation. The removal of non-fibrous, protective, heterogeneous chemical fibre constituents from the surface results in partial initiation of *Agave americana L.* fibre superfluous defibrillation. This can be explained as the difference between the coefficient of expansion and shrinkage of the textile fibres and gummy components instigating the fibrils' separation (Rahman & Sayed-Esfani, 1979:118). There is also evidence of white coloured patches in the separated the *Agave americana L.* fibre ultimate cells of which Rahman & Sayed-Esfani (1979:118) claim to be the lignin-based surface deposit crystals incrusting cellulose microfibrils. Upon close observation of the surface of the manganese peroxidase, pectinase, xylanase and cellulase sequentially biosofted *Agave americana L.* fibre, surface smoothness and defibrillation appeared to be the highest; signifying comparative freedom from hemicelluloses and lignin networking (Jiang *et al.*, 2018:6480-6481, DePrez *et al.* 2018:10).

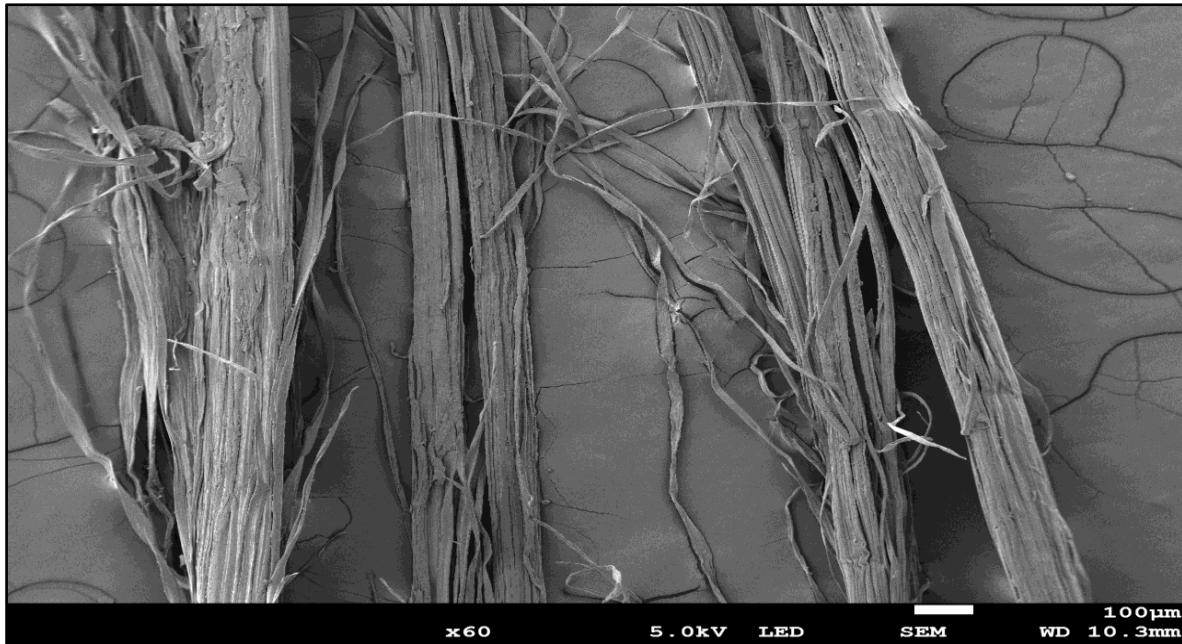
Figure 4.56 depicts the longitudinal SEM micrographs of *Agave americana L.* fibre harvested from the middle leaves of plant; biosoftened with sequential manganese peroxidase, pectinase, xylanase and cellulase respectively.





**Figure 4.56: The longitudinal SEM micrographs of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the middle leaves of plant 1**

The fibre surface exhibited cleanliness, ultimate separation and reduced impurities with the sequential enzymatic biotechnology with four enzymes figure 4.56. Although the fibre bundles seem quite coherent, some fibres were observed; loosened from the fibre bundles with balanced proportion to the degree of enzymatic effectiveness. Treated fibre showed fibre porosity on its surface compared to the raw fibre. This is an indication that the enzymatic bio-softening technology could disturb the cellulose- hemicellulose-lignin network, through the removal of some fibre ultimate. These separated flat, ribbon-like fibre ultimates tend to twist like cotton fibre because of the disintegrated lumens, removal of the protective fibre layer of heterogeneous chemical compounds that result in the *Agave americana L.* fibre defibrillation, more increased uniformity, evenness and softness than the raw fibre.



**Figure 4.57: The longitudinal SEM micrographs of sequential manganese peroxidase, pectinase, xylanase and cellulase biosoftened *Agave americana L.* fibre harvested from the middle leaves of plant 1**

Figure 4.57 emphasizes the fact that when sequential enzymatic biosoftening processes increase in number; fibre surface area and roughness increase as a result of amplified fibre interior exposure. Most surface ridges, longitudinal cracks, fibre defibrillation were observed in SEM micrographs of manganese peroxidase, pectinase, xylanase and cellulase sequentially biosoftened *Agave americana L.* fibre; (figures 4.54-4.57). These happened due to the elimination of the non-fibrous, extra fibre components which cement individual fibrils into fibre bundles. The *Agave americana L.* fibre defibrillation happened as the fibre bundle splits into minute fibre fibrils that detach from its surface primary cell wall and secondary cell wall. Fibre defibrillation leads to fibre reduction in size as observed in figures 4.47-4.57. Thus improves the surface properties but decreases to some extent the mechanical performance of the *Agave americana L.* fibre. Fibre defibrillation probably leads to an increase in disclosure of highly sensitive cellulose groups on the fibre (Vizireanu *et al.*, 2018:8). The surface ridges and longitudinal cracks and what may have appeared as cracks on the *Agave americana L.* fibre surfaces could have been evidence of more fibre defibrillation from the removal of more hemicellulose and lignin.

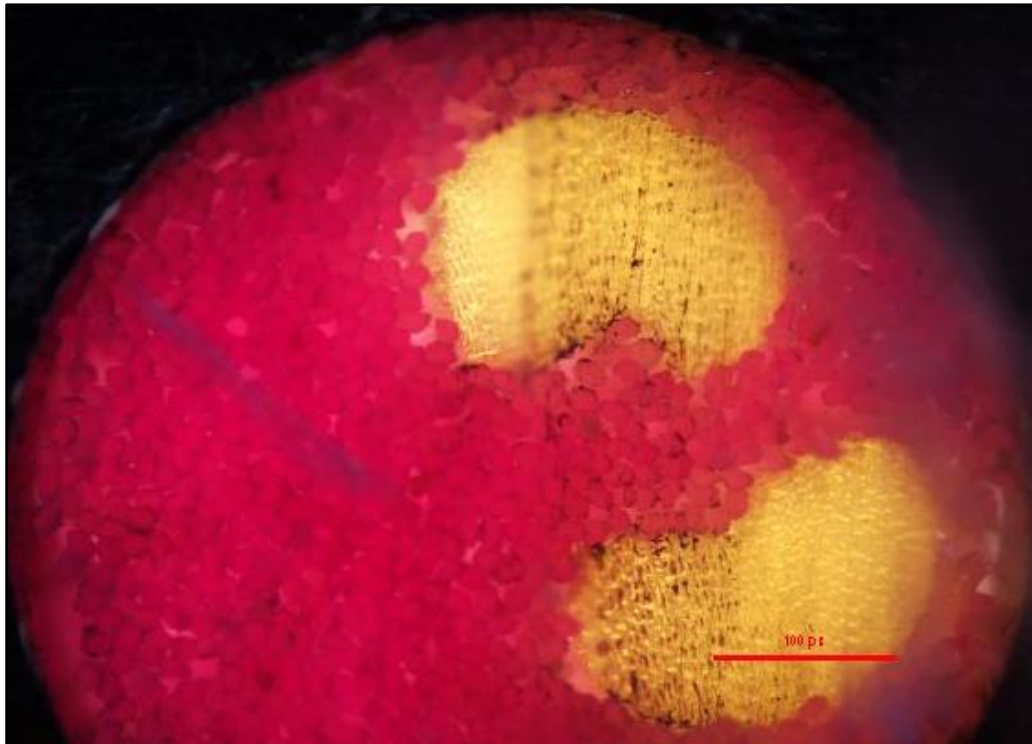
Figures 4.47-4.57 confirm that *Agave americana L.* fibre is not a single fibre *per se*, but a multi-cellular fibre bundle consists of ultimates, like other plant fibres. Most surface impurities and other non-cellulosic components are removed. The sequential enzymatic biosoftened *Agave americana L.* fibre showed a softer, smoother and cleaner surface than the raw fibre.

The critical *Agave americana L.* fibre structure became visible after the removal of extracellular impurities through enzymatic bio-treatment. However, tensile strength and modulus are negatively affected. This has been expected because the fibre becomes thin when; lignin and hemicellulose are removed. The longitudinal views (Figure 4.47-4.57) exemplify that fibre has a somewhat cylindrical shape. The low wax content on the surface of individual cells might have caused it to be visible. The longitudinal surface shows multicellular overlapping micro-fibrils that run parallel to the fibre's axis.

Xylanase enzyme disintegrated hemicelluloses and partially catalysed lignin in the *Agave americana L.* fibre network. Whereas pectinase catalyses pectins to elementarise the fibre. The sequential utilisation of lignocellulolytic enzymes reduced more weight than individual enzymatic biosoftening. This indicated the higher efficiency of sequential enzymatic biosoftening when compared to individual enzymatic biosoftening of *Agave americana L.* fibre. The SEM results designated that the enzymatic biosoftening is a commendable alternative to chemical softening of *Agave americana L.* fibre.

#### **4.7.2 The analysis of *Agave americana L.* fibre transverse morphology**

*Agave americana L.* fibres were observed using a light microscope (Nikon) fitted with camera Nikon DS-fi1 10x enlargement–optip hot-2. To evaluate the cross-sectional view of *Agave americana L.* fibre, the micrographs are shown in figures 4.58-4.61. The cross-sectional shape of the fibre largely impacts its physical and mechanical properties (Omeroglu *et al.*, 2010:1180; Srivastava, 2012:5). Figure 4.58 presents the light microscopic cross-sectional view of the raw *Agave americana L.* fibre extracted from lower leaves of plant 2.



*Figure 4.58: Light microscopic cross-sectional view of the raw Agave americana L. fibre extracted from lower leaves of plant 2*

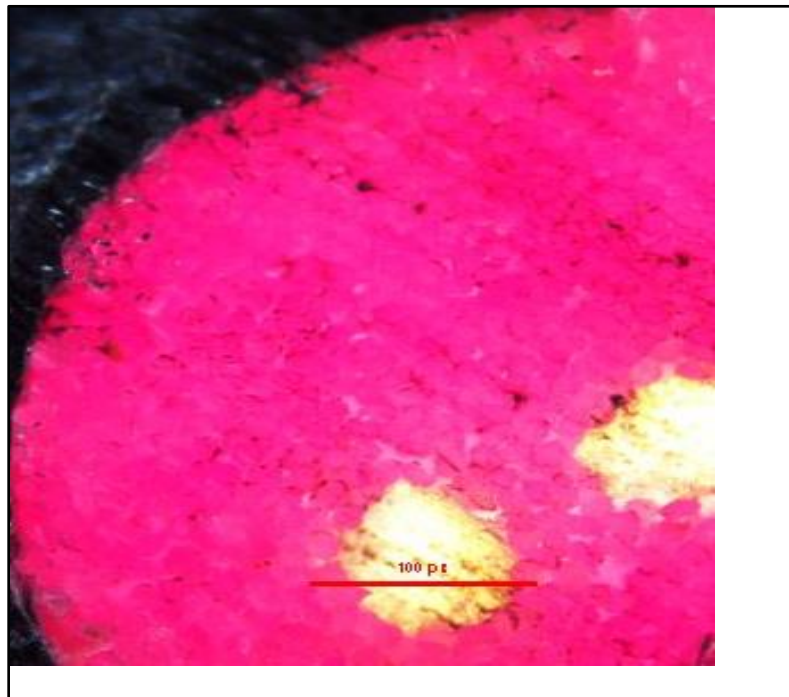
The cross-sectional view of the fibre (the cream coloured part- the pink part is filler of another fibre used to isolate the two parts of the sample) reveals its cross-sectional shape which is one of the most imperative determinants of the fibre physico-mechanical and optical properties (Omeroglu *et al.*, 2010:1180). It is observed from figure 4.58 that the cross-sectional view of *Agave americana L.* fibre depicts that the older fibre has differentiated into a bean (dog-bone or kidney) shape. It also shows that *Agave americana L.* fibre is a multi-cellular fibre bundle consisting of irregular, polygonal, heterogeneous cells that are called ultimates or ultimate cells of which Teli & Jadhav, (2015:3850) believed that they are embedded and glued together by hemicellulose and lignin.

The ultimate cells are overlapping and joined together by a waxy cementing non-fibrous material to form the filament fibre. This is an indication that each individual *Agave americana L.* fibre consists of vascular bundles which are tissue vessels of transportation embedded in thick fibre cell sheaves (Saravanan, 2018:223). It is observed that the fibre diameter was more than 100  $\mu\text{m}$  in agreement with the statement of Hulle *et al.* (2015c:68), who stated that it ranges from 100-150  $\mu\text{m}$ . This indicates that *Agave americana L.* fibre is a relatively thick fibre because it is made up of several cell ultimates of which each has a lumen and separate from neighbouring ultimate by middle lamellae (Reddy *et al.*, 2013:288). This is a typical



characteristic of natural lignocellulosic fibres (Hulle *et al.*, 2015c:71). It is also noted that the fibre cells' lumens are of different sizes. It is from figure 4.58 that the *Agave americana L.* fibre has some middle lamellae residues. This may be due to under- retting which has been caused either by high fibre lignification or insufficient removal of leaf pithy substances during fibre extraction (Bourmaud *et al.*, 2018: 356).

The light microscopic cross-sectional view of pectinase biosoftened *Agave americana L.* fibre extracted from younger leaves of plant 2 is presented in figure 4.59.

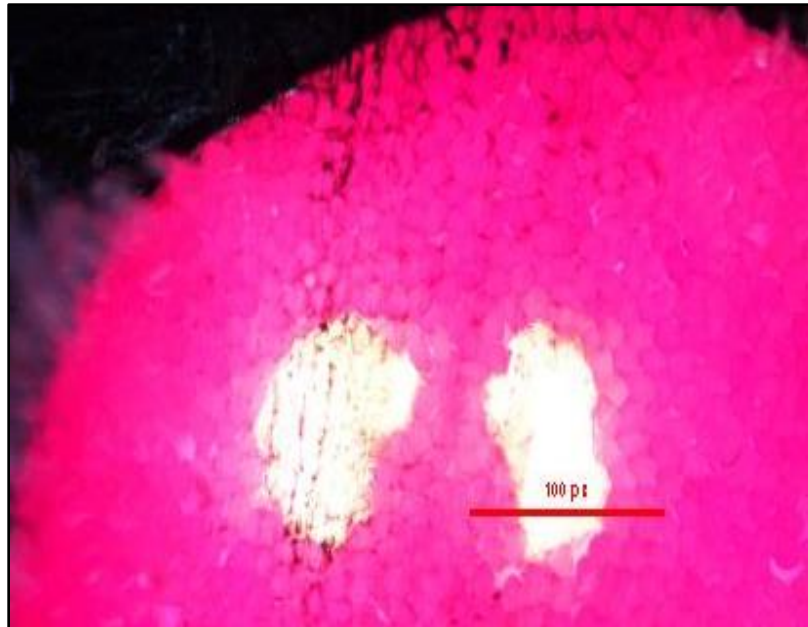


**Figure 4.59:** A light microscopic cross-sectional view of pectinase biosoftened *Agave americana L.* fibre extracted from younger leaves of plant 2

Figure 4.59 illustrates a diameter smaller than 100 pt ( $\mu\text{m}$ ). This might have been attributed to the size of the fibre because it was extracted from the younger leaves. The fibre looks semi-oval shaped with irregular edges. The ultimate cells and their lumens are not easily discernible. One has reason to think that the fibre was too young when harvested; it was at the early growing stage in which the growing cells are surrounded by primary walls before lignification of secondary walls and development of specialized fibre cells. During the early growth stage, plant cells divide to form the middle lamella which is a thin layer containing mainly pectin (Sorieul *et al.*, 2016:5), which is a substrate catalysed with the enzyme pectinase that was used to biosoften the fibre. The young *Agave americana L.* fibre did not have differentiated cells

which would contain walls with distinctive compositions of, specialized cell walls. This implies that the young thin celled fibre was rendered thinner than the raw fibre.

A light microscopic cross-sectional view of xylanase biosoftened *Agave americana L.* fibre extracted from younger leaves of plant 2 is presented in figure 4.60.



**Figure 4.60:** A light microscopic cross-sectional view of xylanase biosoftened *Agave americana L.* fibre extracted from younger leaves of plant 2

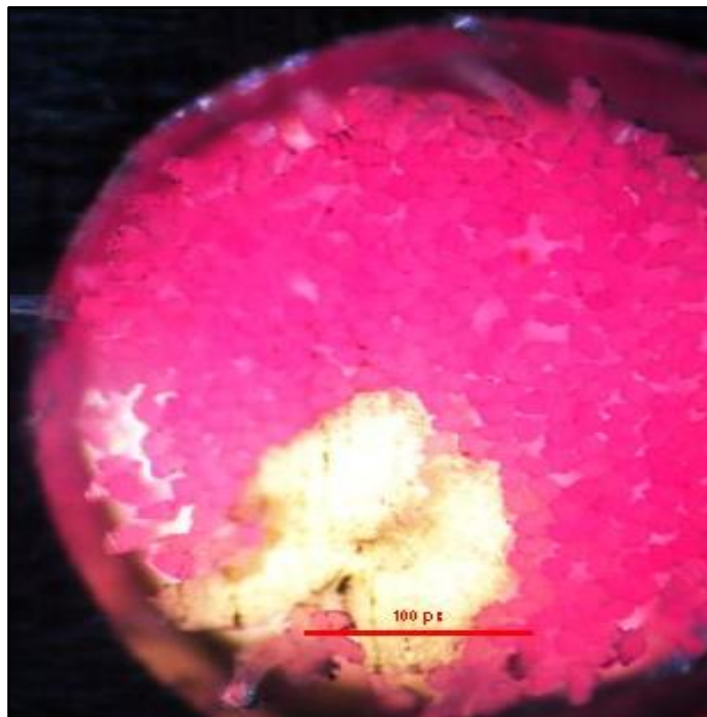
It is observed that the two cross-sectional fibre views shown in figure 4.58 are of different shapes and sizes. Taking in account that it is the same fibre in parts only millimetres from each other, this implies that *Agave americana L.* fibre diameter varies greatly throughout its length from the base; middle and tip sections (Mylsamy & Rajendran 2010:2927) and depends on a number of ultimates that constitutes the cross-section. This examination shows a typical characteristic of natural lignocellulosic fibres. It is also observed from the micrograph (figure 4.58) that the xylanase biosoftened *Agave americana L.* fibre was whiter than the raw and pectinase biosoftened *Agave americana L.* fibre as seen from figures 4.58 and 4.59. The increased fibre brightness indicates that xylanase biobleaching extracted the chromophores associated with lignin-carbohydrate complexes (LCCs). Xylanase biosoftening of textiles is an eco-friendly bleaching process that reduces fibre damage, chemical costs and use (Narpiban *et al.*, 2019:101).

*Agave americana L.* fibre extracted in the current study is generally yellowish-white in colour. The *Agave americana L.* enzymatic biobleaching discoloured natural pigments; mainly



flavonoids and conveyed a brighter and whiter colour to the fibres with less environmental impairment and amounts of rinsing water than those of conventional chemical bleaching. Like all other conventional wet processes; chemical bleaching can decrease the fibre polymerisation degree and damage the fibre considerably. Alternatively, an enzymatic bleaching process would improve fibre quality with less damage, more cost-effective on washing water, energy and time requirements for hydrogen peroxide removal (Narkpiban *et al.*, 2019:101).

A light microscopic cross-sectional view of cellulase-biosoftened *Agave americana L.* fibre extracted from the middle leaves of plant 3 is presented in figure 4.61.



*Figure 4.61: A light microscopic cross-sectional view of cellulase-biosoftened Agave americana L. fibre extracted from the middle leaves of plant 3*

Cellulase biopolished *Agave americana L.* fibre looks thinner than 100 µm with an irregular polygonal shape. The fibre looks brighter than pectinase but less than xylanase biobleached *Agave americana L.* fibre. Bio-polishing process results in substantially reduced fibre diameter. The influence was so significant that it promoted the formation of smaller fibre bundles to improve fibre fineness of *Agave americana L.* fibre. The biopolishing, of *Agave americana L.* fibre was accomplished with cellulase enzymes. Biopolishing of *Agave americana L.* fibre was intended to enhance the physical properties of the fibre. The cellulase biopolishing removed loose fibres. The reaction was expected as the biosoftening was conducted on the cellulosic fibre.

Contrary to that biopolishing brings permanent fibre quality, even after several washing processes; as such it enhances fibre texture, colour and flexural elasticity. The cellulase activity must be controlled to remove the loose fibre surface fibrils only; not to hydrolyse the fibre cellulose backbone. Consequently the fibre is likely to be functional in a textile manufacturing system. Cellulase biopolishing of *Agave americana L.* fibre conferred cooler and softer feel, brighter luminosity of colours. Results obtained showed that there was a noticeable change in both surface modification and mechanical properties of fibre after biosoftening with commercial enzymes. Based on results obtained from a light microscopic cross-sections, enzymatic treatment removed most of the impurities and the superficial loose fibre ends of the treated *Agave americana L.* fibre. The enzyme treatment granted a smoother fibre surface because the impurities were leavened away.

#### **4.8 BENDING LENGTH OF AGAVE AMERICANA L. FIBRE**

The fibre bending length is referred to as the flexibility of fibre falling length. This is because the fibre falls under its weight to a specific value with a specific angle. It is regarded as a property of a textile fibre through which fibre flexibility or stiffness is measured (Chabilendra, 2009:26). The high bending values indicate greater resistance to bending (Hasani *et al.*, 2013:81). Fibres with high bending length are stiff and lack flexibility. They are less comfortable than the fibres with lower bending length. According to Ghosh, *et al.* (2014:3460) fibres with high bending lengths cannot be used for aesthetical and draping textiles. Bending length of raw, water boiled and enzyme-biosoftened *Agave americana L.* fibre was presented in figures 4.62, 4.63 and 4.64, respectively. The bending length of raw *Agave americana L.* fibre is presented in figure 4.62.

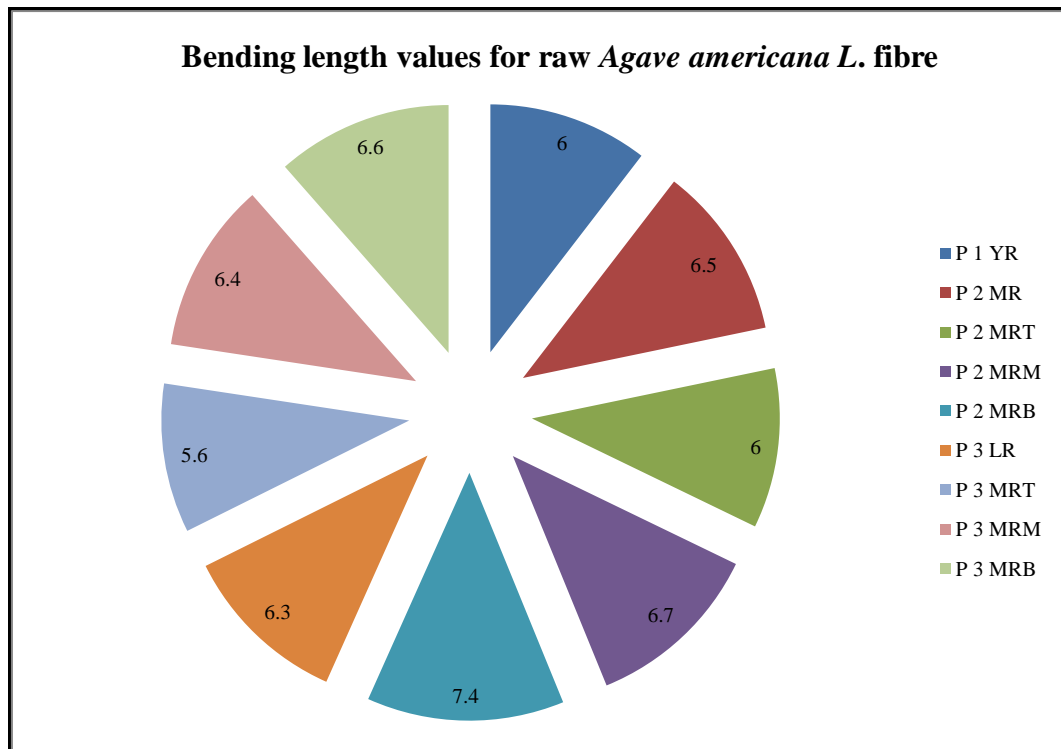
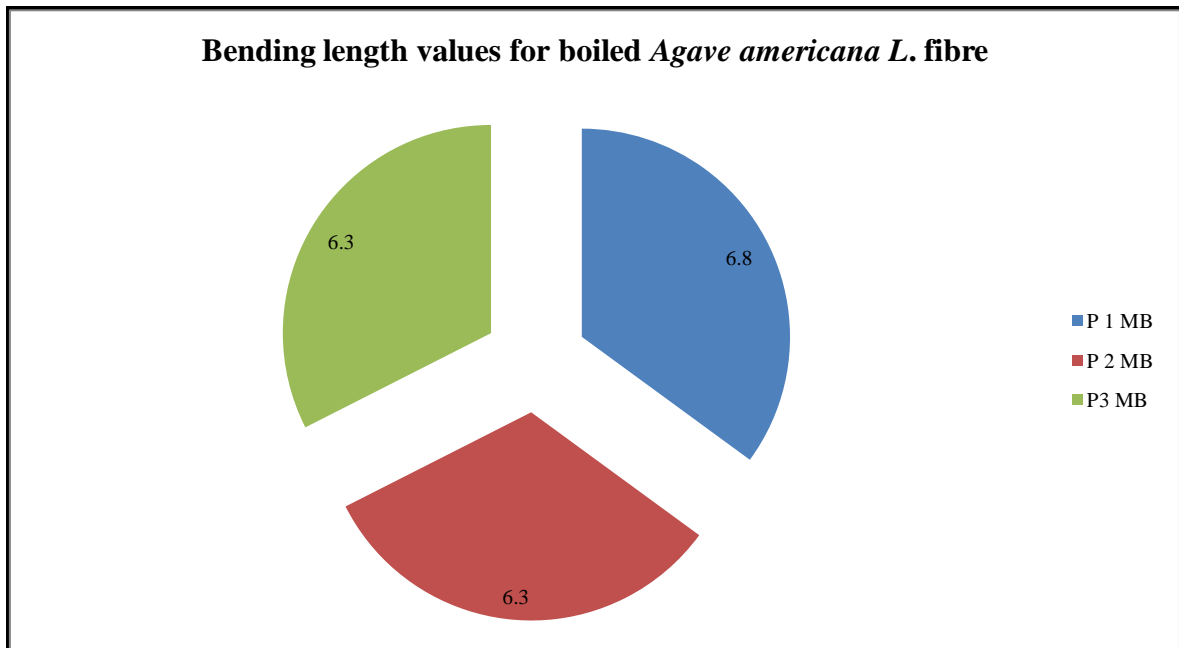


Figure 4.62: The bending length values for raw *Agave americana L.* fibre

It was observed from figure 4.62 that; the lowest bending length determined was 5.6 cm (70% stiffness) for raw fibre obtained from tip part of middle leaves of plant 3. This may be explicated by the fact that the fibre was obtained from the young; still growing part of the leaf where the fibre has mostly the primary cell walls, that are reported to be thin and extensible (Hasani *et al.*, 2013:81). The average bending length of the raw *Agave americana L.* fibre was found to be 6.4 cm (80% stiffness). The highest bending length was found to be 7.4 cm (92.5% stiffness) for water boiled fibre extracted from middle leaves of plant 2.

The bending length of the raw fibre was found higher than that of both boiled and enzyme biosoftened *Agave americana L.* fibre. This can be explained by the structure, organization and the function of fibre cell wall. The physico-mechanical properties of lignocellulosic fibres such as *Agave americana L.* fibre are triggered by extreme cellulose crystallinity, that run parallel to the fibre axis (De Prez, 2018:1). Thus, the *Agave americana L.* fibre bending length behaviour instigates in the cell wall composition, structural arrangement and function of the cell wall (Bourmaud *et al.*, 2018:386). Therefore, it is possible to make a generalisation that *Agave americana L.* fibre, like other natural lignocellulosic fibres; is a complex biopolymer composite that consists of cellulose in the form of helically wound microfibrils, embedded in the cementing matrix of hemicellulose, lignin and other minute proportional components (Srinivasa *et al.*, 2011:2470, Sorieul *et al.*, 2016:5-7).

The bending length of the boiled *Agave americana L.* fibre is illustrated in figure 4.63.



**Figure 4.63:** The bending length values for boiled *Agave americana L.* fibre

The lowest bending length determined was 6.3 cm for both P 2 MB and water boiled fibre extracted from middle leaves of plant 3. The average bending length of the enzymatic biosoftened was found to be 6.5 cm (81.25% stiffness). The highest bending length was found to be 6.8 cm for P 1 MB. There is no difference between the bending lengths of fibre extracted from the middle leaves of plants 2 and 3. But there is a slight increase in bending length resistance from the fibre extracted from the middle leaves of plant 1. The bending length of boiled and enzyme-treated *Agave americana L.* fibre was not significantly reduced when compared to raw fibre. The reduction in stiffness was minimal and resulted in less reduction in fibre bending stiffness even after enzymatic modification of its structure.

The bending length of enzyme biosoftened *Agave americana L.* fibre samples is shown in figure 4.64.

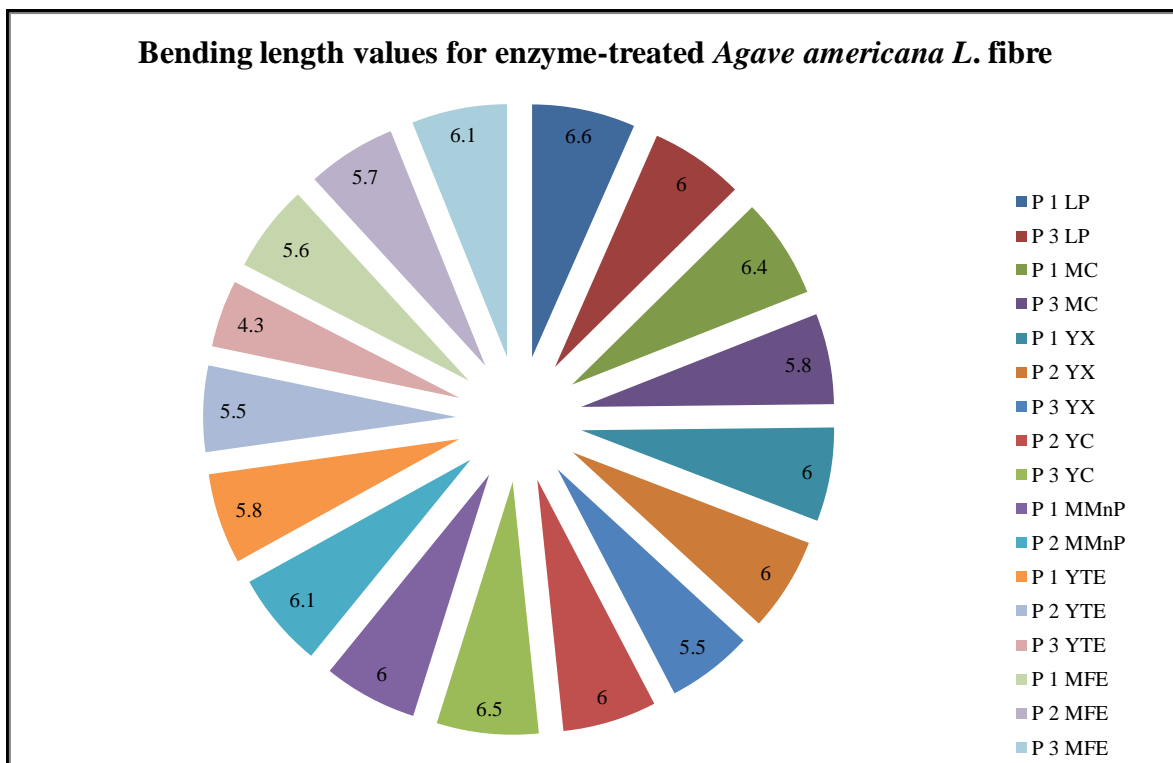


Figure 4.64: The bending length values for enzyme biosoftened *Agave americana L.* fibre

The enzymatic biosoftening polished the *Agave americana L.* fibre’s surface, and thus decreased the bending stiffness. The lowest bending length determined was 4.3 cm (53.75% stiffness) for Sequential pectinase, xylanase and cellulase biosoftened fibre extracted from top (young) leaves of plant. The average bending length of the enzymatic biosoftened fibres was found to be 5.9 cm (73.75% stiffness). The highest bending length was found to be 6.6 cm (82.5% stiffness) for P1 LP.

The bending length of boiled and enzyme-treated *Agave americana L.* fibre was not significantly reduced when compared to raw fibre. The reduction in stiffness was minimal and resulted in less reduction in fibre bending stiffness even after enzymatic modification of its structure. The bending length of the enzymatic biosoftened *Agave americana L.* fibre was found slightly lower than that of both raw and water boiled fibre. Bending results after enzymatic biosoftening of the fibre showed that the commercial enzymes used effectively removed some cementing fibre components that are responsible for the stiffness of raw fibre. Fibre bending length determines other fibre properties such as flexibility, handle, fineness and fibre applications. Fibres with high bending length values are more rigid and inflexible with less bending elasticity; which may be due to high fibre density; while fibres with low bending length values are softer. Fibres with high bending length values are not suitable for use in fine

and soft fabric constructions suitable for clothing since they may be uncomfortable to use close to the body due to their stiffness (Repon *et al.*, 2019:57). It will be more suitable for fabrics where rigidity will be a benefit.

Commonly, the bending length values of enzymatic biosoftened fibres were observed as the lowest, followed by water boiled and raw *Agave americana L.* fibre were the highest. This implies that enzymatic biosoftened *Agave americana L.* fibre has the best advantage as lower bending values imply better handling of fibre. This is likely to occur because of considerable freedom of movement of the fibre during bending (Mettilda *et al.*, 2014:71). In this research study the *Agave americana L.* fibre showed high bending length which is generally not enviable for fibre to be used for garb manufacture as it is the case with the fibre with lower bending length. The higher bending length is enviable for construction of fabrics to be used for heavy-duty purposes (Ghosh & Zhou, 2003:471).

However, the choice and use of Shirley stiffness tester to test the bending length of the *Agave americana L.* fibre had been brought about by the need and access to it. But might have not been a very good choice because it has been designed to characterise the bending length of the fabric, not individual fibres. In most cases, the nature of bending test employed indicates the limitations to characterise the bending length behaviour. It is therefore ideal to realize that a significant number of tests acknowledged as standard in the industry cannot be used for absolute characterization of bending characteristic. This implies that the same bending behaviour can exhibit different values when different theories are tested with different instruments. It is, therefore, reasonable for one to conclude that the Shirley stiffness tester has a stronger correlation with the fabric performance in actual use. The level of deformation or force applied in the test could also have influenced the results obtained (Ghosh & Zhou, 2003:471).

#### **4.9 SUBJECTIVE HAND-VISUAL EVALUATION RESULTS OF THE PHYSICAL CHARACTERISTICS OF AGAVE AMERICANA L. FIBRE**

The traditional textile evaluation method is constituted by the traditional subjective fibre assessment. This refers to the overall perception gathered when textiles are handled and ranked by a panel of both experts and non-experts. These textile feelings are termed the fibre handling constituents. The hand-visual evaluation method is among the most common procedures to



determine any textile fibre quality (Kendra, 2013:5). The subjective hand-and-visual evaluation properties of fibre are important since they determine the textile quality and prospect of fibre that influence the consumer's decision making about where, how and when to use the textile fibre of interest (Hasani *et al.*, 2013:81).

Fibre physical hand characteristics can successfully be examined with subjective assessment. Figures 4.65-4.74 show the subjective scores given to untreated water boiled and enzyme biosoftened *Agave americana L.* fibre. The ten aspects of fibre physical characteristics subjective hand-visual factors described the characteristics of *Agave americana L.* fibre: biosoftening level, fineness, softness, smoothness, density, flexibility, lustre, colour, uniformity and suitability for textile use. Eleven participants took part in the hand-visual evaluation of this study. Some participants were experts in textiles while others were not but all work in the department of Consumer Science at the University of the Free State. They had normal touch and vision.

#### **4.9.1 Fibre biosoftening level**

The quality of *Agave americana L.* fibre can be easily judged by the scoring scheme in which fibre biosoftening rate falls will decide the end-use of the fibre. Figure 4.63 presents the frequency distribution of panel evaluation scores determining *Agave americana L.* fibre biosoftening level.

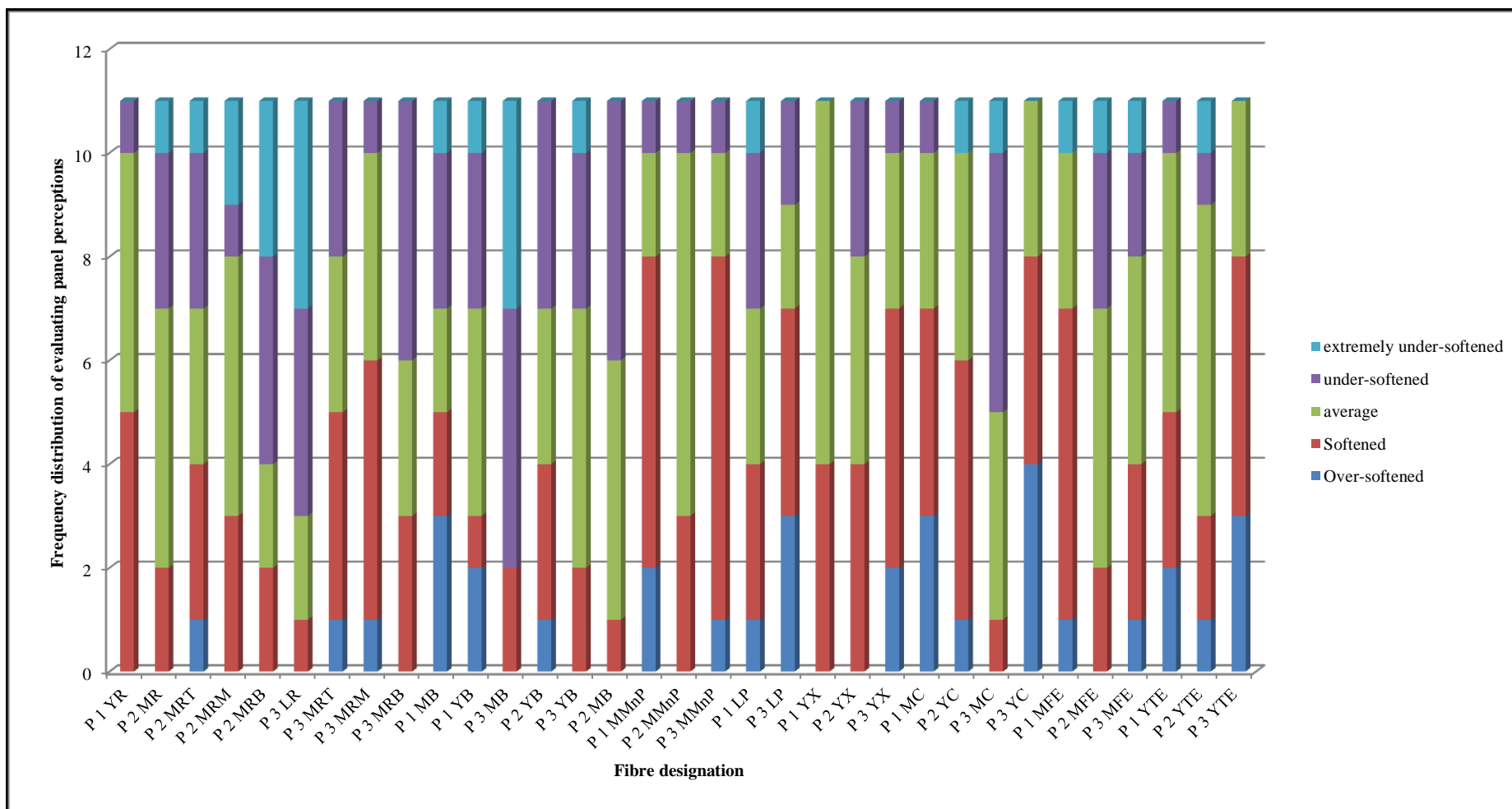


Figure 4.65: Frequency distribution of panel evaluation scores determining *Agave americana L.* fibre biosoftening level

When analysing the frequency distribution of panel evaluation scores determining *Agave americana L.* fibre biosoftening level shown in figure 4.65, it is observed that average scores were most followed by softened which is also followed by undersoftened. The reason for the highest average scores could be the inadequate understanding of fibre properties of some judges. One can infer that they felt comfortable to refrain from both extremes. In general, there is high agreement between the judges when evaluating the *Agave americana L.* fibre biosoftening level in terms of softness properties; according to its three categories: the enzyme biosoftened fibre was regarded as the softest and had highest scores; ensued by water boiled and finally the raw fibre was considered the least biosoftened fibre.

#### **4.9.2 Fibre fineness**

In textiles, fibre fineness defines the size of its diameter (Wolela, 2019:632). The fine fibre is the thin fibre with a small diameter but has larger surface area when compared to its weight. It therefore has a softer texture and better handling than dense coarser fibres. This implies that the finer fibres are of higher quality and are more expensive than their thicker counterparts (Texcoms Textile Solutions (TTS), 2019:15). Figure 4.66 shows frequency distribution scores of panel evaluation determining the fineness of various raw, water boiled and enzyme biosoftened *Agave americana L.* fibre.

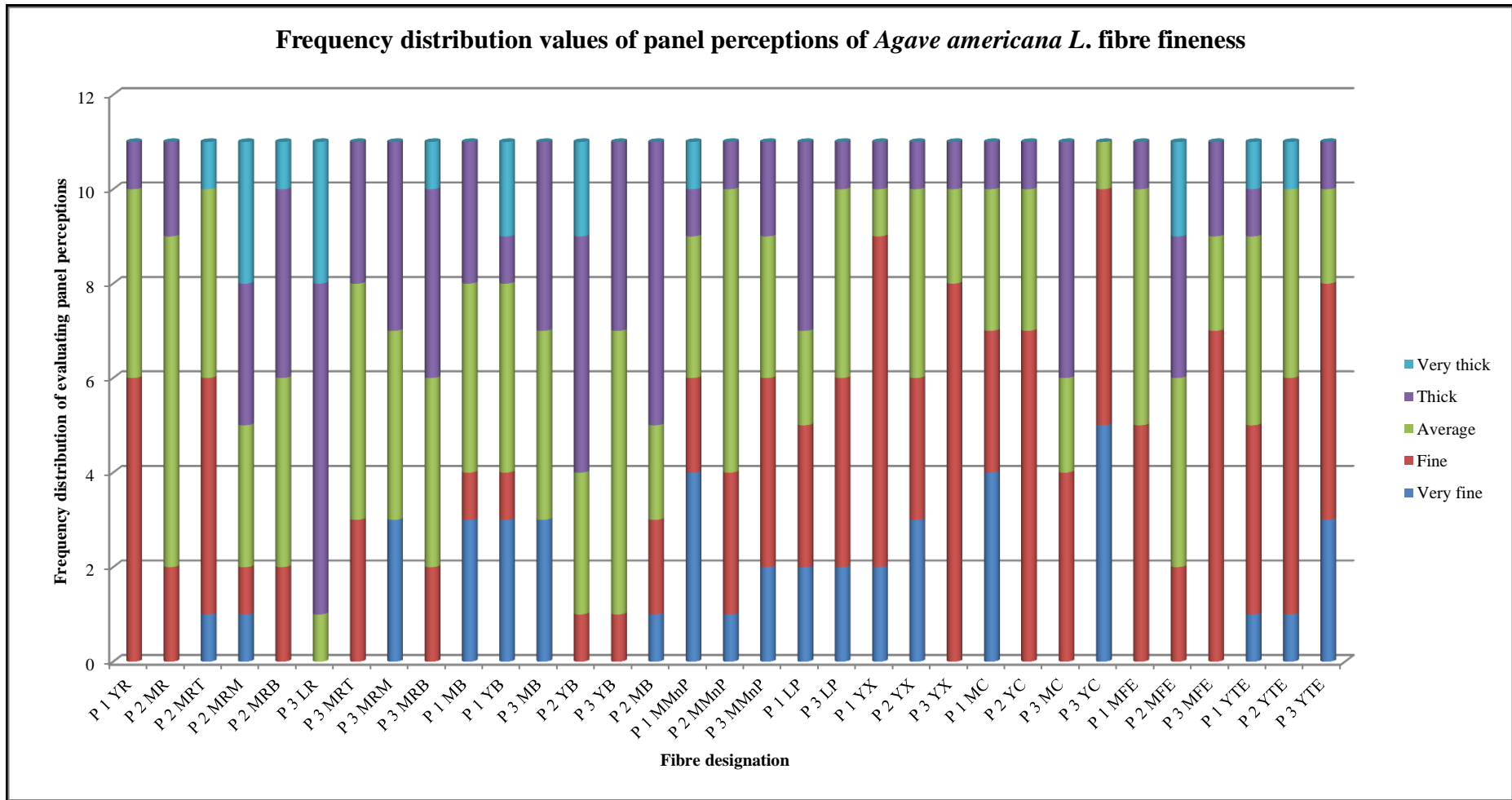


Figure 4.66: Frequency distribution scores of panel evaluation determining *Agave americana L.* fibre fineness

The frequency distribution of *Agave americana L.* fibre fineness (figure 4.66) reveals that the highest very fine and fine scores are observed from the enzyme biosoftened fibre. The very thick, thick and average scores are observed highest from the untreated *Agave americana L.* fibre side, indicating that enzyme biosoftened *Agave americana L.* fibre had the best handle and was the thinnest thus the best quality amongst the three categories. The data from figure 4.64 indicates and confirms that *Agave americana L.* fibre, like other natural plant fibres; differs greatly in fineness depending upon fibre extraction and softening processes, the plant species and the position from which the fibre was obtained from the leaf (Bourmaud *et al.*, 2018:356). The good quality fibre should have the lowest cross-sectional area. Fibre fineness or coarseness affects fibre processing behaviour. Therefore, finer fibres have better quality and handling properties as well as end-uses as compared to thicker fibres (Wolela 2019:633).

### **4.9.3 Fibre softness**

The subjective panel test is one of the most suitable methods to evaluate fibre softness (Wang *et al.*, 2019:781). Fibre softness is an essential end-use property of the textile fibre. It is therefore of utmost importance that textile science researchers and developers are incessantly endeavouring to provide improved fibre softness for textiles. Fibre softness, as a subjective perception of fibre, is difficult to define and quantify. Panel tests have been used to evaluate differences in the softness of raw, boiled and enzyme biosoftened *Agave americana L.* fibre samples. The fibre softness evaluation is considered as a subjective human perception of a texture of fibre that varies from person to person. Fibre softness is conducted with the tactile, visual, auditory, and olfactory senses. Figure 4.67 shows frequency distribution scores of panel evaluation determining *Agave americana L.* fibre softness

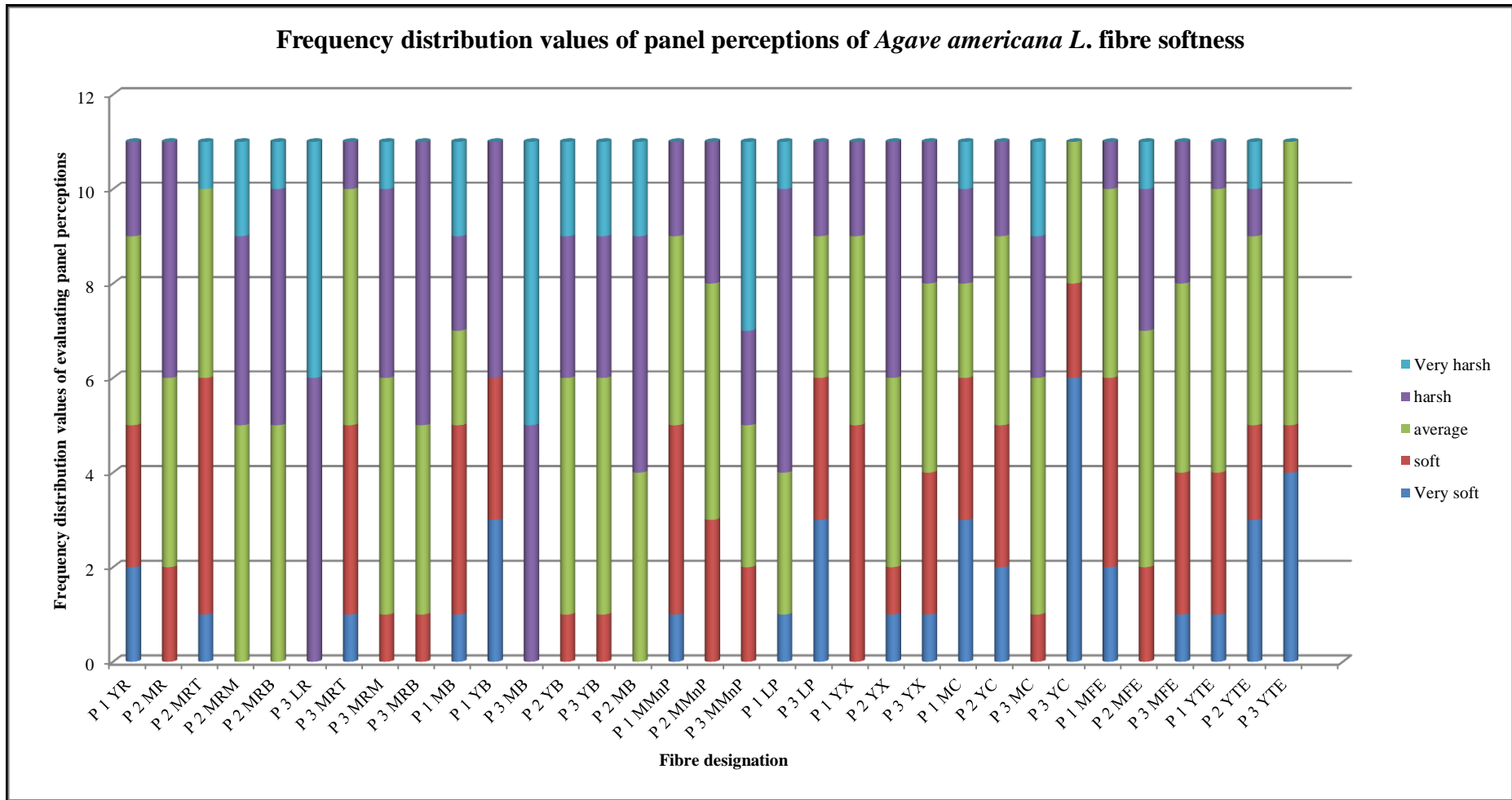


Figure 4.67: Frequency distribution scores of panel evaluation determining *Agave americana L.* fibre softness



Data presented in figure 4.67 shows variation of ideas among the participants when coming to fibre softness. There is no identifiable pattern of alternatives. Panellists disagreed on the majority of alternatives. This indicates that determining fibre softness varied amongst individuals. This reflects critical differences in individual places on the fibre softness. It confirms that fibre softness as a subjective perception is difficult to define and quantify (Wang *et al.*, 2019:781). Panel tests have been used to judge differences in the softness of *Agave americana L.* fibre samples. However, the very soft and soft data is observed mostly from the enzyme biosoftened fibre and very harsh and harsh figures are higher from the raw *Agave americana L.* fibre. The most frequent figures across are observed from the average.

#### **4.9.4 Fibre smoothness**

The *Agave americana L.* fibre smoothness is another important sensitive physical tactile property. The fibre surface smoothness refers to the friction force associated with its surface texture (Moorthy & Kandhavadi 2015:1). Figure 4.68 shows frequency distribution scores of panel evaluation determining *Agave americana L.* fibre smoothness.

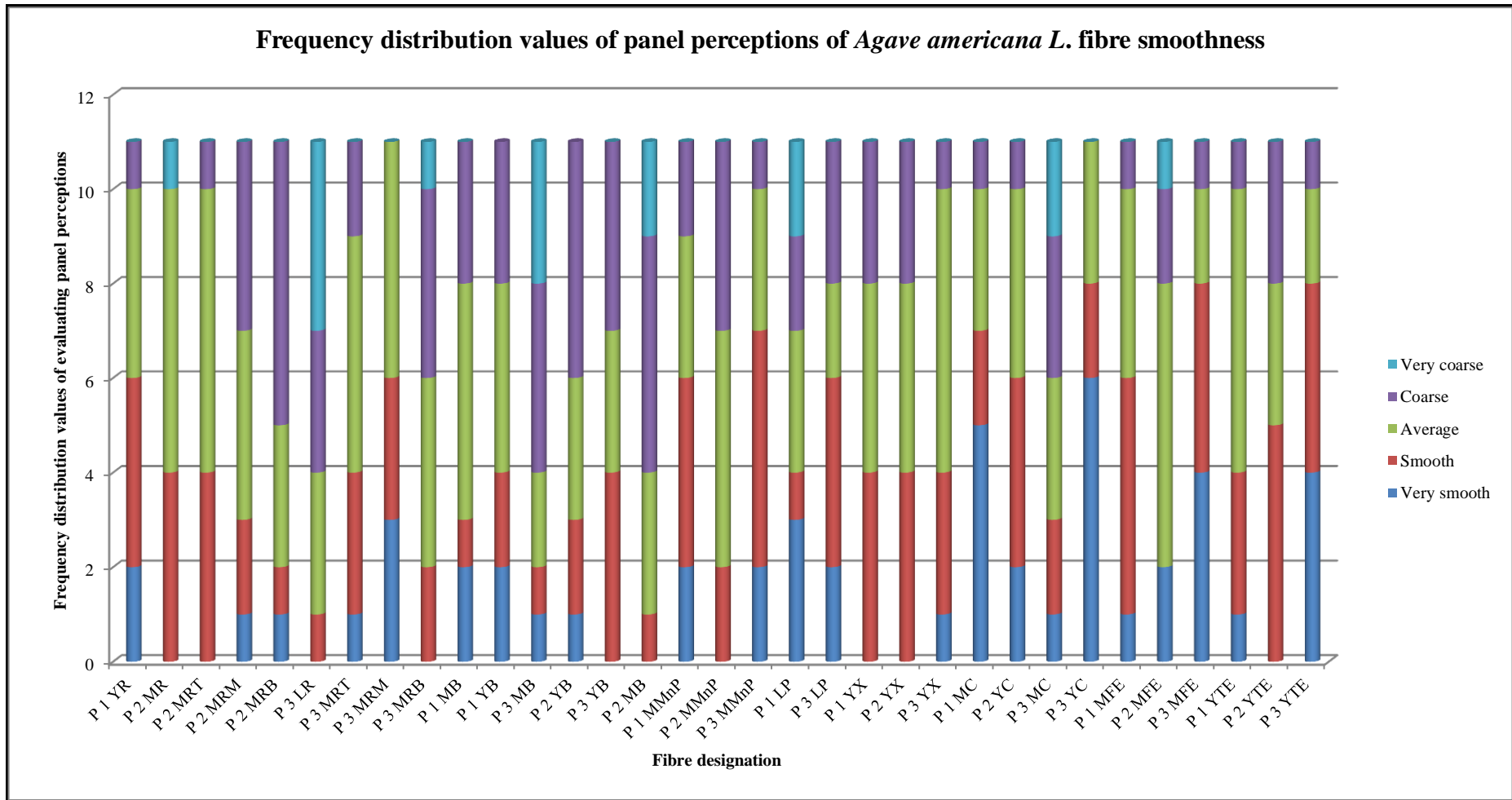


Figure 4.68: Frequency distribution scores of panel evaluation determining *Agave americana* L. fibre smoothness

Most panellists ranked the *Agave americana L.* fibre smoothness as average which means neither coarse nor smooth. Like other fibres, fineness properties very coarse and coarse values were mostly found from the raw fibre side when compared to very smooth and smooth values which are highest and many in the side of enzyme biosoftened fibre. This is the typical behaviour of the lignocellulosic fibres (Hulle *et al.*, 2015c:70).

#### **4.9.5 Fibre lustre**

Fibre lustre is the sheen that is possessed by the fibre. It refers to the rate at which the light is reflected from the fibre surface or the rate of gloss (Gundola & Kistaman, 2008:155-156). Like other plant fibres, the *Agave americana L.* fibre lustre is a surface characteristic that affects the fibre's appearance. The lustre is the nature and amount of light reflected from a surface by a fibre of interest. It defines the natural intensity or dullness of fibre. A smooth-surfaced with more regular cross-sectional shape fibre reflects light in an intensive and uniform manner that creates a high lustre. A rough-surfaced fibre, with uneven, diverse and multi-direction cross-sectional structure, presents lower lustre. A smooth and round fibre also reflects light intensely and delays' soiling as it is the case with multi-lobal fibres (TTS, 2019:28). Figure 4.69 illustrates frequency distribution scores of panel evaluation determining *Agave americana L.* fibre lustre.

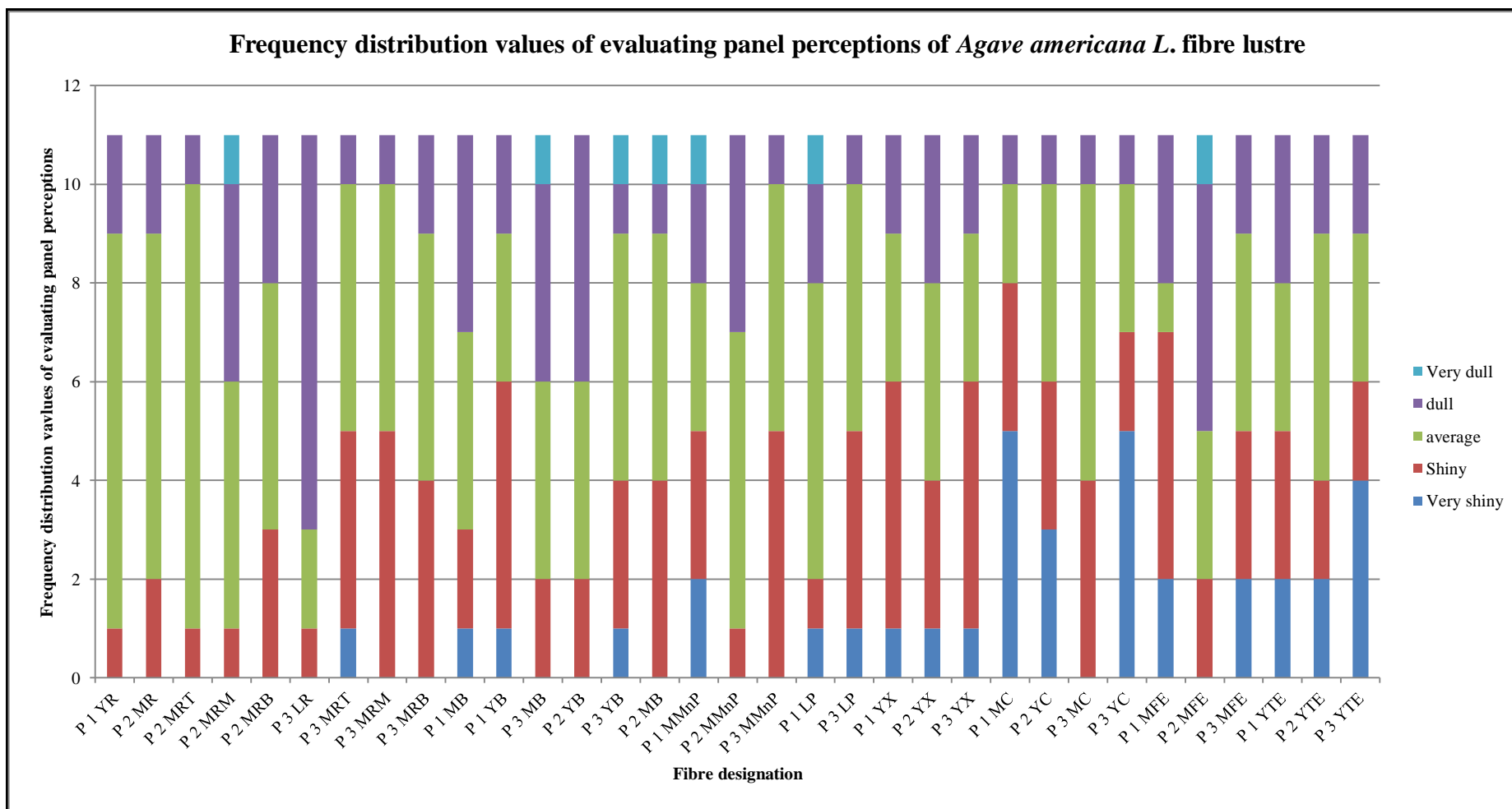


Figure 4.69: Frequency distribution scores of panel evaluation determining *Agave americana* L. fibre lustre

Fibre lustre refers to fibre shininess that occurs when light falls on the fibre and reflected off the surface. Fibre lustre is therefore significantly prominent in its effect on perceived colour. Fibre shape and smoothness are among factors that influence fibre lustre. The relative of the *Agave americana L.* fibre lustre can be influenced by its distinctive physical morphology and chemical composition (Mbugua, 2009:36). The average ranking values are the highest and most frequent of all. The raw fibre has very low values of dull with lesser frequent distribution of very dull than the boiled and enzyme biosoftened fibre. There are higher values and more frequent distributions of very shiny and shiny panellist preferences on enzyme biosoftened *Agave americana L.* fibre than raw fibre. P 1 MC and P 1 YC were the shiniest with 45% panellist preference. Shiny preference values were also higher in the enzyme biosoftened fibre. The very shiny and shiny values were higher for enzyme biosoftened *Agave americana L.* fibre but lower and fewer in the raw *Agave americana L.* fibre. This may be attributed to the fact that the raw *Agave americana L.* fibre has an irregular surface and cross-sectional shape. Hulle *et al.* (2015c:70) explained that a fibre that has an uneven cross-section; as is the case with *Agave americana L.* fibre (illustrated in figure 4.58), reflects light in all directions resulting in a dull appearance. However, this can be improved through proper fibre extraction and softening process as shown in figure 4.69.

#### **4.9.6 Fibre colour**

Fibre colour is highly related to other surface characteristics. Figure 4.70 illustrates the frequency distribution of the evaluating panel's perceptions on the colour of *Agave americana L.* fibre.

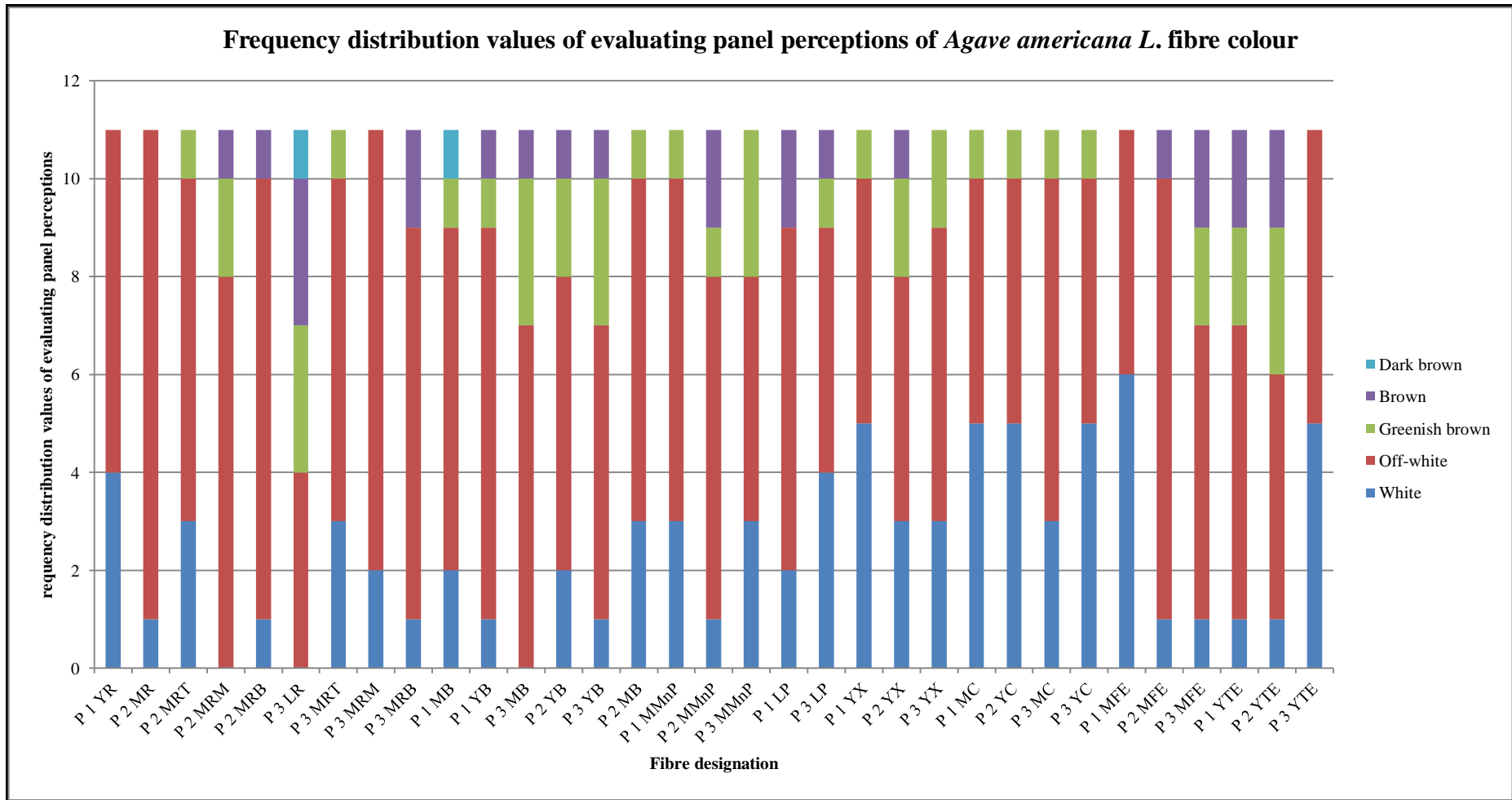


Figure 4.70: Frequency distribution scores of the panel's evaluation with respect to the colour of *Agave americana* L. fibre

The highest panellist agreement was observed in frequency distribution scores for panel evaluating the *Agave americana L.* fibre colour. They agree that *Agave americana L.* fibre is generally off-white to white and that the enzyme biosoftened fibre has higher values for white fibre than raw and water boiled fibre. These results agree with literature that the colour of *Agave americana L.* fibre ranges from yellowish-white to white (Kolte & Deberao, 2012:6) which is subject to the fibre extraction technique and time as well as the post-extraction processing procedure (Hulle *et al.*, 2015c:70).

#### **4.9.7 Fibre density**

The *Agave americana L.* fibre density is the mass of a unit volume of *Agave americana L.* fibre. It helps to explain the thickness of the fibre. It is a measurement that provides the base for the weight of the fibre. It is expressed as cubic cm. The fibre density directly affects fibre flexibility and strength. Figure 4.71 presents the frequency distribution values of the panel's evaluation perceptions on *Agave americana L.* fibre density.



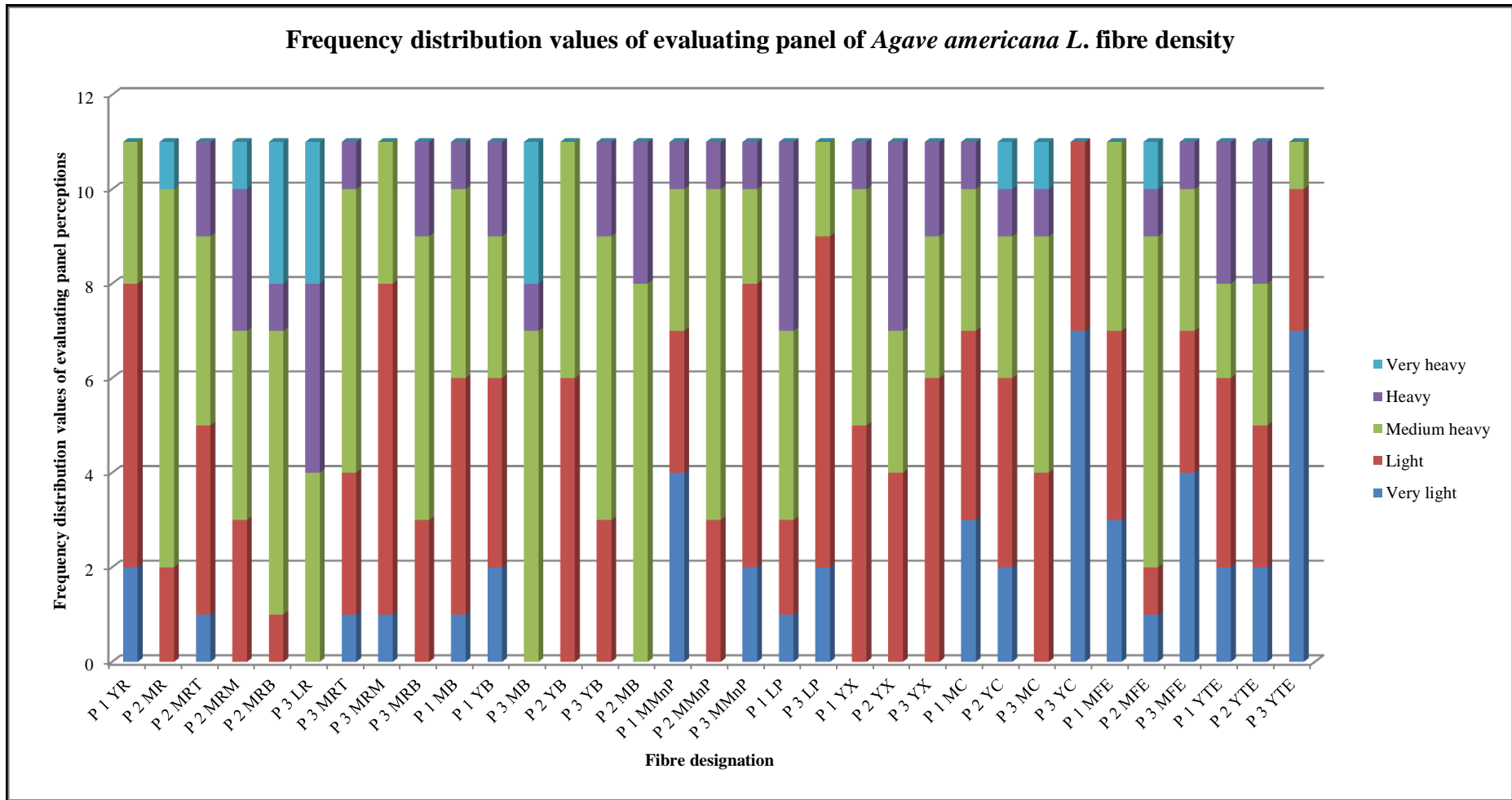


Figure 4.71: Frequency distribution scores of panel evaluation determining *Agave americana L.* fibre density

Values for medium-heavy *Agave americana L.* fibre have the highest frequency distribution; followed by light and very light respectively. P 3 YC is the lightest of all with 63.64% very light and 36.36% light preferences; followed by P 3 YTE with 63.64% very light, 18.18% light and 9.09% medium heavy preference scores there are more and higher, very light and light preference scores for enzyme treated fibre than raw fibre. This is feasible because the enzymatic biosoftening processes removed some fibre impurities which could have added weight to the fibre. The removal of impurities rendered the biosoftened fibre lighter than the raw fibre as results in figure 4.71 confirmed. However, even the raw fibre does not have a very high density if non-cellulosic impurities are adequately removed. This can be explained by the presence of voids, despite its high crystallinity (Saravanan, 2018:24-25), as illustrated in figure 4.71.

#### **4.9.8 Fibre flexibility**

Fibre flexibility is the proportion of stress to strain. It is a property of a fibre with a more flexible structure deflecting further for a given load. In the subjective assessment procedure, the stiffness-softness characteristics are related to the fibre's bending behaviour. The fibre that does not bend easily is normally referred to as stiff fibre, while the one that is less resistant to bending is referred to as flexible fibre. Fibre flexibility is determined by its shape, tensile modulus, density and mainly fineness (Chhabilendra, 2009:23-24). It is the property that is influenced by the presence and amount of lignin in the lignocellulosic fibre, as one of the three main constituents that include cellulose and hemicellulose (Vinodhini & Malathy, 2009:445). A fibre must be pliable enough to qualify for textile construction; so that it can go through repeated bending and flexing during textile processing and end-use with no significant tensile properties (Sinclair, 2015:17). Figure 4.72 shows frequency distribution scores of panel evaluation determining *Agave americana L.* fibre flexibility.

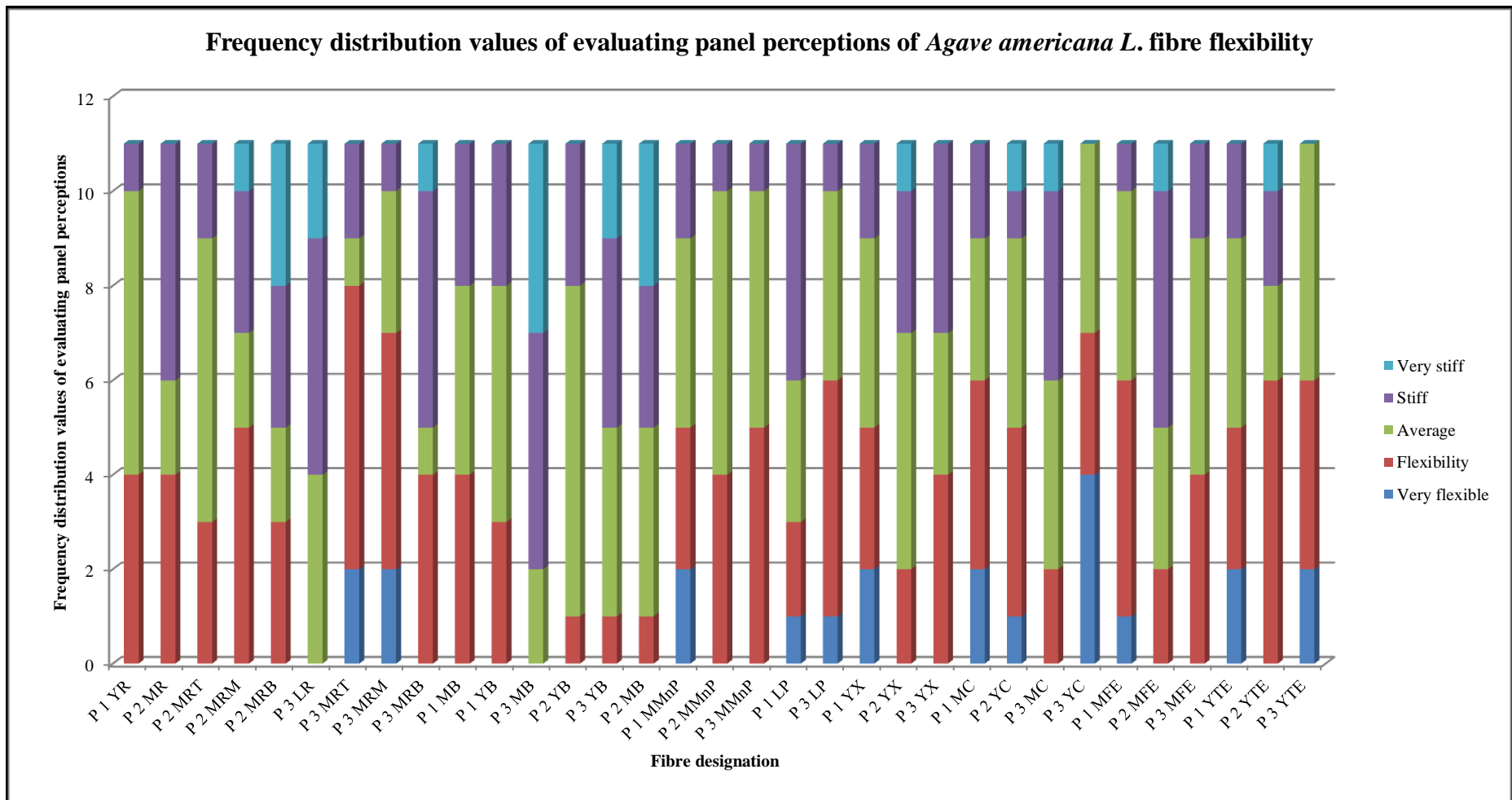


Figure 4.72: Frequency distribution scores of panel evaluation determining *Agave americana* L. fibre flexibility

Figure 4.72 has proven that the *Agave americana L.* fibre flexibility is generally low; especially for raw fibre. The hypothesis that the *Agave americana L.* fibre consists mainly of cellulose molecules that are embedded in the lignin, hemicellulose, pectin and wax matrices (Hulle *et al.*, 2015c:72) and are connected by hydrogen bonds that are fixed tightly to hemicelluloses and lignin which provide the fibre with stiffness may explain this flexibility property. This implies that the flexibility of the fibre is likely to depend on the geometric structure of cellulose microfibrils, their degree of polymerisation and the nature of the crusted and encrusted materials around them which are mainly influenced by the fibre species, growth conditions and fibre extraction method employed (Pecas *et al.*, 2018:1). It is observed from figure 4.72 that the enzymatic biosoftening of the fibre removed some of these non-cellulosic fibre components and decreased fibre rigidity is experienced. The fibre flexibility was improved to some extent as indicated by an increased number of very flexible and flexible frequency values of enzyme-treated fibre.

#### **4.9.9 Fibre uniformity**

Fibre uniformity defines the likenesses and evennesses in length and diameter of the fibre. Fibres suitable for textile processing should be nearly even in shape and size. The fibre with insufficient dimensional uniformity does not qualify to be a textile fibre (TTS, 2019:26). This can be due to fibre cohesion and spinning properties which may be affected negatively. When fibres to be used in yarn construction; have great variations in their properties it may be intolerable to actually construct a yarn or the constructed yarn may have irregular dimensions, shapes and forms, be frail, coarse and unsuitable for textile manufacture. Uniformity in thickness and length is a must-have textile property. However, most plant fibres are characterized by length and width variability due to varying growing conditions (Feigel *et al.*, 2019:2). Figure 4.73 presents frequency distribution scores of panel evaluation determining *Agave americana L.* fibre uniformity.

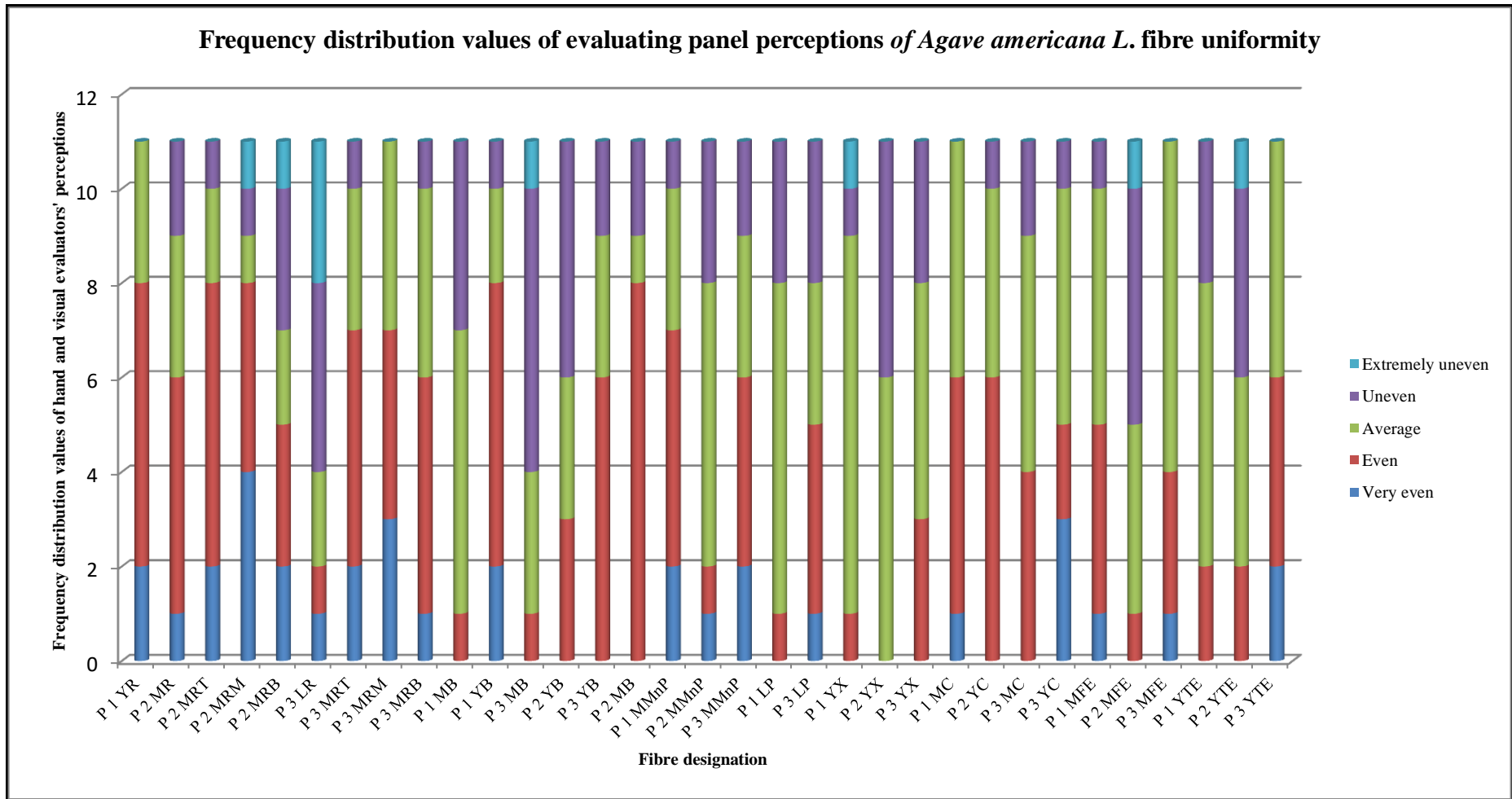


Figure 4.73: Frequency distribution scores of panel evaluation determining *Agave americana* L. fibre uniformity

The results from figure 4.73 reveal that the *Agave americana L.* fibre uniformity was judged differently by most judges. The reason for this discerned difference might be the unsatisfactory judges' perception of the concept of fibre uniformity. The frequency distribution judgment for average values was the highest. However, values for average were higher for enzyme-treated fibre than for raw fibre. This implies that most panellists found the enzyme biosoftened *Agave americana L.* fibre neither even nor uneven. Very even and even values were high in the raw fibre. This is not expected because Hulle *et al.* (2015c:71) claimed that unlike the synthetic fibres, the *Agave americana L.* fibre surface texture, diameter, shape and length are uneven and inconsistent due to growing irregularities, which is a characteristic of natural lignocellulosic fibre.

#### **4.9.10 Fibre suitability for textile usage**

Figure 4.74 presents frequency distributions scores of panel evaluation determining *Agave americana L.* fibre suitability for textile usage.

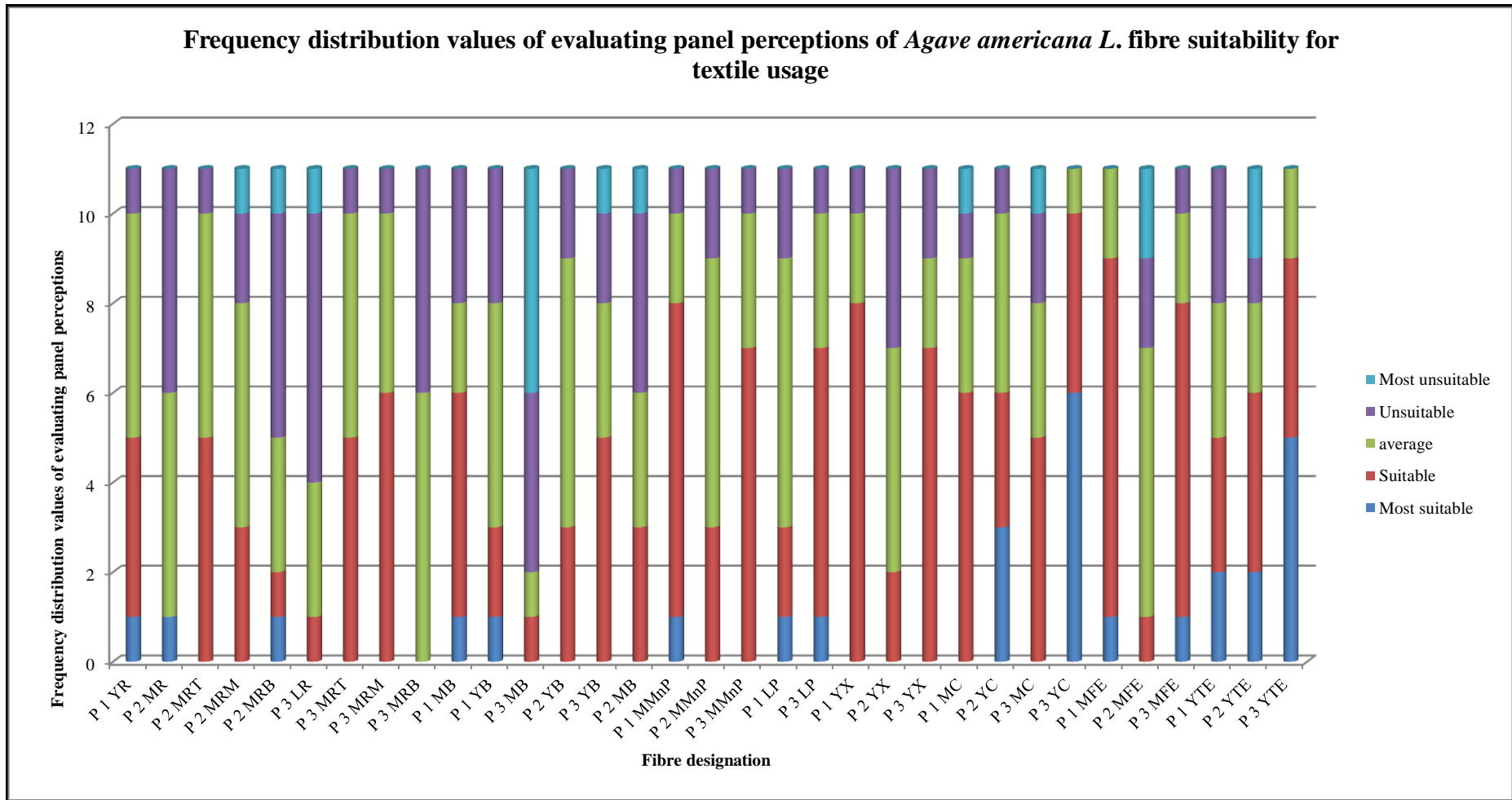


Figure 4.74: Frequency distributions scores of panel evaluation determining *Agave americana* L. fibre suitability for textile usage



There is a consensus among the panelists that *Agave americana L.* fibre is suitable for textile use. This is observed from the result in figure 4.74. The enzyme biosoftened fibre samples showed the highest frequency values. The highest frequency value is 8 out of 11 for the younger fibre from plant 1 treated with xylanase and the fibre extracted from the middle leaves of plant 1 biosoftened with four different enzymes applied in sequence namely P 3 MFE, P 3 YX, P 1 MnP and P 2 M MnP. Looking at the evaluated innate and introduced physical properties of *Agave americana L.* fibre, one can infer that *Agave americana L.* fibre is suitable for textile fibre use because it possesses certain physical characteristics that include high strength, good colour and lustre which qualify it to be converted into various textile products (Asim, 2015:3).

Water boiling pre-treatment and enzymatic biosoftening; further improved its textile characteristics such as fineness, softness, smoothness, flexibility and density to mention a few. However; further research has to be conducted to improve it for use to manufacture softer clothing that can be used next to human skin because there is still room for improvement on these properties that include flexibility, softness and smoothness. *Agave americana L.* fibre is readily available (Kendra, 2013:3) in Lesotho because it grows at no or minimal cost everywhere in the country, enzymatic biosoftening conducted in this research study has proven its improved softness, smoothness, pliability, density colour and lustre.

## CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

### 5.1 CONCLUSIONS

This chapter gives the overall conclusion on the results and discussion that emerged from the study. Major results that were presented in the previous chapter are emphasised herein as well as some vivacious inferences and commendations for future research. The overall aims of this research study were to develop a sustainable and eco-friendly method of fibre extraction, explore enzymatic biosoftening processes, investigate the inter-intra plant fibre variability and the physico-mechanical textile characteristics of *Agave americana L.* fibre in order to satisfy the mounting demand for natural fibres, sustain petroleum fibre resources and diversify its functionality in the textile industry. The conclusions and recommendations were made from the results acquired from the empirical procedures for the fibre extraction, enzymatic biosoftening, variability and physico-mechanical properties of *Agave americana L.* fibre.

**H0<sub>1</sub>: Hand harvesting of *Agave americana L.* plant is impossible due to its sharp leaf marginal and tip spines.**

The hypothesis is nullified because the *Agave americana L.* leaves were successfully hand harvested for fibre extraction. Even though; the researcher had to be careful and wear appropriate protective equipment when harvesting and preparing *Agave americana L.* plant leaves for treatment to avoid being stung and skin irritations by the leaf sap. The appropriate sharp cutting equipment are used accordingly. Hands were washed after handling the partially degraded *Agave americana L.* plant.

**H0<sub>2</sub>: Triangulation water retting is an ineffective and inefficient fibre extraction technique to degrade the non-cellulosic *Agave americana L.* leaf biomass to release fibre bundles**

The hypothesis is nullified because the triangulation water retting of *Agave americana L.* leaves to extract fibre was conducted effectively and efficiently on comparable time with the soft natural cellulosic fibres. It also produced a comparable good quality fibre. It was to some extent easy and speedy; to extract the fibre from decomposed non-cellulose leaf biomass components with a gentle hammering of the under-retted fibre that ended up with less or no

fibre damages. The triangulation water retting produced an improved fibre yield, with brighter colour, and softer and smoother texture than the conventional open whole leaf water retting method. It is an improvised method and a combination of ribbon, insulated, closed tank water retting techniques. The triangulation water retting of *Agave americana L.* leaves occurred under cloudy and rainy weather conditions. It speeded-up the retting of the voluminous *Agave americana L.* leaves which would otherwise delay and produce lower quantity and quality fibre. The triangulation retting is also feasible in every season.

**H0<sub>3</sub>: The triangulation water retting is an environmental-hazardous *Agave americana L.* fibre extraction method.**

The hypothesis is nullified because the triangulation water retting is a combination of several modified, controllable techniques that fast-track the retting process and reduces environmental pollution and contamination. The retting drums were cleaned. The tap water used for retting was also clean and safe to use. The *Agave americana L.* leaves were first stripped for easy water penetration and leave contents dissolution in water to hasten the retting process. The retting drums were closed with tight fitting lids to reduce chances of humans inhaling the smell from the fermenting leaf contents. The triangulation water retting process is simple biotechnology that is more environmental-friendly and less health hazardous than a conventional water retting.

**H0<sub>4</sub>: The age of *Agave americana L.* leaves negatively affect the retting and fibre extraction processes**

The hypothesis is accepted because; the retting and extraction of *Agave americana L.* fibre were affected by the age of the plant leaves. The older leaves are more resistant to the retting process than the younger leaves. This then resulted in reduced separation rate of the shive from the older leaf fibres which reduced fibre extraction efficiency and fibre quality. The older leaves have accrued more lignin and other extra-cellular contents that render a challenge to the retting microorganisms to hasten the process which in turn affects fibre extraction negatively.

**H0<sub>5</sub>: The depth of retting water negatively affects the *Agave americana L.* leaves retting and fibre extraction processes**

The hypothesis is accepted because; the depth of retting water is another factor that negatively affects the retting and extraction efficiency of *Agave americana L.* fibre. Retting of *Agave americana L.* fibre was faster and most efficient at the surface level in the retting water and gradually decreased with the increase in depth. The more under-retted parts of the leaves were those in the bottom of the barrels. This then resulted in excessive fibre damage due to the difficulty and slow fibre separation from the non-cellulosic biomass and imperfect removal of sticky substances such as pectin substances which affected the fibre processing efficiency and time, fibre yield, and quality.

**H0<sub>6</sub>: *Agave americana L.* fibre is not a potential sustainable alternative textile fibre to the synthetic fibres.**

The hypothesis is nullified because the results showed that the *Agave americana L.* fibre is a lignocellulosic fibre extracted from the local, natural and wild plant. The textile fibre sustainability is an emerging concept that provides development opportunities to natural fibres like *Agave americana L.* fibre. The *Agave americana L.* fibre is a renewable and biodegradable potentially sustainable alternative to non-renewable and non-degradable synthetic fibres, if its potential characteristics can be harnessed. It divulges the physico-mechanical properties that qualify it to become a potential textile fibre. It is likely to be transformed into sustainable and viable textile fibre processes and products. From the ecological and socio-economic point of view, *Agave americana L.* fibre is a potential new, textile fibre that can satisfy the growing demands for sustainable textile fibres that can be an alternative to non-degradable and non-renewable synthetic fibre.

**H0<sub>7</sub>: *Agave americana L.* fibre is not a potential textile fibre**

The hypothesis is nullified because the results showed that the *Agave americana L.* fibre possesses some desirable physico-mechanical textile fibre properties. It also has some properties that are undesirable for it to become a textile fibre but can be improved to upgrade it. The raw *Agave americana L.* fibre has good tensile strength and a desirable length-to-width ratio to be eligible to become a futuristic textile fibre. However, it is coarse, harsh and rigid in texture, but it can be softened (with enzymes and boiling water), to achieve the desirable softness and flexibility properties for the prospective textile fibre use.

The raw *Agave americana L.* fibre colour is dependent upon the fibre extraction time and processing technique, but it ranges from off-white to yellowish rust but it can be whitened

with enzymes to improve the fibre aesthetic value. The level of judges' agreement at panel assessment of the fibre biosoftening rate, fineness, softness, smoothness, lustre, colour, density, flexibility, uniformity and suitability for textile use; was high enough to conclude that *Agave americana L.* fibre is a potential textile fibre that can be enzymatically softened and upgraded to textile fibre for diverse uses.

**H0<sub>8</sub>: *Agave americana L.* fibre is an inconsistent and heterogeneous multi-cellular fibre composite**

The hypothesis is accepted because results indicated that *Agave americana L.* fibre exists as a natural lignocellulosic fibre composite consists of minute numerous single micro-fibrils referred to as ultimate fibres or just ultimates. The *Agave americana L.* fibre is a multi-cellular fibre bundle consists of irregular, polygonal, heterogeneous cells. The ultimate fibre cells are overlapping and detained together by waxy and sticky non-fibrous constituents to form the filament fibre. This implies that each individual *Agave americana L.* fibre is a relatively thick vascular fibre bundle which is tissue vessels of transportation embedded in thick fibre cell sheathes. The *Agave americana L.* fibre has a lumen that is separated from neighbouring ultimate by middle lamella.

**H0<sub>9</sub>: There is insignificant intra-plant variability of *Agave americana L.* fibre physico-mechanical properties**

The hypothesis is rejected because the results showed a wide variability in physico-mechanical properties. The fibre quality and quantity obtained from different leaves obtained from the same level of a plant have different physico-mechanical properties. *Agave americana L.* fibre quality and quantity are innately variable within individual plants, leaves at the same and different levels and ages. The variability is evident in the morphology, size, texture and tensile properties of the fibre from the different parts of a fibre (base, middle and tip), between different fibres extracted from same leaves, extracted from different leaves at different levels (lower, middle and top) from the same plant. The intra-plant fibre variation is due to climatic and seasonal acclimatization conditions, soil type and fertility rate, fibres growing in varied parts of a plant.

**H0<sub>10</sub>: There is insignificant inter-plant variability of *Agave americana L.* fibre physico-mechanical properties.**

The hypothesis is nullified because the results portrayed great variations in the morphology, size, texture and tensile properties of *Agave americana L.* fibre. The variations in the physico-mechanical properties can be explained by the chemical and structural disparity between the different fibres extracted from different plants and their layers that include variation in cellulose content against lignin and hemicellulose proportions and microfibril angle along the cell axis.

**H0<sub>12</sub>: Enzymatic biodelignification is an inefficient wet processing textile technology to improve the texture of *Agave americana L.* fibre to qualify to be a textile fibre**

The hypothesis is nullified because the results showed that manganese peroxidase biodelignification removed the *Agave americana L.* fibre surface impurities and debris and had it softer than when it was raw. The enzymatic delignification is considered as an effective sustainable biotechnology to degrade lignin and soften the fibre. The enzymatic biodelignification is an effective, efficient, eco-friendly and a substrate specific biotechnology that removes lignin to soften and upgrade the *Agave americana L.* fibre for textile use.

**H0<sub>13</sub>: The enzymatic bioscouring of *Agave americana L.* fibre is an infeasible alternative to conventional scouring procedure for lignocellulosic fibre softening**

The hypothesis is nullified because enzymatic bioscouring of *Agave americana L.* fibre with pectinase was accomplished effectively; under mild and eco-friendly conditions to remove non-cellulosic fibre substances such as wax, fats, dirt and others from the fibre without negatively affecting the fibre strength; as it would be the case with conventional chemical bioscouring. The *Agave americana L.* fibre texture was softer in pectinase bioscouring compared to harsh feel in untreated fibre. The elimination of non-cellulosic fibre substances from the fibre was designated by reduced fibre weight and strength when compared to those of the raw fibre.

**H0<sub>14</sub>: The enzymatic biobleaching of *Agave americana L.* fibre is not expected to decolourise natural pigments and to confer a pure white appearance to the fibres**

The hypothesis is nullified because the *Agave americana L.* fibre was biobleached with xylanase. The xylanase brightened the fibre colour. The improved fibre colour after enzymatic treatment is indicative of the removal of non-cellulosic impurities that which

otherwise impart discolouration and dullness to the fibre. The enzymatic biobleaching brightens and improves fibre lustre and appearance.

**H0<sub>15</sub>: Bio-polishing of *Agave americana* L. fibre with cellulase is an ineffective biosoftening biotechnology**

The hypothesis is nullified because the results indicated that the enzymatic biopolishing *Agave americana* L. fibre with cellulase enzyme removed the impurities and individual loose fibre ends which protruded from the surface of the untreated fibre to render it a smooth surface finish. The fibre lost weight and became smoother and softer when compared to the raw fibre. This happens because cellulase enzyme catalyses and removes the lignocellulosic, protruding fibre micro-fibrils to softens the fibre.

**H0<sub>16</sub> Sequential enzymatic biosoftening is ineffective when compared to an individual enzymatic biosoftening of *Agave americana* L. fibre**

The hypothesis is nullified because the enzymatic wet processes applied to biosoften the *Agave americana* L. fibre were proved effective and the sequential enzymatic biosoftening processes resulted in higher weight loss percentage than individual enzymatic biosoftening processes. The fibre weight loss percentage was an indication of the effectiveness and efficiency of the enzymatic biosoftening processes. Weight loss percentage implied the effective elimination of non-fibrous impurities from the fibre. The *Agave americana* L. fibre weight loss increased spontaneously with increase in a number of sequential enzymatic biosoftening processes.

The highest weight loss percentage was obtained with the sequential enzymatic biodelignification, bioscouring, biobleaching and biopolishing using MnP, pectinase, xylanase and cellulase enzymes respectively. Thus, better improved the *Agave americana* L. fibre softness. It makes sense to conclude that the fibre weight loss increased spontaneously with increased number of enzymes and the treatment time used. The individual enzymes are substrate specific. Thus each enzyme works on one fibre component and leave the rest intact.

**H0<sub>17</sub>: Water boiling pre-treatment is an ineffective the *Agave americana* L. fibre softening process**



The hypothesis is nullified because the boiling water treatment improved the texture of the fibre to a certain extent; but at a lower rate than the enzymatic biosoftening technology. The weight loss percentage for water boiling treatment ranges within the acceptable weight loss percentage that can improve the texture of the lignocellulosic fibre. Water boiling pretreatment of *Agave americana L.* fibre is considered as a potential and accessible technology that can be applied to function as a wetting and a softening agent.

**H0<sub>18</sub>: The physical properties of *Agave americana L.* fibre are negatively affected by controlled enzymatic biosoftening processes**

The hypothesis is nullified since; the enzymatic biosoftening had a considerable effect on physical properties of *Agave americana L.* fibre. The *Agave americana L.* fibre is a coarse and hard fibre to bend because it is a lignocellulosic fibre which has high lignin content in its composition. The enzymatic biosoftening reduced its innate coarseness, rigidity and hard feel. Enzymatic biosoftening has affected almost all physical properties of the fibre. However, the changes are of varying magnitudes. Some changes especially uniformity and bending length, were less favourable; some were encouraging like fibre colour, lustre, smoothness, softness, fineness, density biosoftening rate and suitability for textile use.

Nevertheless, the fibre can be used to manufacture aesthetic textile items which do not require graceful draping and evenness. The fibre aesthetic and textile performances are acceptable even with these drawbacks. However, there is a need for more research for it to further biosoften it to qualify for manufacture of extreme soft, next to skin apparel. The fibre physical textile properties are acceptable and have potential to improve with enzymatic biotechnology and must further be conducted under more accurate and good controlled conditions, concentrations and time frames.

**H0<sub>19</sub>: The tensile properties of *Agave americana L.* fibre are negatively affected by enzymatic biosoftening processes**

The hypothesis is accepted since; the enzymatic biosoftening has had a considerable effect on the tensile properties of *Agave americana L.* fibre. Enzymatic biosoftening has affected almost all the tested tensile properties of the fibre. Even though the changes are of varying magnitudes too. The improved enzymatic biosoftening rate results in reduced tensile strength and Young's modulus properties when compared to those of the raw fibre. However, the plant fibre mechanical properties normally have an inverse relationship with the must have

fibre softness properties of a textile fibre. This indicates that even though mechanical fibre properties were negatively affected but that improved the softness properties of the fibre.

The research study showed that the strength reduction in *Agave americana L.* fibre is related to fibre diameter reduction. The fibre strength reduction is an undesirable fibre feature. Nevertheless, the fibre can be used in textiles to manufacture textile items that will not be subjected to severe strain. The fibre strength performance is acceptable even with these drawbacks. It must be noted that due fibre fineness and the enzymatic biosoftening of *Agave americana L.* fibre must be conducted under more accurate and good controlled condition concentrations and time frames. The *Agave americana L.* fibre strength properties are affected to some extent but that have not compromised the fibre quality for textile use since other textile fibre properties are improved.

As a result, *Agave americana L.* fibre can offer a sustainable alternative opportunity as fine and flexible to a certain extent, biodegradable and lengthy natural fibre. *Agave americana L.* fibre is decomposable and thus it is regarded as eco-friendly. Enzymatic biotechnology was found efficient enough to biosoften the *Agave americana L.* fibre. It is a safe and environmental-friendly procedure that is commendable for sustainable textile *Agave americana L.* fibre

**H<sub>020</sub>: Enzymatic biosoftening of *Agave americana L.* fibre is not an ecological alternative biotechnology to chemical auxiliaries in textile processing**

The hypothesis is nullified because the results have proven that the enzymatic biosoftening of *Agave americana L.* fibre is an environmental-friendly, biotechnology that can improve the physical textile properties. Enzyme biotechnology is an emergent ecological biotechnology; alternative to chemical auxiliaries in textile processing. Enzymatic biosoftening is conducted under mild environmental conditions. It shortens processing times, consumed little energy and water. The enzymatic biotechnology minimizes pollutions because it uses very few and mild chemicals. Enzymes are non-toxic and reduce pollution in textile production safe handling, storage, use and disposal make enzymes best choice catalysts for the textile bioprocessing; because it is risk-free and eco-friendly, the characteristics which are progressively essential for textile producers to decrease the textile production and processing pollution. work

Enzymatic biosoftening of *Agave americana L.* fibre is an emergent alternative, save and eco-friendly biotechnology that can substitute harsh chemicals, which when the disposed without pre-treatment pose ecological distresses. Biosoftening of *Agave americana L.* fibre using the lignocellulolytic enzymes is the potential sustainable textile biotechnology alternative to chemical textile technology which is harsh, hazardous and environmentally-insensitive.

**H0<sub>21</sub>: The effectiveness and efficiency of the enzymatic biosoftening of *Agave americana L.* fibre is inversely proportional to the fibre tensile strength**

The hypothesis is approved because the results revealed that the enzymatic biosoftening of *Agave americana L.* fibre increased inversely with the fibre weight loss percentage. The extent of fibre weight loss indicated the extent of fibre softness and fineness. The increase in weight loss percentage indicated the tensile strength decrease. The fibre softness and strength are essential properties that influence other basic properties of a textile. Fibre softness must be maximised to increase the functional and aesthetic performances of the fibre. Results show that *Agave americana L.* fibre was softened and this was indicated by weight loss.

Conversely, the excessive weight loss is not necessary because it would mean that all the lignin from the fibre is removed, the total removal of lignin would imply complete defibrillation of *Agave americana L.* fibre which would result into shorter ultimates with excessive strength loss. It is therefore ideal to improve fibre softness to improve consumer perception of the textile in which the fibre of interest is used while striving to improve and/ or maintain sufficient and effective textile fibre strength. However, it is generally accepted that fibre softness properties are reversely correlated to fibre strength properties. As strength upsurges, softness declines or vice versa.

**H0<sub>22</sub>: The commercial enzymes are incapable of degrading the non-cellulosic polymers substrates of *Agave americana L.* fibre as a means for bio-softening and up-grading it**

The hypothesis is nullified the commercial lignocellulolytic enzymes showed the ability to impart softness to the *Agave americana L.* fibre. The following procedures biodelignification, bioscouring, biobleaching and biopolishing were applied to biosoften *Agave americana L.* fibre with the following commercial enzymes: MnP, pectinase, xylanase and cellulase respectively; in single and sequential applications. Although enzymatic biosoftening of *Agave americana L.* fibre with commercial enzymes showed the potential and prospects for best textile products, under specific substrate, low energy costs and mild operating conditions;

which could not be achieved with chemical processes. However; the commercial enzymes are expensive. The high cost is a limiting factor for further exploration of *Agave americana L.* fibre biosoftening with commercial enzymes to improve the fibre textile properties.

**H<sub>023</sub>: The fibre bending length of *Agave americana L* fibre is naturally low and is not improved by the enzymatic biosoftening processes**

The hypothesis is rejected because the enzymatic biosoftening removed some of these natural cementing non-cellulose impurities which impart fibre stiffness and coarseness. Thus, improved fibre fineness, softness, smoothness lustre and supple were obtained when compared to raw fibre. It is therefore reasonable for one to think that it improved its spinability which is suitable for diverse textile uses depending upon the intensiveness of enzymatic biosoftening and combination of biosoftening processes.

## **5.2 RECOMMENDATIONS**

These recommendations are based on the opinions expressed in the results analysis of the present study. It is essential to further do research on sustainable *Agave americana L.* fibre extraction and biosoftening processes. The results of this research express the possibility of more biosoftening exploration to increase its utilisation rate and further reduce the chemical agent consumption in the textile industry. It is recommended to intensify research on sustainable, *Agave americana L.* fibre extraction and biosoftening technologies as good alternatives to conventional non-environmental methods.

Thus, any future study should investigate more intensely on advance practices that reduce retting water requirements, but develop environmentally safe and improved fibre retting technologies. Striping the *Agave americana L.* leaves several times can support the approach to alternative technology to whole leaf retting through water and time saving, futuristic fibre extraction biotechnology which assist to produce high quality and yields with very much reduced pollution, while still efficient and cost effectiveness.

The surface area of the retting tanks to be used should be wide and the leaves of *Agave americana L* plant should not be submerged in water at the level deeper than 60 cm. It is also recommended that other environmental-friendly fibre extraction methods that include ribbon retting backed up with double retting and/or with inoculation of bacterial culture or

enzymatic retting may be more feasible eco-friendly techniques to produce outstanding quality of fibre. The use of warm water at a temperature more or less 35°C to ret *Agave americana L.* fibre, is recommended for future triangulation water retting to increase the speed with which the leaves fermentation and retting can occur.

In future study research of *Agave americana L.* plant retting to extract fibre; it is recommended that microbial community, temperature, depth and pH of retting water be evaluated as they may be other key factors that define the quality and quantity of retted fibre. Research on textile technology and machinery developments should be conducted because that can assist in effective and efficient *Agave americana L.* plant harvesting and fibre extraction to increase development prospects.

It is recommended that additional research on *Agave americana L.* plant and fibre be deepened to identify the zero waste characteristics and future alternative uses of the plant and fibre in order to diversify their functions. The *Agave americana L.* fibre mixing and blending with other sustainable textile fibres should also be explored in order to improve its textile performance properties to diversify its uses. Investigating its textile use after biosoftening is of paramount significance for the sustainable textile development. The *Agave americana L.* fibre possesses high tensile properties and the physical properties that can be improved to provide a wide variety of uses in textiles. Additional research on *the* chemical properties of *Agave americana L.* fibre is crucial to increase opportunities for diversifying the fibre future uses that may contribute to economic activity.

The enzymatic biosoftening of *Agave americana L.* fibre has been proven to diminish the exploitation of excessive water, energy and chemicals. The enzymatic biosoftening turns into sustainable fibre processing, non-polluting water and energy conserving, safe and healthy tool for textile scientists and workers and communities. However, the commercial enzymes are very expensive. It is therefore recommended that other microbial enzymatic biosoftening processes be explored to reduce costs. Enzymatic biosoftening is a potential sustainable technology that can assist to improve the textile properties of *Agave americana L.* Enzymatic biosoftening of an *Agave americana L.* fibre is not only a sustainable technology which could trigger the production of good quality fibre.

It is also recommended that other methods and technologies like biological biosoftening of the *Agave americana L.* fibre, which could be as well-thought-out sustainable, safe and

alternative enzymatic biotechnology have to be investigated. More research studies have to be conducted that investigate the eco-socio-economic sustainability impact of *Agave americana* L. fibre in its whole life cycle starting from its crop growing to the end-products disposals and to intensify its indebtedness to the people in general against untenable materials.

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