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ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

CHARACTERIZATION, VALORIZATION AND OPTIMIZATION OF ENSET */ENSETE VENTRICOSUM/* FIBERS FOR PAPER PULP PRODUCTION

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A PhD Dissertation Submitted to the School of Chemical and Bio Engineering in Partial Fulfillment of the Requirements of the Degree of Doctor of Philosophy (Process Engineering Stream)

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DECLARATION

I hereby declare that this dissertation is my own, original work. It is being submitted for the Degree of Doctor of Philosophy, to Addis Ababa University Institute of Technology, Addis Ababa, Ethiopia. It has not been submitted previously for any other degree or examination in this or in any other institution. Due citation and acknowledgement has been made properly.

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This is to certify that the dissertation entitled as **"Characterization, Valorization and Optimization of Enset *Ensete Ventricosum* Fibers for Paper Pulp Production"** submitted to Addis Ababa Institute of Technology, School of Chemical and Bio Engineering for partial fulfillment of the requirements for the degree of Doctor of Philosophy (Process Engineering Stream) complies with the regulations of the university and meet the accepted standards with respect to its originality and quality.

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ABSTRACT

Characterization, Valorization and Optimization of Enset /Ensete Ventricosum/ Fibers for Paper Pulp Production

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December, 2018

Enset /*Ensete ventricosum* [Welw.] Cheesman/ is exploited as a food crop in Ethiopia where it represents an important staple food. The plant is harvested and large amounts of biomass residues are originated, mainly from the pseudo stem (i.e., fiber bundles obtained from the leaf sheaths after being scrapped to produce starchy food) and the inflorescence stalk. These materials were studied in relation to their summative chemical composition, composition of lignin, lipophilic and polar extracts. Moreover, their structural characteristics, in view of their valorization, were scrutinized. The analytical studies were performed with the aid of FTIR, GC/MS and Py-GC/MS. The fiber bundles are aggregates of mainly long and slender fibers with low ash, extractives and lignin contents (3.8%. 4.4% and 10.5% o.d. based respectively) and high holocellulose and α-cellulose contents (87.5% and 59.6% o.d. based respectively). The hemicelluloses in the fibers are mostly highly acetylated xylans and the lignin is of the H-type (H:G:S, 1:0.7:0.8). This lignin composition is in line with the FTIR peaks at 1670 cm^{-1} and 1250 cm^{-1}. The inflorescence stalk has high ash content (12.3% in the main stalk and 24.6% in fines) with a major proportion of potassium, and high extractives, low lignin and α-cellulose contents (25.9%, 5.8% and 17.9% respectively). The stalk includes numerous starch granules in the cellular structure with the predominant presence of parenchyma. The potential valorization routes for these materials are clearly different. The fiber bundles could be used as a fiber source

for paper pulp production with the possibility of a prior hemicelluloses removal while the inflorescence stalk has nutritional value for food and fodder.

Based on the above result, the Enset fiber was further delignified by sulfur-free ethanol-alkali pulping. Response surface methodology with central composite experimental design was used to evaluate the effects of four independent process variables (pulping temperature, time, ethanol concentration and alkali concentration) on pulp yield, kappa number and color of the resulting pulp. The major parameters influencing pulp yield and kappa number were found to be the alkali concentration followed by temperature. For the predicted optimal pulping conditions, the pulp yield was 69.9% with 4.9 kappa number and 64.5% brightness. The ethanol-alkali pulp obtained at center conditions was characterized by viscosity and strength properties and compared with Kraft pulp, showing comparable burst, tensile strength and tear strength. Results suggest that the valorization of the Enset fibers for production of well delignified, high yield and resistant pulp is a promising approach.

The effects of un-catalyzed and alkali-catalyzed (0.1% NaOH) hydrothermal pre-treatment on the fractionation of the Enset fiber bundle material were made to recover hemicellulose sugars prior to ethanol alkali pulping. The treatments were analyzed at 130ºC, 150ºC, 180ºC, and 200ºC and the composition of the solids regarding lignin and monomeric sugar and of the liquid phase were evaluated. Temperature-dependent mass loss was observed in both catalyzed and un-catalyzed treatment. The liquor was rich in oligosaccharides with a maximum recovery achieved with the alkali-catalyzed hydrothermal treatment at 130ºC for GOS (12.4 g/100 g of original dry sample) and at 180ºC for XOS (3.4 g/100 g of original dry sample). The fibrous nature of the material was preserved after the treatment. Hence, the solid residue was further delignified by

using ethanol reinforced alkali pulping at near optimum point (40% ethanol conc., 15% alkali conc., 140^{o}C, 60 min). At the optimum point, the maximum pulp yield of treated Enset fiber was found to be 61.0% per original sample (69.6% per treated sample) which is comparable to pulping of original sample 69.1%. Therefore, the hydrothermal treatments can be applied as a first fractionation step in the valorization of Enset fibers, allowing the subsequent use of the solid as a fiber source for pulping.

Keywords: *Agricultural residue, Enset fibers, Characterization, Extractives, Valorization, Ethanol-Alkali Pulping, Response Surface Methodology, hydrothermal pre-treatment, alkali-catalysis, XOS*

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LIST OF SYMBOLS AND ACRONYM

SYMBOLS

alk	Alkali Concentration
bt	Brightness
C	Carbohydrate
Cel	Cellulose
^{o}C	Degree Celsius
eth	Ethanol
G	Guaiacyl Lignin
H	Hydroxyphenyl Lignin
K	Kappa Number
kg	Kilo Gram
Lig	Lignin
m	Meter
min	Minute
mm	Mill Meter
µm	Micro Meter
P	Protein
S	Syringyl Lignin

T	Temperature
t	Time
W	Whiteness
Y	Pulp Yield

ABBREVIATIONS

ANOVA	Analysis of Variance
ATR	Attenuated Total Reflectance
ADLI	Agricultural Demand Led Industrialization
BSTFA	Bis(trimethylsily)-Trifluoroacetamide
CE	Catechin Equivalent
CIF	Cost Insurance and Freight
CSA	Central Statistical Agency
CTMP	Chemo Thermo Mechanical Pulp
CW	Cold Water
DPPH	2,2-diphenyl-1-picrylhydrazyl hydrate
EIMS	Electron Impact Mass Spectra
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
GC-MS	Gas chromatography–mass spectrometry

GOS	Glucose Oligosaccharide
HW	Hot Water
ID	Identified
NI	Not Identified
NPS	Undetermined Phenolic
Py-GC/MS	Pyrolysis Gas Chromatography/Mass Spectroscopy
RT	Retention Time
TAPPI	Technical Association of the Pulp and Paper Industry
TEAC	Trolox Equivalent
TMP	Thermo Mechanical Pulp
TMS	Trimethylsilyl
TRS	Total Reduced Sulfur
XOS	Xylose OligoSaccharide
UV	Ultraviolet Spectroscopy
USD	United State Dollar

CHAPTER ONE

1. Introduction

1.1.Background

Paper is a web of fibers derived from raw materials containing cellulose fibers, generally wood and non-wood from which lignin and other non-cellulose components are separated by chemical or mechanical pulping (Sridach, 2010). It is a multipurpose material with several uses and as a result it turns into a vital need in our day-to-day life.

The global consumption of papermaking fibers is anticipated to increase from 325 million tons in 2000 to 570 million tons by 2020 with annual growth rate of 2.8% (Chandra, 1998; Feria et al., 2012). This fiber demand is most widely satisfied from the harvest of forest. Wood constitutes approximately 90% of virgin pulp fiber used by the world's paper and board industry (Sridach, 2010). The growth in pulp and paper production requires massive cut down of trees, which in turn leads to deforestation. Hence, it is becoming problematic to satisfy an increasing demand of natural fiber from conventional fiber sources. This demand requires a search for new and previously unexploited sources of cellulose fiber (Sridach, 2010).

In this case, non-wood species are viewed as alternative sources of cellulosic fibers, especially in regions that are poor in forest resources. There is an increasing tendency of using non-wood fiber sources even in countries with adequate wood supply due to fast growth of fiber demand and

environmental considerations. In this regard, the future use of non-wood fiber source in pulp and paper making raw material is expected to grow further.

Non-wood fibers are often obtained from agricultural wastes and industrial dedicated plants. There are many studies that have been carried out over many years to investigate the use of annual plants and agricultural wastes as alternative sources of fiber. wheat straw (Sun et al., 2004), bagasse (Agnihotri et al., 2010; Novo et al., 2011), *Leucaena diversifolia* (Feria et al., 2012), rice straw (Ho et al., 2012), bamboo (Chaurasia et al., 2016), vine stem (Mansouri et al., 2012), abaca fiber (Ramadevi et al., 2012), tobacco residue (Shakhes et al., 2011), banana fiber, leaf and pseudo stem (Cordeiro et al., 2004; Li et al., 2010; Oliveira et al., 2007; Reddy Marella et al., 2014), and giant reed (*Arundo donax L.)* (Shatalov and Pereira, 2007) are some of the agricultural residues and industrial plants that have been investigated so far. The utilization of non-wood fibers is environmentally sound to produce pulp and paper compared to the clear-cutting of rain forests or natural forests and plantation forests. Over the last few years, technological advancement in almost all the fields of papermaking have made non-wood materials more competitive with wood as a raw material for papermaking.

The other environmental consequence related to pulp and paper production is the severe environmental impact of conventional pulping processes. The conventional pulping processes produces large amounts of highly polluted waste water, especially sulphite and sulphate based processes and also give lower pulp yield with higher amount of residual lignin (Akpakpan et al., 2012; Saberikhah et al., 2011). Due to this reason, the need for new processes that can reduce the unpleasant effects is gaining attention as an alternative to conventional pulping processes (Demirbas, 2009; Shatalov and Pereira, 2014). Accordingly, the less polluting pulping processes

that use pure or solution of organic solvents are the current interest (Demirbas, 2009). Organosolv pulping is a chemical pulping method in which delignification of the biomass is carried out with an organic solvent or in organic solvent-water-alkali mixture system (Akpakpan et al., 2012; Saberikhah et al., 2011).

Low environmental impact, higher pulp yield, lower kappa number, ease of bleaching, easy solvent recovery, recovery of lignin and sugars for profitable utilization and low capital for a new plant are the main advantages of organosolv pulping method over the conventional methods (Akpakpan et al., 2012; Demirbas, 2009; Saberikhah et al., 2011). The reactions taking place during alkali organosolv cooking are similar to those in the corresponding conventional processes (Kraft, soda, and alkaline sulphite). Like conventional pulping process, the major cooking parameter influencing the ethanol-alkali pulping is alkali concentration (Akpakpan et al., 2012; Neiva et al., 2014).

In the present study, it has been attempted to investigate the suitability of Enset residues for pulp and paper production by conducting morphological and chemical characterizations of the residues. In addition, sulfur free ethanol alkali pulping was conducted to delignify Enset fiber and comparison was made with conventional Kraft pulping of Enset fiber.

1.2.Problem statement

At the national level, the Ethiopian government has developed a policy that promotes agro based industrialization, known as agricultural demand led industrialization (ADIL)(Altenburg, 2010). Of these, one is paper pulp industry that requires huge tones of fibrous input. To satisfy the enormous amount of fiber requirement, it becomes stringently necessary to look for alternative fibers sources other than wood. In this regard, non-wood fibers, such as agricultural residues and

perennial plants, can be considered as an effective alternative source of cellulose fiber for producing pulp and paper sheets with acceptable properties (Sridach, 2010).

Agricultural residues such as wheat straw, bagasse, cotton stalk and Enset fiber are possible non-wood fiber sources for paper pulp production in Ethiopia. However, there is insignificant use of agricultural residues as industrial raw materials in Ethiopia since there is lack of awareness and know-how.

Enset crop is a plant used as an important staple food in Ethiopia, where it is cultivated in large plantations (Nurfeta et al., 2008a; Forsido et al., 2013). More than 20 percent (>15 million) of Ethiopian populations in southern parts depend on Enset for food, fiber, fodder, construction materials and medicines (Mohammed et al., 2013; Ayele and Sahu, 2014). Domestic Enset is primarily grown to produce a starchy food from the pseudo stem and corm (Mohammed et al., 2013). The Enset food production process leaves huge quantities of residues, mostly a fibrous material that remains after the leaf sheaths that constitute the pseudo stem of the plant are scraped to collect a starchy liquid used traditionally as food. After washing and sun-drying, the material appears as long threads of fibrous cellular aggregates that are used in local handicrafts e.g. sacks, ropes or mats (Brandt et al., 1997; Mohammed et al., 2013). These residues are abundant natural resources and can be potential source of cellulosic fiber (Ayele and Sahu, 2014).

CSA (2017) reported that, more than 100 million Enset plants have been annually harvested to produce starchy food. Based on this report, it can be estimated that around 150,000 tons of fiber are produced per year in Ethiopia. The availability of these large quantities of fibrous residues has triggered the interest in their valorization.

1.3.General objective

The general objective of this dissertation was to assess the possible ways for complete valorization of Enset residues, as raw material for paper pulp industry.

1.3.1. Specific objectives

The specific objectives were

- Analysis of structural features and summative chemical compositions of Enset residues in terms of lignin, polysaccharides, extractives and ash including detail analysis of extractives and lignin composition.
- Determination of the effect of ethanol reinforced alkali pulping parameters (temperature, time, and ethanol to water ratio and alkali concentration) on delignification of Enset fiber bundle based on pulp quality parameters such as yield, kappa number and color.
- Comparison of ethanol reinforced alkali pulping with conventional Kraft pulping of Enset fiber bundles based on pulp yield and pulp quality parameters.
- Pretreatment of Enset fiber bundles using un-catalyzed and alkali-catalyzed hydrothermal pretreatment at different temperature to fractionate hemicellulose sugar prior to ethanol-alkali pulping.

1.4.Structure of the dissertation

Chapter 1 gives a short background of the dissertation and presents the objectives of the dissertation together with its problem statement and this overview of all chapters.

Chapter 2 presents the main concepts and earlier works within the scope of this dissertation. Related works from adjacent fields are also briefly stated.

Chapter 3 reports the chemical and morphological characteristics of Enset residue. Summative chemical compositions, lipophilic extraction composition, phenolic content and anti-oxidant properties, ash composition and nitrogen content and lignin pyrolysis of Enset residues is presented in this chapter. Moreover, this chapter briefly discusses structural characterization of Enset residues.

Chapter 4 presents the effect of ethanol - alkali pulping process conditions and most favorable pulping parameters are optimized by using response surface methodology, central composite design. Comparison of conventional kraft pulping methods with ethanol alkali method is reported in this chapter. Pulp produced by using Kraft method is characterized using yield, kappa number and viscosity. In addition, the prepared hand sheet is characterized using its physical property and brightness.

Chapter 5 reports on the bio-refinery potential of Enset fiber to fractionate valuable hemicellulose sugar. The effects of alkali-catalyzed and un-catalyzed hydrothermal pretreatment prior to ethanol alkali pulping of Enset fiber was reported based on pulp quality parameters.

Chapter 6 provides short conclusion of the dissertation. It states the main conclusions of the research, based on the findings of chapter 3, chapter 4 and chapter 5. Furthermore, this chapter points out some remarkable areas where future work can be performed.

Appendixes are attached with supplementary data and pictures. List of publication out of this research is given in Appendix A. Photographs of Enset fiber extraction, some laboratory equipments used in this research and the resulting pulp and paper is provided in Appendix B, C and D respectively. The pyrolysis products from Enset fiber bundle and inflorescence stalk are in Appendix E.

CHAPTER TWO

2. Literature review

2.1.Global pulp and paper production and demand

The manufacturing of pulp, paper and paper products ranks among the world's largest industries. The global production of paper and cardboard stood at approximately 407 million tons in 2014. More than half of that production was attributable to packaging paper, while almost one third was attributable to graphic paper. In 2015, the regional distribution of pulp and paper production was as follows: Asia-Pacific, 195 million tons (48%); Europe, 104 million tons (26%); Northern America 83 million tons (21%); Latin America and the Caribbean, 21 million tons (5%); and Africa, 4 million tons (1%) as shown in figure 2.1 (Roth et al., 2016). In terms of future capacity, trends in the paper and board industry show a stable production over the next five years.

North America, Europe and China accounted for 28%, 26% and 15% of the global paper and board consumption in 2005, respectively. However, there are drastic regional differences in paper and board consumption. Per capita consumption in developed countries is close to 300 kg/cap, whereas in Africa the consumption is less than 17 kg/cap(Chen et al., 2012).

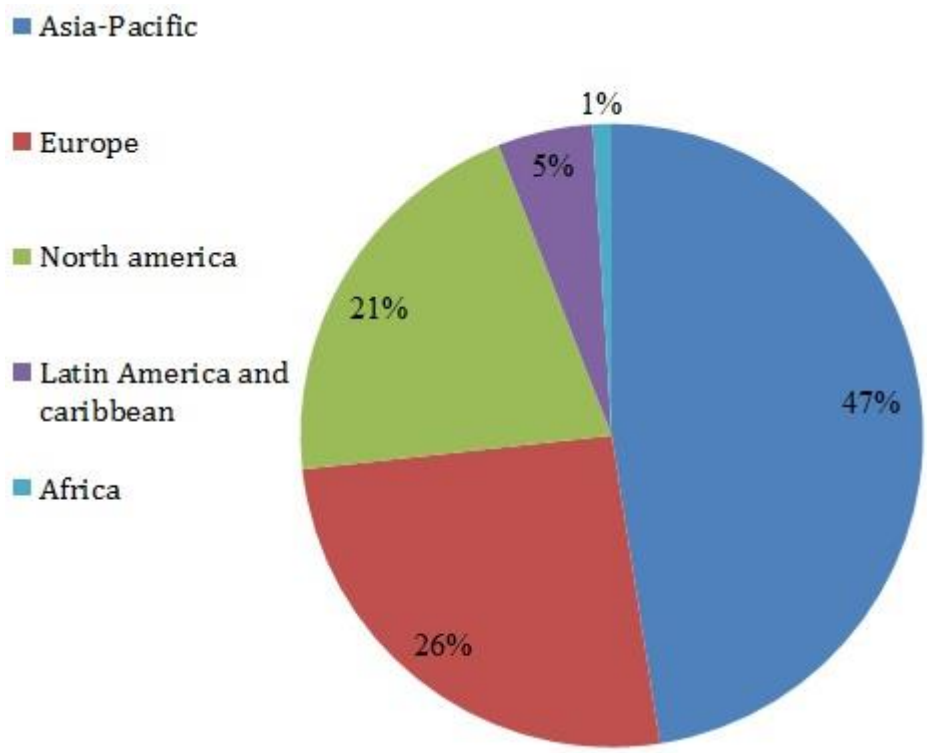

Figure 2.1: *Global paper and paper board production in 2015 (Roth et al., 2016)*

2.1.1. Pulp and paper manufacturing and demand in Ethiopian

In Contrary to the extremely rising global demand and production of paper and paper products, paper production in Ethiopia is at its infant stage where there are only very few paper mills producing a maximum annual output of 35 thousand tones in aggregate which is less than 0.01% of global production. The huge capital investment and absence of pulp mill processing the raw material in the country are considered as the major reasons for these limited number of paper mills. These make the local production of paper inefficient and less competitive when compared to other sectors in the industry, which significantly affects the overall economic growth of the country (Fenta, 2010).

According to the CSA report from 2009 to 2016, the imported amount of paper and pulp by Ethiopia increased from nearly 82 thousand tons to 154 thousand tons and from 4 thousand tons to 8 thousand tons respectively (Table 2.1) (CSA, 2009-2016).

Table 2. 1: *Pulp and paper imported by Ethiopia between 2009 and 2016*

Year	Gross weight of imported pulp (kg)	CIF value (USD)	Gross weight of imported paper (kg)	CIF value (USD)
2009	4,060,624.00	2,200,849.78	82,217,748.96	84,651,044.37
2010	1,765,788.16	1,632,848.18	76,512,841.77	82,450,928.73
2011	6,763,884.00	5,668,217.546	86,112,164.82	101,833,766.21
2012	8,689,498.00	6,759,688.00	103,951,203.64	116,359,837.88
2013	10,311,278.00	8,059,794.46	109,789,963.25	132,301,772.92
2014	5,140,705.60	4,214,596.83	139,078,195.66	127,237,688.80
2015	7,323,832.40	6,025,699.99	110,747,096.32	135,953,748.31
2016	7,949,797.12	6,310,023.24	154,157,617.08	167,529,679.09

Source: CSA, 2009-2016

2.2.Pulp and paper production process

In the pulp and paper industry, fibrous raw materials are converted into pulp, paper, and paperboard. In general, the principal steps in pulp and paper production are raw material preparation, pulping, bleaching, chemical recovery, and papermaking (Chen et al., 2012). The general procedure for pulping can be summarized in Figure 2.2. The overall technology of pulp and paper making deals with release of fibers found in wood or plant matrix followed by

unifying the fibers to form the paper web. The pulp can be converted to various types of products with diverse applications (Ek et al., 2009).

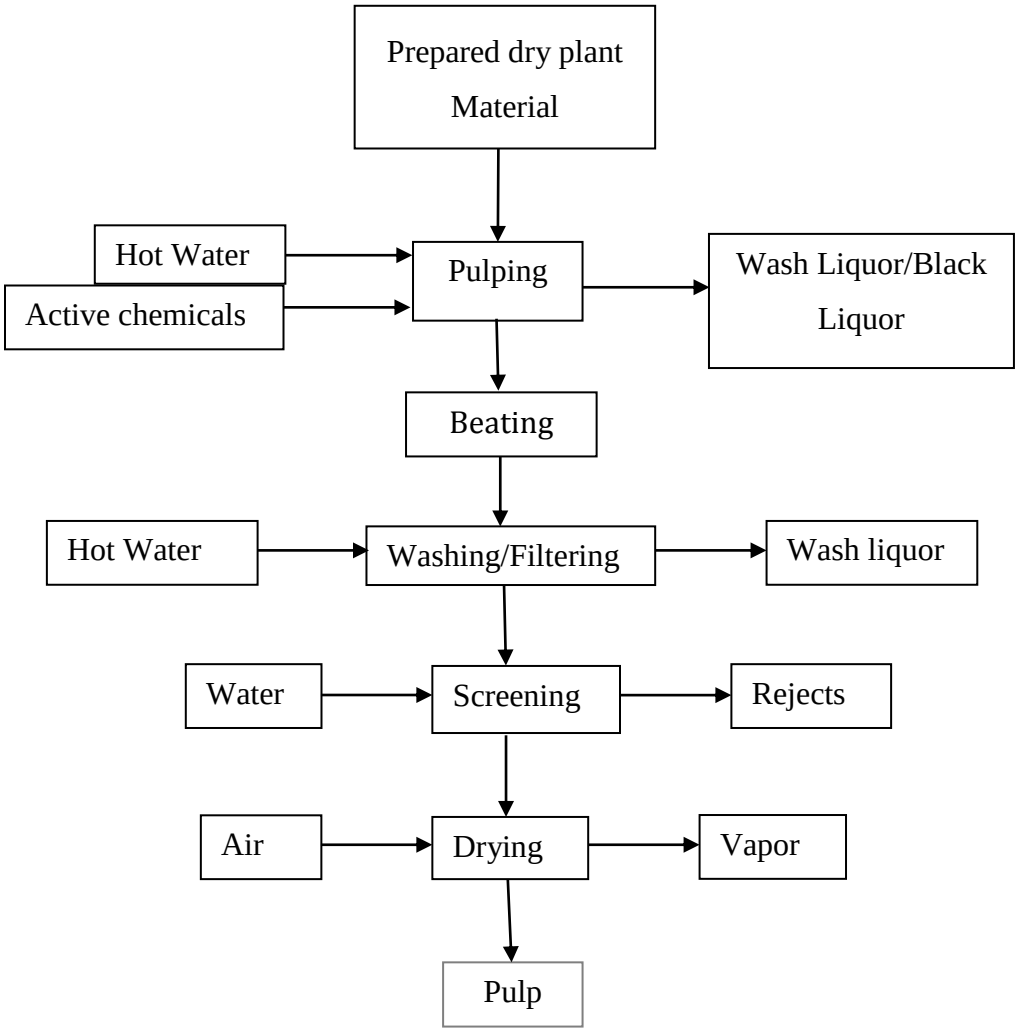

Figure 2.2: *General production steps for pulping (Omar Lwako, K., 2013)*

2.2.1. Pulping process

Pulp consists of fibers, usually acquired from wood. The pulping processes aim first and foremost to separate the fibers from the wood matrix. Various forms of pulping technologies are used in the fiber separation stage (Ek et al., 2009; Sridach, 2010; Rousu et al., 2016) .

Pulping process can be classified in two broad categories; mechanical pulping and chemical pulping. Mechanical methods demand a lot of energy, but on the other hand the yield of the process is high. In chemical pulping, only approximately half of the wood becomes pulp, the other half is dissolved (Ek et al., 2009; Sridach, 2010; Rousu et al., 2016).

2.2.1.1.Mechanical pulping

In mechanical pulping the fibers in the wood are released by grinding wood or wood chips. In this process, some easily water soluble carbohydrates and extractives are washed out, but on the whole the pulp yield is little affected. The pulp yield for mechanical pulp is about 90 to almost 100 %, depending on type of mechanical pulping method used. The fibers resulting from this process are stiff and mostly un-collapsed. In addition, mechanical pulp has large quantity of smaller material called fines. These are smaller particles, such as broken fibers, material from the fiber surface, and give the mechanical pulp its specific optical characteristics.

However, the fines negatively affect pulp strength, since strength is enhanced if the pulp consists of a higher portion of long fibers. To reduce fine formation, the lignin in the middle lamella is softened by increasing temperature. The chips can be pre-treated with steam before feeding to refiner. This type of mechanical pulping is called thermo-mechanical pulp (TMP). The other option is soaking the chips in a sodium sulfite solution where the lignin becomes sulfonated and the lignin softening temperature is decreased. This type of mechanical pulping is called Chemo-

Thermo Mechanical Pulp (CTMP), where the chemical treatment is followed by a steam pre-treatment (Ek et al., 2009).

2.2.1.2.Chemical pulping

In chemical pulping process the lignin is removed as much as possible and thereby release the fibers without damaging of the fiber strength (Sridach, 2010; Rousu et al., 2016). The pulp chemicals degrade and dissolve away the lignin and leave behind most of the cellulose and hemicelluloses in the form of intact fibers. No pulping chemicals are entirely selective to lignin; also there is loss of carbohydrates of the wood in varying extent. Approximately half of the wood material is dissolved during chemical pulping. The delignification is therefore terminated with some lignin remaining in the pulp to prevent more drastic carbohydrate loss. The amount of lignin left in the pulp is predicted by determining the kappa number of the pulp (Ek et al., 2009; Sridach, 2010).

Chemical pulp fibers are more flexible than mechanical pulp fibers because mechanical pulping does not dissolve the lignin. Chemical pulp fibers make bond better to each other when forming the paper and offer good strength properties. There are various types of chemical pulping methods. The main chemical pulping methods are Kraft, sulfite, soda and organosolv pulping processes (Kamoga et al., 2013). Depending on the required paper grades, the dark pulp can be subjected to a bleaching process which leads to brighter (whiter) paper, with better contrast, cleanliness by removing impurities and improve aging by removing the residual lignin that turns to yellow.

2.2.1.2.1. Kraft pulping

Kraft pulping (also known as sulfate process) is the dominant chemical pulping method globally. It involves treatment of wood chips with a mixture of sodium hydroxide and sodium sulfide, known as white liquor that breaks the bonds that link lignin to the cellulose (Fengel and Wegener, 1989). The active cooking species are OH^- and HS^-. The hydrogen sulfide ion is the main delignifying agent and the hydroxide keeps the lignin fragments in solution (Ek et al., 2009).

The name "Kraft cooking" is derived from the German and Swedish word meaning strength. It was first used in the context of pulp from the sulfate process with high lignin content. These pulps have relatively high strength and are used for packages such as linerboard and paper bag. Kraft pulp, refers to all pulp processed by the sulfate pulping method either unbleached or bleached grades (Ek et al., 2009).

The main reasons for the dominance of the Kraft process are its versatility in dealing with different raw materials coupled with superior pulp quality and a more efficient cooking chemicals recovery method. Kraft pulping produces a stronger pulp, but it increases pressure on the environment by releasing sulfur containing compounds known as total reduced sulfur compound (TRS), sulfur dioxide, suspended solids, and polluted wastewater (Sridach, 2010).

2.2.1.2.2. Soda pulping

Soda process, the first process to manufacture chemical pulp, was invented by Hugh Burgessin in 1851, using sodium hydroxide as a cooking chemical.

Soda pulping can be performed by addition of small amount of anthraquinone (AQ), which acts as a pulping catalyst. It has been reported that the modified soda pulping by addition of AQ is attributed to higher retention of hemicellulose. As a result, higher pulp yield is obtained compared to soda and Kraft pulping. Soda-AQ pulping is more environmental friendly pulping process compared to Kraft pulping. Moreover, uniform pulps with lower rejects at constant kappa number and lower consumption of alkali has been obtained (Akgül and Tozluo, 2010). Addition of AQ to soda pulping can raise the delignification rate and protect cellulose degradation, which was demonstrated by the quantity of screened pulp yield (Agnihotri et al., 2010).

2.2.1.2.3. Sulfite pulping

In the sulfite pulping, sulfurous acid (H_2SO_3) and bisulfite ions ($H_2SO_3^-$) are the active chemicals to degrade and dissolve lignin. Sulfite pulping can be performed at a pH ranging from 1–2 in acid sulfite pulping to 7–9 in neutral sulfite pulping. The pH of cooking chemicals is adjusted to desired acidity using different forms of sulfite salts of Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}. The sulfite process produces wood pulp which is almost pure cellulose fibers by using various salts of sulfurous acid to extract the lignin from wood chips.

Sulfite pulping processes are suitable only for species with low extractive contents (i.e., those low in tannins, poly phenols, pigments, resins, fats, and the like) because of the interference of these substances with the sulfite pulping process.

Sulfite pulp is light colored and can sometimes be used without bleaching if high brightness is not required. Unbleached sulfite pulp is often blended with ground wood and other high-yield mechanical pulps for strengthening newspaper stock. Sulfite pulp is easily bleached to very

bright pulps for writing and printing paper (Fengel and Wegener, 1989). Sulfite pulping has been in a steady decline for many years due to the environmental concerns and the inferior physical properties of the pulp (Sridach, 2010).

2.2.1.2.4. Organic solvent pulping

Organic solvents (organosolv) can be used in delignification, either as the sole degrading chemical or as reinforcement chemical in sulfite, sulfate or soda processes. Different types of solvents have been tried so far such as ethanol, methanol, glycerol, and acetic acid.

Yields of organosolv pulps can be higher than yields of conventional pulps at equal kappa number. Tensile and tear strengths of organosolv pulps fall between the corresponding values of Kraft and sulfite pulps. The organosolv pulping methods have considerable potential in terms of delignification selectivity and papermaking quality (Johansson et al., 1987).

The use of aqueous ethanol for delignification of wood was first proposed by Kleinert and Tayentha in 1931. The great conceptual advantage of using an organic solvent for lignin removal lies in the fact that such a process offers the possibilities for a more efficient utilization of the cellulosic feedstock. The dissolved lignin together with other dissolved components such as extractives can be recovered in an un-degraded form, for instance by evaporating the solvent after the digestion process. These components can then be used as chemical feedstock for further valorization in a multitude of ways.

2.2.2. Paper making

After liberating the fibers from the wood, they have to be consolidated again to form a web of paper. This is achieved on a paper machine where very dilute slurry containing up to 5% of fibers is sprayed on to a moving wire, web. Apart from fibers, the slurry may contain fillers, retention aid and wet strength additives.

The wire is an endless woven wire cloth with a mesh size allowing the water to be drained, but retaining the fibers on the wire. From the wire, the paper web enters the pressing section of the paper machine where water is pressed out by squeezing the paper web between steel rolls. Further to increase the dryness of the paper, it is dried in the drying section usually consisting of cylinders with steam within. At the end of the paper machine, the web is reeled.

2.3.Hemicellulose extraction prior to chemical pulping

The state of art pulp mill has been modified into integrated forest product bio-refineries using the technology and infrastructure already present in pulp and paper industries. This frame work presents tremendous opportunity to produce co-product in addition to valuable cellulose fiber including fuel grade ethanol, chemicals and additional energy, thus resulting in increased economic returns (Huang et al., 2010; Walton et al., 2010).

During chemical pulping, the major fraction of hemicelluloses is degraded mostly into low-value hydroxyl acids. However, extracting the hemicellulose under near neutral, acidic or mild alkaline conditions prior to pulping allows utilization of the hemicelluloses in higher value oligomeric or monomeric form (Walton et al., 2010). Hemicellulose extraction prior to chemical pulping improves pulp mill operations by reducing cooking times and increasing production capacity for pulp mills, which are limited by the recovery-furnace throughput (Walton et al., 2010).

Pre-extraction of hemicellulose using water/steam as the only solvent is cheap and environmentally friendly and results in simpler downstream processes compared to dilute acid and alkaline pre hydrolysis. Water pre-extraction is also called auto-hydrolysis because the hydrolysis and subsequent dissolution of the wood is catalyzed by acetic acid released from O-acetyl groups in the polysaccharides, which lowers the pH of the extract to 3-4 (Huang et al., 2010).

Alternative pre-extraction methods using alkaline chemicals to maintain the pH of the final extraction liquor at near-neutral pH have been reported. The hemicellulose pre-extraction experiments resulted in liquid solutions containing dilute oligosaccharides, acetic acid liberated from the hemicellulose polymers, lignin, organic acid degradation products, and residual salts from the alkaline chemicals used (Huang et al., 2010).

2.4.Crop residue as paper making raw material

Non wood fibers that can be used for papermaking segmented in to three broad categories: non woody fiber crops (industrial crop), crop residues and natural- growing plants (Sridach, 2010; Kaur et al., 2017). Industrial crops, such as hemp, sugarcane and kenaf, can produce high quality pulps with relatively higher cost of raw materials (Sridach, 2010). Naturally growing plants are also used to produce high quality pulps. This includes bamboo and some grass fibers, such as elephant grass, reed and sabai grass (Kaur et al., 2017).

Crop residues are the secondary products of the principal crops such as rice straw, corn stalk and wheat straw. They are characterized by low raw material price and moderate quality (Sridach, 2010). The use of crop residues in pulping and papermaking might be desirable because it avoids

the need for disposal, which currently increases farming costs and causes environmental deterioration through pollution, fires, and pests (Chandra, 1998).

The specific morphological and chemical characteristics of crop residue have significant effect on the technical issues in paper pulp production (Sridach, 2010). Analysis of fiber morphology and chemical composition of plant material has been used to assess the paper making potential of various species. The amount and composition of compounds differ among different plant species and even among plant parts as shown in figure 2.3 and they affect the pulping properties of the plant material.

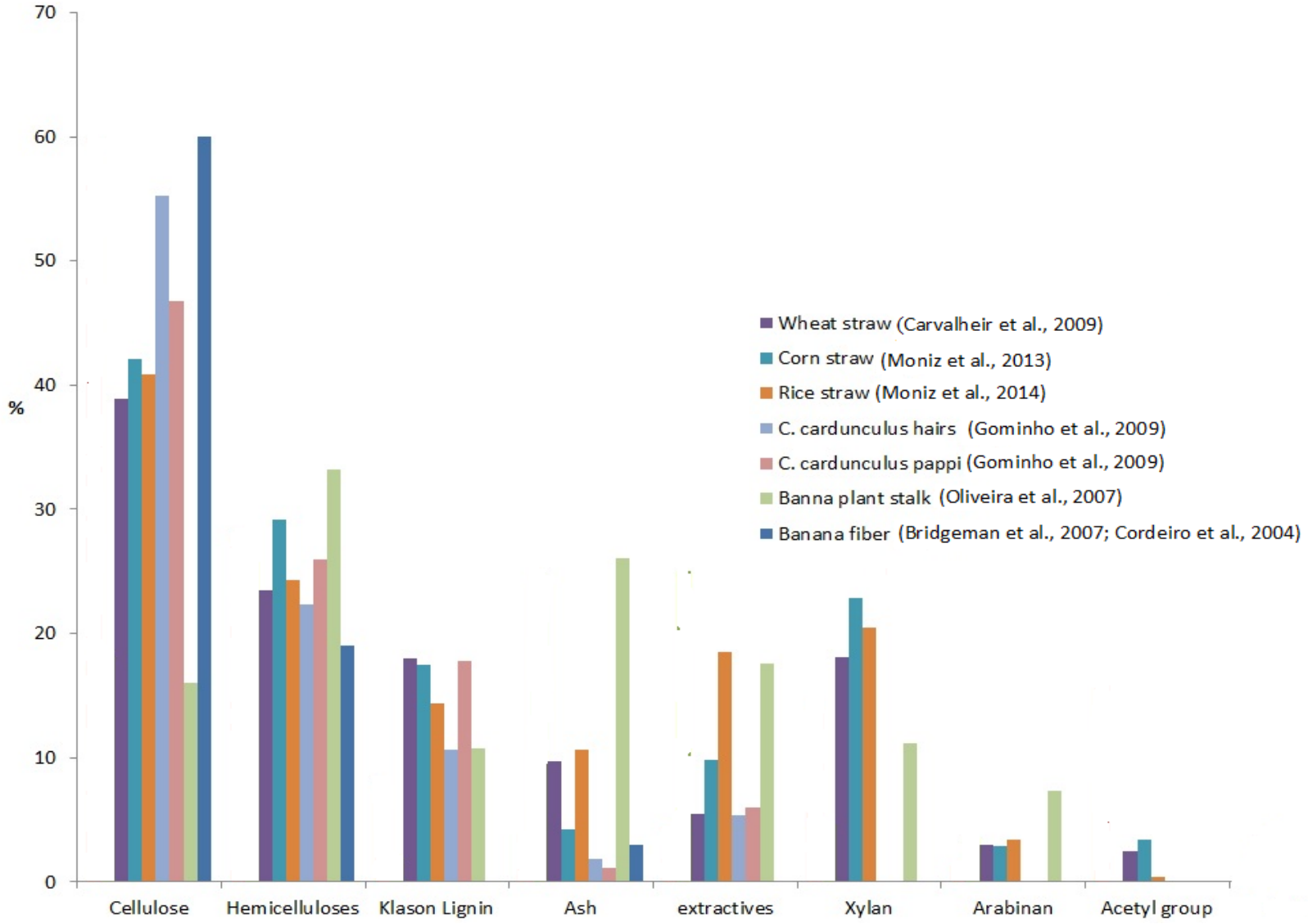

Figure 2. 3: *Graphical comparison of chemical composition of different non wood material*

2.5.Lignocellulosic biomass

Natural lignocellulosic biomass mainly consists of three types of substances: cellulose, hemicellulose, and lignin. The polysaccharides, cellulose and hemicellulose are associated with each other and generally contribute to more than 70% of the total biomass. Also, smaller amounts of pectin, protein, extractives and ash are found. The structure of these materials is very complex and the chemical composition of lignocellulosic biomass differs with the plant species. The existing bond among cellulose, hemicellulose, and lignin is mainly hydrogen bond.

2.5.1. Cellulose

Cellulose is the most common polysaccharide in nature and consists of repeating units of cellobiose, two glucose anhydride units (Figure 2.4). It is the principal component of plant fibers and the largest naturally occurring homo polymer (Kamoga et al., 2013). It is a glucan polymer consisting of D-glucopyranose where the glucose units rotated 180^0 to each other and linked together by β-(1,4) glycosidic bonds which gives the linear structure (Rowell, 2005). The number of glucose units in a cellulose molecule (i.e. the degree of polymerization) reaches 9,000–10,000 in plant and possibly as high as 15,000 in cotton (Rowell, 2005). The glucose units in cellulose molecules aggregate each other with hydrogen bonding and form micro-fibrils, which are the building blocks of fibrils, and in turn build the cellulose fiber (Sjostrom, 1993). Hydrogen bonding between the cellulose molecules results in high strength of the cellulose fibers (Kamoga et al., 2013).

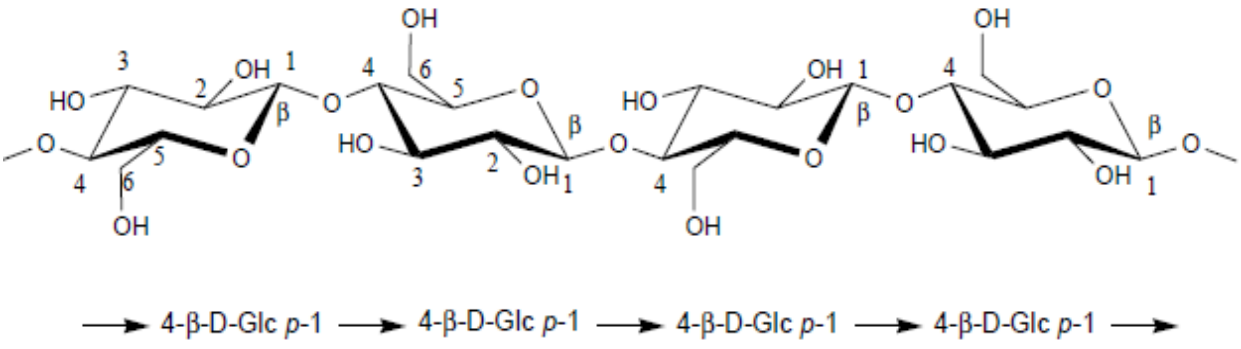

Figure 2.4: *Structure of cellulose (Rowell, 2005)*

Cellulose is the major wood component comprising about 40- 50% of the dry wood weight. Under normal conditions, cellulose is extremely insoluble in water, which is of course necessary for it to function properly as the structural framework in plant cell walls (O'Sullivan, 1997).

The cellulose content can be correlated with the yields of unbleached pulps. The alpha or true cellulose content of a fibrous material does not affect directly its pulpability, but the higher the alpha-cellulose content of a material, the higher the yield of fully delignified, bleached chemical and semi chemical pulps (Sjostrom, 1993).

2.5.2. Hemicellulose

When extractives (compounds which are soluble in cold water or in neutral organic solvents) and lignin are removed from wood, it yields a fibrous product termed holocellulose, which represents the sum total of cellulose and other polysaccharides. The later one is usually termed as hemicelluloses (polyoses).

Hemicelluloses, highly branched heterogeneous polysaccharides, are the second most abundant natural polysaccharide after cellulose. They are composed of a wide variety of sugars such as,

pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose, and rhamnose), and sugar acids (glucuronic acid, 4-O-methyl glucuronic acid, and galacturonic acid) (Sjostrom, 1993). The principal component of hemicelluloses in softwood is glucomannan, while in hardwood and herbaceous plants such as grasses and straw is xylan, which contains xylose and uronic acid (Fengel and Wegener, 1989).

Hemicelluloses are linked to cellulose via intermolecular hydrogen bonding and van der Waals forces, and to lignin via cinnamate acid ester linkages, and to other hemicelluloses through covalent and hydrogen bonds (Sjostrom, 1993). The degree of polymerization in hemicellulose is lower than cellulose, having an average of about 100–200 and the molecules are highly branched (Rowell, 2005). The branching nature in hemicellulose arises beyond the linkage that exist the β -(1-4) glyocsidic bond there are also β-(1-2), β-(1-3) and β-(1-6), which varies depending on the hemicellulose components (Fengel and Wegener, 1989). The uronic acids in the hemicellulose components structure are always found in branching side. Due to the combination of several sugar units and its amorphous structure, the hemicellulose is more soluble in water and therefore easily degraded by dilute acid or base than cellulose. They are often regarded as the most thermo-chemically sensitive components among the key constitutions of lignocellulosic biomass (Hendriks & Zeeman, 2009; Agbor et al., 2011). In the lignocellulosic materials, the cellulose and lignin are intimately linked, because hemicellulose acts as matrix of the cell wall component (Fengel and Wegener, 1989). The amount of hemicelluloses in lignocellulosic materials can range from 20 to 40% of the dry biomass weight depending on the type of species (Rowell, 2005).

In chemical pulping most of the hemicelluloses are dissolved or degraded, but usually between 3-15% remain in the pulp. The presence of hemicelluloses in the finished pulp increases the pulp yield, inter fiber bonding and the strength of paper in terms of tensile, burst and fold.

2.5.3. Lignin

The other most abundant and important polymeric organic substance in the plant world is lignin. In the tree it provides stiffness to the wood fibers and binds them together. This macromolecule plays a vital role in providing mechanical support to bind plant fibers together. Lignin also decreases the permeation of water through the cell walls of the xylem, thereby playing an intricate role in the transport of water and nutrients. Moreover, lignin plays an important function in a plant's natural defense against degradation by impeding penetration of destructive enzymes through the cell wall (Sjostrom, 1993).

Lignin is an amorphous hetero-polymer, built up from phenyl propane units linked together by ether and carbon–carbon bonds including coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (Buranov and Mazza, 2008). The respective aromatic constituents of these alcohols in the lignin polymer are called guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) (Figure 2.5).

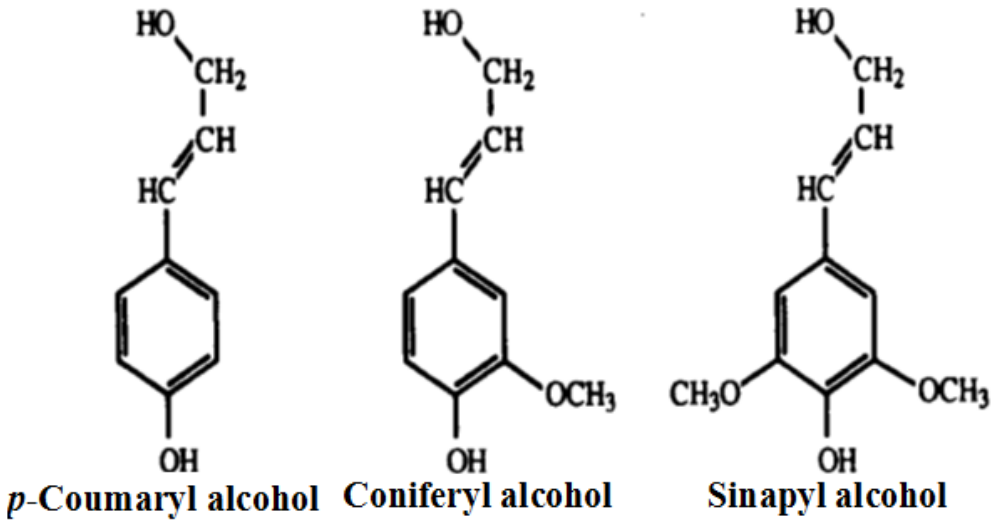

Figure 2. 5: *Lignin building blocks (Santos et al., 2013)*

The principal component of softwood lignin is typical guaiacyl unit whereas in hardwood the ratio of syringyl and guaiacyl unit can range from 1.1-3.1 depending on species (Santos et al., 2014). In contrast, the lignin of herbaceous plants contains all three units (S, G, H) in significant amounts with different ratios (Buranov and Mazza, 2008). Guaiacyl is largely a polymerization product of coniferyl alcohol. Guaiacyl-syringyl lignin is a copolymer of coniferyl and sinapyl alcohols. Hydroxyphenyl propanol lignin structures are typically found in grasses and non-wood plants.

The lignin content of hardwoods is usually in the range of 18–25 %, whereas the lignin content of softwoods varies between 25 - 35 % (Sjostrom, 1993; Rowell, 2005). Herbaceous biomass usually has a lower lignin content (10-25%) compared to woody biomass (Buranov and Mazza, 2008).

2.5.4. Ash and extractives

Extractive includes cutin, suberin, tannins, waxes and minerals found in the cell walls. Cutin and waxes are attached to the epidermal walls on plants surface. Cutin appears to be embedded in wax and pectin; these components serve as diffusional barriers that impede ruminal digestion of the intact tissue. Suberin is a functional component of cell walls. The polyesters that appear in suberized tissue can be esterified with phenolic monomers, oligomers or lignin.

Ash is a term generally used to refer to inorganic substances such as silicates, sulfates, carbonates, or metal ions. It is fraction of inorganic compounds constitutes of Ca, K, Mg, P, Mn, Fe, Si, Al and Na salts and the actual composition varies between wood species and is also influenced by the local compositions of the soil (Theliander et.al., 2002). Silicon is an important inorganic element in plant cell walls and mainly present in the form of silica in the walls of epidermal cells and leaf hairs.

2.5.5. Morphological characteristics of lignocellulosic material

The fiber dimensions (fiber length, fiber diameter, lumen width and wall thickness) are essential parameters of lignocellulosic materials because they are associated with various structural, physical and chemical properties of the plant. They are also very good indicators to decide the material suitability for different end products. They affect many wood-product processing, like drying process, resistance to cutting and machining and pulpwood quality. Fiber dimensions are also related to different pulp quality indices like Runkel ratio, slenderness ratio, rigidity coefficient and flexibility coefficient (Malik and Abdelgadir, 2015; Ogunjobi and Elizabeth, 2011; Ogunjobi et al., 2014).

Fiber length has positive correlation with burst strength, tensile strength, tear strength, and folding endurance. Long fiber lengths are preferable for manufacture of quality paper. Long fibers give more drainablity and less uniform sheet structure. Thin cell walls positively affect flexibility, burst and tensile strength of paper (Kiaei et al., 2014; Ogunjobi et al., 2014). The most important parameter indicator used to evaluate suitability of any raw material for pulp and paper production is the Runkel ratio which is twice the ratio of wall thickness to lumen diameter. The standard value for this ratio being one (1), satisfactory pulp strength is usually obtained when the Runkel ratio is below the standard value. Low Runkel ratio means thin fiber wall and larger fiber lumen width. Thin fiber wall is desirable for high quality, dense and well-formed paper. Paper manufactured from thick walled fibers will be bulk with coarse surface. Moreover, large lumen size positively affects the beating of pulp, which involves the penetration of liquid into spaces within the fiber. Thus, fiber with high Runkel ratio value will be stiff, less flexible and will form bulkier paper of low bounded area (Ogunjobi et al., 2014). The other important parameter is the slenderness ratio. If it is lower than 70 then fibers are invaluable for quality pulp and paper production (Malik and Abdelgadir, 2015).

Other calculated pulp properties are flexibility ratio and rigidity coefficient. The strength properties of paper such as tensile strength, bursting strength and folding endurance are affected mainly by the way in which individual fibers are bonded together in paper sheet. The degree of fiber bonding depends largely on flexibility and compressibility of individual fibers (Ogunjobi and Elizabeth, 2011). The coefficient of flexibility, usually expressed in percentage, is derived from the ratio of lumen width to its fiber diameter. Coefficient of flexibility gives the bonding strength of individual fiber and by extension the tensile strength and bursting properties. In

general, there is a positive relationship between slenderness ratio and folding endurance, and between flexibility coefficient and burst, and breaking length and tear resistance.

2.6.Enset plant

Enset /*Ensete ventricosum* (Welw.) Cheesman/ is a monocarpic, tall, perennial herbaceous plant. It belongs to the order Scitamineae, family Musaceae, and genus Ensete. It is commonly called false banana for its close resemblance to large thick, single-stemmed banana plant (*Musa* sp.) (Brandt et al., 1997). Enset plant has an underground stem structure known as corm and bundle of leaves like a banana plant (Ayele and Sahu, 2014). It, however, does not bear edible fruits and it is not categorized as common banana plant (genus *Musa*).

Enset is usually larger than a banana plant. It reaches up to 13 meters with a pseudostem up to one meter in diameter and dilates at the base to a circumference of 1.5 to 3.0 m and length of 2 to 5m. The leaves are more erect than the of a banana plant, have the shape of a lance head and maybe 4 to 6 meters long and nearly 0.6 to 0.9 meter wide (Brandt et al., 1997). The plant has an adventitious root system, an underground stem structure known as a corm. The corms are 0.7-1.8 m long and 1.5-2.5 m in diameter at maturity (Tsegaye and Struik, 2001). The above ground part of Enset pseudostem is formed by a bundle of clasping and overlapping leaf sheaths (Figure 2.6).

The proportion of the different components of the Enset plant (% dry matter) was reported as lamina and midribs of leaves 15-17%, leaf sheaths 45-51%, stalk 9-11% and corm 26-29% (Fekadu and Ledin, 1997) but a higher variation range was reported among different varieties and age of the plant e.g. leaf lamina 6-16%, leaf midribs 4-21%, pseudo stem 46-60% and corm 10-30% (Nurfeta et al., 2008a).

As the plant grows older and matures, an inflorescence grows at the apex and a stalk develops along the inner part of the pseudostem, and the flower emerges at the top and drops out (Figure 2.6). The pseudostem and leaf midribs colors vary considerably; some are purple to dark red but most are light green with variegated brown patches.

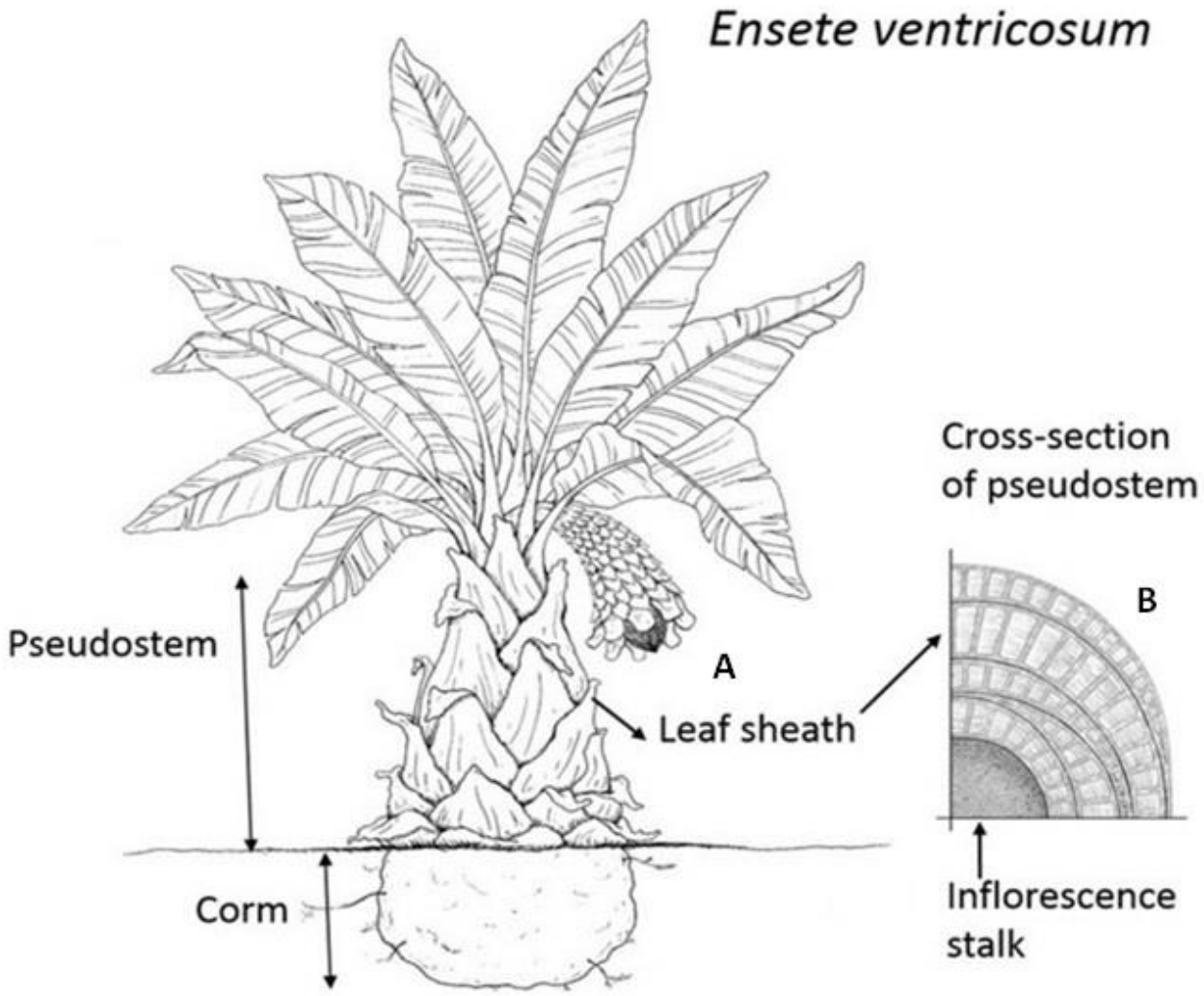

Figure 2.6: *Enset plant (A) and a cross-section of the Enset pseudo-stem (B)*

Enset is cultivated in south-western part of Ethiopia and covers considerable land area within the private holdings with an estimated area over 300,000 hectares in the highlands (1100-3000 m a.s.l) of central, southern and southwestern parts (CSA, 2011). An average temperature 10-21^{o}C and rainfall of 1100-1500mm per annum is conducive for the growth of the plant. It grows well in most sufficiently fertile and well-drained soil types (Garedew et al., 2017; Tsegaye, 2002). Wild Enset is common in central, eastern and southern Africa and Asia (Nurfeta et al., 2008a; Shumbulo et al., 2012).

Enset is a drought-resistant plant; failure of rain can only stop the growth but not kill the plant, as it has a large accumulation of moisture in its pseudo stem. As a result, famine rarely occurs in areas where Enset is widely grown. Enset leaves are fed to cattle during the dry season. It is also being used as an ornamental plant in Ethiopia and elsewhere.

Enset plant is domesticated only in Ethiopia and it is hardly known as a food plant elsewhere (Brandt et al., 1997; Quinlan et al., 2015; Tsegaye, 2002). More than 20% of Ethiopian population (>15 million) found in the highlands of southern and southwestern Ethiopia depends upon Enset for their food, fiber, animal forage, construction material and medicines (Addis, 2005; Ayele & Sahu, 2014; Brandt et al., 1997).

2.6.1. Enset plant distribution and production in Ethiopia

According to CSA data, around 2.27% of area under major crops cultivation is covered by private Enset plantation. SNNPR and Oromia regions are the two major Enset growing regions in Ethiopia. SNNPR produces over 65% of Enset production in Ethiopia (Table 2.2) (CSA, 2017, 2016). In these regions, Enset is cultivated mainly for a starchy food and livestock feed. In addition, every part of Enset plant is thoroughly utilized in one way or another. Sidama, West

Arsi, Gedio, Keffa, Jimma, Guji, Gurage, Kembata, Hadiya, Kefa, and related ethnic groups are the main Enset Producing zones in Ethiopia (Table 2.2) (CSA, 2017, 2016, 2015).

Table 2.2: *Production of Enset plant in major Enset growing Zones*

Regions and Zone	**Number of harvested Enset Plant**		
	2014/2015	**2015/2016**	**2016/17**
Ethiopia	**98,002,435**	**112,522,152**	**123,479,334**
Oromia region	**32,413,904 (33.0%)**	**35,263,883 (31.33%)**	**40,552,565 (32.8%)**
West Aris Zone	14,814,415	18,120,920	22,648,764
Jimma Zone	6,701,638	5,934,782	6,167,640
Guji zone	5,629,993	5,495,623	5,039,865
Southwest Shoa Zone	2,434,948	2,474,999	2,126,260
Borena Zone	1,199,014	1,024,398	1,433,124
West Shoa Zone	831,436	861,516	818,704
SNNPR	**65,583,936 (67%)**	**77,032,108 (68.45%)**	**82,829,054 (67.0%)**
Sidama zone	33,645,024	39,092,279	44,527,648
Gedio Zone	8,946,755	7,776,231	8,618,486
Kaffa Zone	3,947,893	6,263,281	8,238,934
Gamo Gofa Zone	4,150,823	2,977,505	2,429,388
Gurage Zone	2,776,806	2,894,268	4,011,142

Hadya Zone	2,952,017	2,703,039	3,356,956
Dawro zone	2,445,856	2,455,935	3,001,691
Wolayita Zone	1,754,093	2,014,068	1,699,304
Sheka Zone	592,717	6,385,174	1,941,581
Silite Zone	897,734	767,020	780,813
Segen People Zone	746,603	813,126	649,737
Benchi machi Zone	498,116	412,976	668,890
Konta Special Woreda	224,002	230,987	507,711
Yemi Special Wereda	278,108	282,845	388,345
Other Regions	**0**	**0.22%**	**0.2%**

Source: CSA, 2015, 2016, 2017

2.6.2. Food and non-food use of Enset plant

The Enset plantations are usually harvested after the appearance of the inflorescence at an age of about 6-7 years, although the plant can be harvested at any stage if there is a shortage of food or cattle feed (Nurfeta et al., 2008; Shumbulo et al., 2012). Over 100,000,000 Enset plants are harvested every year from all over the country for food and non-food use (CSA, 2015, 2016, 2017) (Table 2.2).

In Ethiopia, domestic Enset is primarily cultivated to produce a starchy food from the pseudostem and the corm (Karlsson et al., 2013). The major foods obtained from Enset are Kocho, Bulla and Amicho (Garedew et al., 2017). The time for maturation of Enset plant depends upon climatic condition, clone type, management such as the use of fertilizers. Hence

the flowering time varies from 3 years to 15 years but on the average around 6 to 7 years (Ayele and Sahu, 2014).

Harvesting of Enset includes cutting the leaf sheaths of the pseudostem into pieces, scraping the leaf sheaths pulp (parenchymatous tissue) from the cut pieces, pulverizing the corm, mixing the pulverized corm with the scraped leaf sheaths pulp and fermenting the mixture for a period 2 to 6 months. 'Kocho' is the main food product obtained by fermenting the mixture. Part of the starchy liquid called 'bulla' obtained by squeezing the mixture can also be consumed after it is allowed to settle for 4 to 5 hours or more resulting in thick paste. The freshly cooked corm is locally called 'Amicho' and can be consumed in a similar way as Irish potato. Thus, the plant is considered as a field bank of the food. The pseudostem, corm, and stalk of inflorescence constitute the most important source of food for humans, whereas the whole parts of the plant except the roots are used to feed livestock. The pseudo stem, the main food source, is rich in soluble carbohydrates (80%) from this the largest proportion is starch (65%) but has low protein content (4%). The average, the total production of Enset based food is in the form of Amicho (28,009,778 quintals), Kocho (31,625,631 quintals), and Bula (1,100,606 quintals) in year 2017 (CSA, 2017).

Different types of residues are disposed of commonly during food preparation of Enset. The fiber, the leaf and inflorescence stalk are the main solid residues which are not utilized for Enset based foods preparation. The fiber bundle, with a hair-like structure, locally called "Kacha" is collected after scraping of the leaf sheath and leaf bases around the pseudostem. Over centuries the Enset fibers have been extracted from the leaves of this plant as major material for the weaving, ropes and cord production, as well as for baskets production (Diriba et al., 2013;

Yirmaga, 2013). Enset fiber obtained from the leaf sheaths has an excellent structure, and its strength is equivalent to the fiber of abaca, a world-class fiber crop (Bezuneh and Feleke, 1965; Brandt et al., 1997). It is used to make bags, ropes, cordage and mats.

Mizera et al., (2016) reported circular cross section of Enset fiber and specified its dimension. From this analysis, Enset fiber shows high strength and rigidity and in proportion to its own density exhibits exceptional structural properties. With regard to its biodegradability and recyclability as well as to its mechanical behavior the fiber produced from Enset could be used as a raw material for construction material, paper and packaging, textile industry and so on (Mizera et al., 2016; Müller et al., 2017).

The dried leaf sheaths and midribs are used as packing and wrapping material, in fences and house construction. The leaf blades are used as a traditional plate for serving cooked foods, for wrapping several kinds of materials such as fermented Enset, butter and chat to prevent them from drying too much. Local people also believe that some Enset clones have various medicinal properties for different illnesses such as bone fracture and diarrhea for both human and animals (Addis et al., 2010).

Moreover, Enset has important ecological functions such as producing organic matter, creating a nutrient reservoir in the soil, controlling erosion, thus contributing to the stability and continuity of farming (Shack, 1963; Pijls et al., 1994).

2.7.Process condition optimization

Mathematical models based on delignification kinetics to predict the extent of pulping reaction are complex and impractical when more than two independent variables considered (Neiva et al., 2014; Shatalov and Pereira, 2014). Now days, such complexity is avoided by using an experimental design. Response surface methodology is one of experimental design optimization method that allows analysis of the simultaneous effects of multiple independent variables while requiring a small number of experiments (Bas and Boyaci, 2007). Concepts and techniques of Response Surface Methodology (RSM) have been extensively applied in many branches of engineering, especially in the chemical and manufacturing areas (Zangeneh et al., 2002; Cassettari and Mosca, 2013). Therefore, it is frequently applied in chemical and biochemical processes including delignification studies (Jiménez et al. 1999; Neiva et al. 2014).

RSM has been used to optimize pulping conditions of various raw materials to estimate different dependent variables of pulp and paper (e.g. yield, kappa number, strength properties and brightness) as a function of different independent operational variables such as temperature, time and alkali concentration (Neiva et al., 2014; Shatalov and Pereira, 2014).

2.8.Concluding Remarks

The global production of paper and paper related material increased with annual growth rate of 2.8%. Tremendous variation has been observed among regions of the world on the annual production of paper products. The annual paper production of Ethiopia only covers less than 0.01% of global production. This is due to the required huge capital investment and huge tons of fibrous raw materials. Enormous amount of foreign currency have been used to the import of pulp and paper products to the country.

Agricultural residues are one of sustainable raw material for paper production. The chemical and morphological characteristics of agricultural residues are different for different species. Paper pulp making potential of various non-wood plants and agricultural residues have been investigated so far (Agnihotri et al., 2010; Chaurasia et al., 2016; N. Cordeiro et al., 2004; Feria et al., 2012; Ho et al., 2012)

Enset is one of staple food in Ethiopia, and large amount of residues are discarded without significant commercial value. It is estimated that about 150 thousand tons of Enset fibers are discarded annually. However, resource characterization and valorization for paper production has not been previously studied. Mizera 2016 studied the Enset fiber behavior for industrial application as reinforcement in composite material. Moreover, any pulping methods for Enset fiber bundles delignification have not been tried before.

CHAPTER THREE

3. Characterization of Crop Residues from Enset Plant

3.1.Introduction

Enset is a monocarpic tall perennial herbaceous plant very similar and close relative to banana plant. It is a drought resistant plant that is cultivated in over 200,000 hectares in wider range of altitude 1100-3000 m a.s.l of central, southern and south-western parts of Ethiopia. An average temperature of 10-21^{o}C is conducive for the growth of the plant (Baker and Simonds, 1953; Brandt et al., 1997). It is estimated that Enset-based foods provide double to 20 times more calories than cereals (Gabel and Karlsson, 2013; Quinlan et al., 2015).

Enset food crop produces several residual by-products, mainly fibers from the pseudo stem and the inner inflorescence stalk (Figure 3.1). In fact, the scraping of the parenchymateous tissue of the lower sheath part of the leaves that make up the pseudo stem, gives a solid residue of a fibrous nature that is used traditionally to make sacks, bags, ropes, mats, and sieves (Brandt et al., 1997; Gabel and Karlsson, 2013). These fibers are cellular clusters with diameters ranging from 100 to 400 μm and have been studied regarding their tensile behaviour pertaining to their industrial application as natural fibers and as reinforcement in composite materials (Mizera et al., 2016, 2017). However, currently large quantities of these residues are available without significant commercial value.

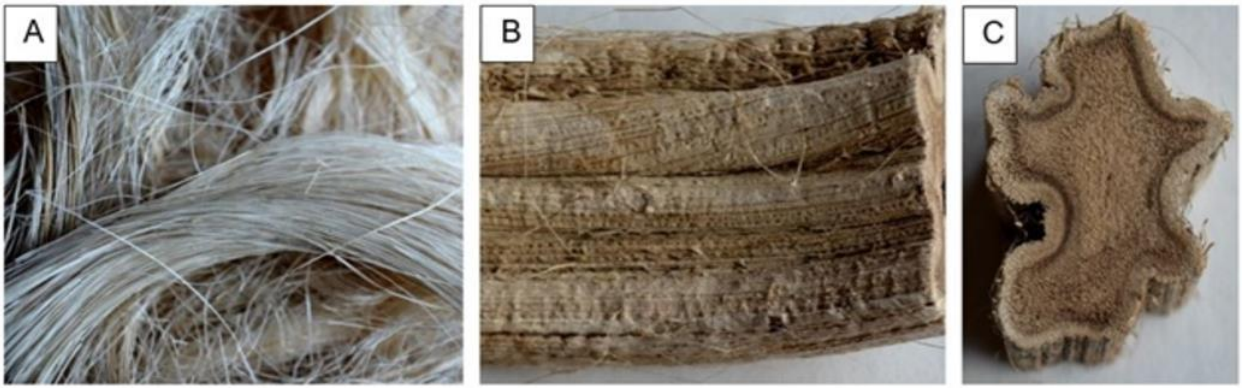

Figure 3.1: *Enset fiber bundle (A), longitudinal view of stalk (B) and cross sectional view of stalk (C)*

In this work, the Enset crop residual products remaining from the food production are analyzed in view of their valorization. Although some reports exist on the mineral and nutritional value of Enset plant parts mainly targeting their use as livestock feed (Fekadu and Ledin, 1997; Nurfeta et al., 2008b; Zewdie et al., 2008), detailed chemical composition with regard to their valuation is not known. This study was focused on both the fiber bundles obtained from the scraping of the pseudo stem as well as the inflorescence stalk remaining after leaves sheath removal. The structural features of the materials were observed and their chemical composition thoroughly analyzed in terms of lignin, polysaccharides, extractives and ash, including a detailed analysis of extractives and lignin composition. The overall objective was to design valorization procedures for these residual materials that will strengthen the local economies and provide full-resource crop utilization.

3.2.Materials and methods

3.2.1. Sampling

Residues obtained from the exploitation of Enset for food production were used in this study: the fibers obtained from the scraping of the leaf sheaths, the leaf and the inflorescence stalk (Figure 3.1). The detail characterization was done only on the fiber and the inflorescence stalk. The samples were collected from a household Enset plantation located in Wolkite, Gurage zone, Ethiopia ($08^0$17'N; $37^0$47'E, 1920 m of altitude) upon communicating with the owners.

The samples were fractionated separately using a knife mill (Retsch SM 2000) with an output sieve size of 6 mm^2 and 10 mm^2. The particle size distribution was determined by using a vibratory sieving apparatus (Retsch AS 200 basic) for 10 min shaking time with different sieve sizes.

3.2.2. Summative chemical composition

The chemical summative analysis was carried out using 40-60 mesh (0.250 to 0.450 mm) particle size fractions. All the chemical determinations were made on duplicate samples.

The inflorescence stalk was analyzed separately in two fractions: the main fraction (here called stalk main) and the fines (called stalk fines). For chemical analysis, the stalk main of the stalk was further pulverized to obtain particles between 40-60 mesh.

Ash content was calculated gravimetrically according to TAPPI standard method (T211 om-93). Extractives were determined by successive extraction of samples using dichloromethane, ethanol and water for 6 h, 16 h and 16 h, respectively, based on TAPPI T 204 om-88. The extractives solubilized by each solvent were determined gravimetrically and reported as percent of the

original sample. Cold and hot water solubility and 1% NaOH solubility of Enset residues were determined based on TAPPI standards T207 cm-99 and T212 os-58, respectively.

The Klason lignin and acid soluble lignin were determined based on TAPPI T222 om-98 and TAPPI UM 250, respectively. 350 mg of extractive free sample was treated with 72% sulfuric acid at 30 ^{o}C for 1 h, where after it was diluted to 3% and autoclaved for 2 h at 120^{o}C and 1.2 bar. The solid residues were filtered, oven dried and weighed as Klason lignin. The filtrates were used for acid soluble lignin determination by UV-spectrophotometer at the wavelength of 250 nm. The monosaccharaides including neutral sugars and uronic acids as well as acetates were quantitatively determined in the hydrolysis liquor by High Performance Anion Exchange Chromatography.

The holocellulose content of extractive-free samples was determined by the chlorite method (Rowell, 2005). One gram of sample was placed in an Erlenmeyer flask and 32 ml of distilled water was added. While slowly shaking, 0.750 g of $NaClO_2$ and 0.3 ml of acetic acid were added and the flask was covered with watch glass and boiled at 70 °C for 1 h. This procedure was repeated three times. After cooling, the sample was filtered using filter flask and washed with 50% cold water and acetone until sample was free of acid. The insoluble portion was dried at 105°C for 4 h, cooled in a desiccator and weighed repeatedly until a constant weight was obtained.

The α-cellulose was determined by treating 250 mg of the holocellulose with 1.25 ml of 17.5% NaOH and stirring with glass rod. In 5 min intervals, 0.6 ml of 17.5% NaOH was added two times. After 30 min the fiber suspension was diluted with 6.6 ml of distilled water and was filtered with a coarse crucible. Finally, it was washed thoroughly with 12.5 ml of 8.3% NaOH

and 100 ml distilled water, and soaked in 1.9 ml of 10% acetic acid for 5 min. The neutralized α-cellulose was washed with distilled water and dried at 105^{o}C and the yield calculated in relation to the original material. The hemicelluloses fraction was calculated by difference between holocellulose and α-cellulose content.

3.2.3. Composition of lipophilic extractives

The lipophilic extracts that were solubilized with dichloromethane were recovered after solvent evaporation and dried under vacuum at room temperature overnight. Aliquots about 2 mg were taken and derivatized prior to analysis. The samples were dissolved in 100 μL of pyridine and the compounds with hydroxyl and carboxyl groups were trimethylsilylated into trimethylsilyl (TMS) ethers and esters, respectively, by adding 100 μL of bis(trimethylsily)-trifluoroacetamide (BSTFA). The reaction mixture was then heated at 60^{o}C for 30 min and immediately analyzed by GC–MS by injecting in a GC–MS Agilent 5973 MSD with the following conditions: Zebron 7HG-G015-02 column (30 m, 0.25 mm ID, 0.1 μm film thickness), injector 280^{o}C, oven temperature program 50^{o}C (1 min), rate of 10^{o}C min^{-1} up to 150^{o}C, rate of 4^{o}C min^{-1} up to 300^{o}C, rate of 5^{o}C min^{-1} up to 370^{o}C, rate of 8^{o}C min^{-1} up to 380^{o}C (5 min). The MS source was kept at 220^{o}C and the electron impact mass spectra (EIMS) was taken at 70 eV of energy.

The compounds were identified as TMS derivatives by comparing their mass spectra with a GC–MS spectral NIST and WIELY library and by comparing their fragmentation profiles with published data. For semi-quantitative analysis and a full scan to find all possible compounds, the area of peaks in the total ion chromatograms of the GC–MS analysis was integrated and their relative proportions expressed as area percentage. Each aliquot was injected in triplicate and the results are presented as their mean.

3.2.4. Phenolic content and anti-oxidant properties of polar extracts

Approximately 0.5 g of samples was extracted with 20 ml of 1:1 ethanol-water mixture for 60 min at 50^{o}C in an ultrasonic bath (Branson 2200 Scientific Support, Inc., Hayward, CA, USA). The insoluble material was oven dried at 60^{o}C overnight and at 105^{o}C for 2 h, and the extraction yield was calculated as percent mass loss of the initial material. The filtrates were stored at 4^{o}C for further analysis of phenolic extract composition and antioxidant activity.

The total amount of soluble phenolic was estimated by the Folin–Ciocalteu method using gallic acid as standard (Singleton and Joseph, 1965). An aliquot (100 µl) of the extract was mixed with 4 ml of Folin-Ciocalteu reagent and 4 ml of a 7% Na_2CO_3 solution respectively with 6 min interval. After 15 min of incubation in a bath at 45°C, absorbance was read at 760 nm. A calibration curve was built with gallic acid as standard. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of extract (Singleton and Joseph, 1965; Sartori et al., 2016).

Total flavonoids were quantified by an aluminum chloride colorimetric assay using catechin as a standard (Zhishen et al., 1999). An aliquot of about 1.0 ml of the extract was mixed with 4.0 ml of distilled water followed by 0.3 ml of a 5% sodium nitrate solution. After 5 min, 0.3 ml of a 10% aluminum chloride solution was added to the mixture. After 5 min, 2.0 ml of 1 M sodium hydroxide solution was added, and the total volume was adjusted to 10.0 ml with distilled water. The absorbance was measured at 510 nm, corrected by a blank and the results were expressed as mg of (+)-catechin equivalents (CE) per gram of extract. All used reagents and solvents were supplied by Sigma-Aldrich, St. Louis, MO, USA (Zhishen et al., 1999).

The proanthocyanidins content (condensed tannins) determined by the vanillin-H_2SO_4 method using catechin as a standard (Abdalla et al., 2014). An aliquot (1.0 ml) of the extract was mixed with 2.5 ml of 1.0% (m/v) vanillin in absolute methanoland then with 2.5 ml of 25% (v/v) sulfuric acid in absolute methanol for vanillin reaction with the polyphenols in the extract. The blank solution was prepared in the same procedure without vanillin. Absorbance were recorded at 500 nm after 15 min, and the results were expressed as mg of CE per gram of extract (Sartori et al., 2016).

The antioxidant activity was assessed by the DPPH free radical assay (Sharma and Bhat, 2009) following a procedure described before (Sartori et al., 2016). The antioxidant activity was expressed as the amount of extract required to reduce 50% of the DPPH concentration (Inhibition concentration IC_{50}) values and also in terms of Trolox equivalents (TEAC) on dry extract base (mg Trolox mg^{-1} dry extract).

First, different dilutions of the initial extract and of a Trolox solution (0.2 mg ml^{-1}) in methanol were prepared. An aliquot of 100 µl of each methanolic solution of the extract and of Trolox was added to 3.9 ml of a DPPH methanolic solution (24 µg ml^{-1}). The blank sample consisted of 100 µl of methanol added to 3.9 ml of DPPH solution. After 30 min incubation at in the dark, the Absorbance was measured at 515 nm. All measurements were done under dim light (Sharma and Bhat, 2009).

The radical scavenging activity of each sample was calculated by the DPPH inhibition percentage as follows.

$$I\% = [(Abs_0/Abs_1)/Abs_o] \times 100 \quad \text{(Eq 3.1)}$$

where Abs_0 is the absorbance of the blank solution and $Abs_1$was the absorbance in the presence of the extract at different concentrations.

The IC_{50} inhibiting concentration was obtained by plotting the inhibition percentage against the extract concentration. The scavenging effect on the DPPH radical of the extract was also expressed as the Trolox equivalent antioxidant capacity (TEAC) calculated from the calibration curve with the Trolox solution concentrations and the percentage of scavenging effect on the DPPH radical as:

$$TEAC\ (mg\ of\ trolox\ equivalent/mg\ of\ extract) = [{}^{(\%\ inhibition_{sample} + n)}/_{s \times m}] \ldots\ldots$$

(Eq 3.2)

where n and s represent the intercept and the slope of the Trolox calibration curve for DPPH and m is the sample amount in mg dry basis (Miranda et al., 2016).

3.2.5. Ash composition and nitrogen content

The ash obtained by combustion in a muffle furnace at 500°C was analyzed for macro- and micro-element concentrations according to TAPPI standard method (T 266 om-02). The ash was dissolved in HCl and the concentrations of Ca, Mg, Fe, Cu, Mn, Zn, Na, and K were determined by atomic absorption spectrophotometry in a Pye Unicam SP-9 apparatus (Cambridge, UK) equipped with a GF95 graphite furnace.

Nitrogen was determined by the Kjeldahl method (Jackson, 1958) in detector equipment (Herdon, VA, USA) and was expressed as protein content using a conversion factor for crop residue of 4.4 (Chen et al., 2017).

3.2.6. FTIR spectroscopy of Enset residues

1 mg of extractive-free samples were ground with a Retsch MM200 mixer ball mill for 10 min and dried at 105°C for 24 h. Samples were analyzed in ATR mode FTIR in the 400 to 4000 cm^{-1} range with a resolution of 4 cm^{-1} and 64 scans accumulation.

3.2.7. Analytical pyrolysis (Py-GC/MS)

The extractive-free samples were dried and ground in a Retsch MM200 mixer ball mill for 10 min, and 0.20 mg of each sample was pyrolysed in a 5150 CDS apparatus fitted to an Agilent GC 7890B with a mass detector system 5977B installed with a ZB-1701 fused-silica capillary column (60 m x 0.25 mm i.d. x 0.25 µm film thickness). The chromatograph oven program was: 40 ^{o}C held for 4 min, 10 ^{o}C min^{-1} to 70^{o}C, 5 ^{o}C min^{-1} to 100 ^{o}C, 3 ^{o}C min^{-1} to 265 ^{o}C, held for 3 min, 5 ^{o}C min^{-1} to 270 ^{o}C, held for 9 min. The compounds were identified by comparing their mass spectral fragmentation with Wiley and NIST2014 libraries. The peak molar area of each identified compound was calculated, summed and the percentage of each compound was calculated. The percentage of hydroxyl phenol (H), guaiacyl (G) and syringyl (S) derived products were separately summed and the S/G and H/G/S ratios were calculated.

3.2.8. Anatomical characterization

The samples were impregnated with DP1500 polyethylene glycol, and transverse sections of approximately 17 µm thicknesses were cut with a Leica SM 2400 microtome stained and mounted (Quilhó et al., 2004).

For fibers length determination, samples were macerated in 1:1 solution of 30% H_2O_2 and CH_3COOH at 60 °C for 48 h and stained with astra blue. A light microscope (Leica DM LA) was used, photomicrographs were taken with a digital camera (Leica DFC 320) and image acquisition

was performed with Leica software Qwin V3.5.0 (Leica Micro systems Imaging Solutions, Cambridge, UK).

The samples were also observed by scanning electron microscope using a Hitachi-TM3030 Plus table top microscope.

3.3.Results and discussions

3.3.1. Structural characterization

The studied materials have very distinct characteristics (Figure 3.1) that show different application potential. The fiber bundles show a yellowish white appearance with a rough surface and some stiffness (Figure 3.1 A). Their cross-sectional view is roughly elongated to circular with approximately 0.1-0.2 mm diameter. SEM photographs show that each thread is a compact cellular cluster of mostly long cells (Figure 3.2 A) that are contained by an enveloping membrane (Figure 3.2 B). This was also seen in microscopic thin cross-sections and Figure 3.2 C shows the disrupted membrane and fragments of the cellular aggregates. A longitudinal cut along the fiber thread shows different cell types, including the predominant fibers as well as vessels and parenchyma (Figure 3.2 D).

The fiber bundles are compact cellular structures (Figure 3.2 A-D) corresponding to the fibro-vascular bundles of the leaf sheaths, as described in reference plant anatomy books (Fahn, 1974). The parallel lying fibers in the bundles (Figure 3.2 A, D) indicate their ability for use as a fibrous source. One option could be the direct use of the bundles for rug and mat weaving, as it is already done traditionally, or as a fiber component in composite panel formulations as recently researched (Mizera et al., 2015, 2016, 2017). The fiber bundles that were mechanically

characterized in those works showed similar cross-sectional dimensions (0.2 mm) and cellular structures as observed in the present work. Another option for the use of the fiber bundles may be their dissociation into individual fiber cells to be used for paper production, as it has been researched for the banana fibers (N Cordeiro et al., 2004; Oliveira et al., 2007; Rahman et al., 2014).

Fiber length was measured from the cellular fibers obtained after dissociation of the bundles (Figure 3.2 E). Only the fibers with the two ends clearly visible were considered for measurement. Results show that fibers length varied from 2.65 mm to 11.37 mm (mean 5.29 mm), width from 15.3 to 18.1 µm, lumen width from 7.0 to 11.2 µm, and cell wall thickness from 2.3 to 4.6 µm.

With the above data, Enset fiber is expected to produce strong paper because of the positive correlation between fiber length and burst strength, tensile strength, tear strength, and folding endurance. In addition, the thin cell walls of Enset also positively affect flexibility, burst and tensile strength of paper (Ogunjobi et al. 2014; Kiaei et al. 2014).

The inflorescence stalk has a hard cortex around the central cylinder (Figure 3.1 B-C) with a predominant presence of parenchyma where fibro-vascular bundles are embedded (Figure 3.2 F). The presence of starch in the parenchyma cells was very high featuring elongated to circular granules with dimension ranging approximately from 10 to 30 µm (Figure 3.2 G). This structure is similar to that of floral stalks of other plants e.g. of *Cynara cardunculus* (Quilhó et al., 2004). This remarkable feature together with other chemical features allow considering a nutrition related application.

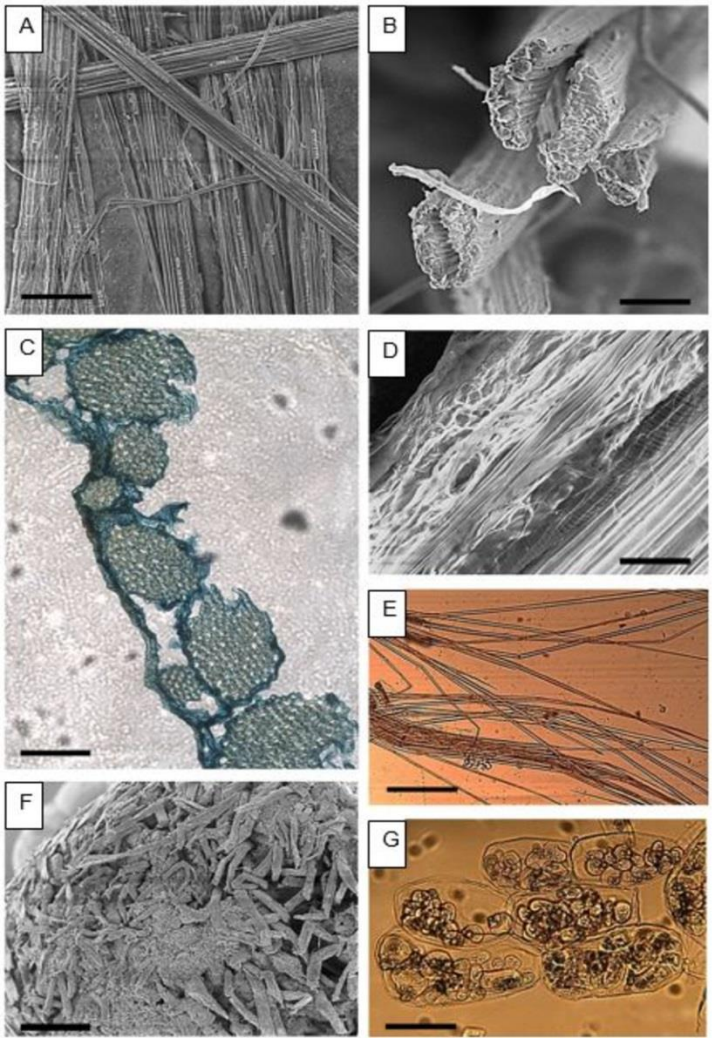

Figure 3. 2: *Structural features of Enset fiber bundles and pseudo stem*

Where (A)SEM longitudinal observation of various fiber bundles, scale bar 200 µm (B) SEM observation of the cross section of fiber bundles, scale bar 100 µm (C) optical microscopy observation of a thin cross section of one fiber bundle, scale bar 100 µm (D) SEM observation of a longitudinal cut of one fiber bundle; scale bar 50 µm (E) optical microscopy observation of dissociated fibers from one fiber bundle, scale bar 200 µm (F) SEM observation of a cross-section of the pseudo stem; scale bar 400 µm (G) optical microscopy observation of dissociated cells of the pseudo stem, showing starch granules scale bar 50 µm.

Fiber dimensions and derived values of Enset and their comparison with other lignocellulosic material are summarized in table 3.1. In the present study, the Runkel ratio of Enset was found to be 0.76, indicating thin fiber walls which are suitable for paper production favoring good sheet binding formation with high pulping quality (Kiaei et al., 2014; Malik and Abdelgadir, 2015).

The dimensional analysis shows that Enset fiber exhibits fairly good properties for paper pulp production in all aspects. The slenderness ratio of Enset, which was found to be 316.76 is above the recommended value of 70. The value is greater than most of conventional paper making raw materials and comparable with bamboo fiber as shown Table 3.1. Paper tear resistance increases with increasing fiber slenderness. This means paper made from Enset residue will have high tear strength and therefore it is suitable for wrapping and packaging purposes (Ogunjobi et al., 2014).

Table 3. 1: *Morphological Characteristics of Enset fiber and their comparison with other lignocellulosic material*

Morphological Characteristics	***Enset * fiber***	**Sugarcane Bagasse [(a)]**	**Wheat Straw [(b)]**	**Bamboo [(c)]**	***Musa paradisica* [(d)]**	***Eucalyptus globulus* Tree [(e)]**	***Vitex Donian* Tree [(f)]**	***Kenaf* [(g)]**
Fiber length (L), mm	2.65-11.37	1.51	1.14	3.11	2.21	0.98	1.48	2.9
Fiber width (D), µm	15.30-18.10	21.40	19.32	8.03	22.20	18.80	21.90	28.16
Lumen diameter (d), µm	7.00-11.20	6.27	10.54	4.35	n.a.	n.a.	12.70	6.08
Cell wall thickness (w), µm	2.30-4.60	7.74	4.39	6.98	n.a.	4.90	4.90	11.04
Slenderness ratio (L/D)	316.76	70.56	59.00	387.29	99.54	52.12	67.57	105.0
Runkel ratio (2w/d)	0.76	2.46	0.83	3.20	n.a.	n.a.	0.77	0.76
Flexiblity coefficient (d×100/D)	54.50	29.29	54.55	54.17	n.a.	n.a.	57.99	57.00
Rigidity coefficient (2w/D)	0.41	0.72	0.72	1.73	n.a.	0.52	0.45	n.a.

**Current study, a: (Agnihotri et al., 2010) b: (Kasmani and Samariha, 2011) c: (Kamthai and Puthson, 2005) d: (Rahman et al., 2014) e: (Miranda et al., 2012) f: (Ogunjobi et al., 2014) g: (Udohitinah and Oluwadare, 2011), n.a. Not Available*

3.3.2. Chemical composition

The chemical compositions of the Enset fiber bundles obtained from scraping of the leaf sheaths, and of the inflorescence stalk are given in Table 3.2. The chemical compositions of both materials were very different in proportion and composition of the structural and non-structural components. The corresponding results for these two materials are discussed separately.

The fibers have a comparatively low content of extractives (4.4%), of which more than 90% were polar compounds that are soluble in ethanol and water. The lignin content was found to be around 10.5%. The fibers are characterized by very high holocellulose content (87.5%) and the large proportion is α-cellulose (59.6%). The monomeric composition of the polysaccharides (Table 3.2) consists mainly of glucose (51.9% molar proportion) while the hemicellulosic units comprise mainly xylose (14.3% molar proportion), a large proportion of acetyl groups (29.8% molar proportion) with smaller contents of arabinose, galactose, mannose and uronic acids. Considering the molar composition given in Table 3.2, for 100 hemicellulosic sugar units (without glucose), xylose corresponds to 81 units, uronic acids to 13 units, arabinose to 7 units and mannose and galactose to 5 units. The hemicelluloses have a high acetylation: 1.7 acetyl groups per sugar monomer were detected.

Table 3. 2: *Summative chemical composition (mass % o.d. material) and monosaccharide composition (molar % of total monosaccharaides) of the Enset fibers and stalks*

Chemical Composition (%)	**Fiber**	**Stalk main**	**Stalk fines**
Ash	3.80	12.30	24.60
Extractives	4.44	25.92	25.01
Dichloromethane	0.43	1.05	0.81
Ethanol	1.11	7.43	5.95
Water	2.90	17.44	18.25
Lignin	10.53	5.75	3.39
Klason lignin	8.22	3.62	2.11
Acid soluble	2.31	2.13	1.28
Holocellulose	87.47	46.73	46.73
α-cellulose	59.59	17.03	17.03
Protein	0.66	3.78	3.21
Monosaccharide (% molar)			
Arabinose	1.29	5.37	3.46
Galactose	0.48	1.71	1.07
Glucose	51.87	80.59	89.94
Xylose	14.25	8.64	4.59
Rhamnose	0.00	0.00	0.00
Mannose	0.32	0.54	0.33
Galacturonic acid	1.29	0.91	0.63
Glucuronic acid	0.69	1.73	1.07
Acetyl	29.82	0.00	0.00

The main inflorescence stalk has high extractives content (25.9%), mainly corresponding to polar compounds that were soluble in ethanol and water (Table 3.2). The lignin content was low (5.8%), as well as that of holocellulose (46.7%) and α-cellulose (17.0%). The monomeric

composition of stalk polysaccharides is dominated by glucose (80.5% molar proportion), while the hemicellulosic units comprise xylose, arabinose, galactose, mannose and uronic acids, but no acetyl groups. The fines obtained from the inflorescence stalk had a similar composition to the main fraction with only a higher content of ashes (24.6%, Table 3.2). The sugar units obtained by hydrolysis were predominantly glucose that comprised over 80% of the molecules. This means that a considerable proportion of glucose should result from the hydrolysis of starch which is conspicuously present in the Enset stalk (Fig 3 G). Enset is a starch producing plant and Enset starch has high proportion of the branched amilo pectin component (Wondimu et al., 2014). The hemicelluloses in the Enset inflorescence stalk were mostly xylans but in comparison with the fibers had higher proportion of arabinose and no acetyl groups were found (Table 3.2). It had lower xylan content than that of corn straw (Moniz et al., 2013), rice straw (Moniz et al., 2014) and wheat straw (Carvalheiro and Silva-fernandes, 2009) with comparable arabinan content.

The results are compared with data obtained from literature on other lignocellulosic materials in Table 3.3. The cellulose content was the highest among the other lignocellulosic materials presented in Table 3.3. A comparison with the fibers extracted from the banana pseudo stems shows somewhat comparable hemicellulose, holocellulose and α-cellulose compositions as represented in Figure 3.3 (Cordeiro et al., 2006; Li et al., 2010). For banana leaf sheaths, Li et al. (Li et al., 2010) reported 72.7% holocellulose and 39.1% cellulose and carbohydrate composition of glucose 71.8%, xylose 11.2%, arabinose 7.3%, galactose 2.0%, mannose 0.6%, galacturonic acid 7.1%. The predominance of xylose units in the composition of hemicelluloses in banana leaf

sheaths was also reported (Cordeiro et al., 2004; Oliveira et al., 2007). Starch is present also in the banana plant floral stalks in amounts of 26.3% (Oliveira et al., 2007).

Table 3.3: *Comparison of the Chemical Composition of Enset Residue with Other Paper making Raw Materials*

Chemical composition	**Cellulose %**	**lignin%**	**Ash%**	**Ethanol extractives %**	**Cold water extractives %**	**Hot water extractives %**	**1% NaOH extractives %**
Fiber *	59.59± 0.99	8.22± 0.90	3.8± 0.28	1.11± 0.075	2.90± 0.45	6.67± 0.507	22.75± 0.75
Leaf *	37.96± 0.68	18.93± 0.95	11.75± 0.75	19.09 ±0.84	26.58± 0.44	16.66± 0.65	49± 0.09
Pseudo stem *	17.03± 0.94	3.62± 0.96	12.3± 0.19	7.43± 0.875	17.44± 0.19	14.96± 0.36	49.75± 2.25
Bagasse fiber [(a)]	42.34± 0.36	21.7± 0.35	2.10± 0.03	1.85± 0.01	3.02± 0.02	7.42± 0.05	32.29± 0.1
Abaca fiber [(b)]	68.32	8.50	5.10	n.a.	n.a.	n.a.	n.a.
Vine stem [(c)]	35.0	28.1	3.9	11.3	8.2	13.9	n.a.
Banana pseudo-stem[(d)]	39.12	8.88	8.20	3.05	n.a.	n.a.	n.a.
Banana leaf stalk/leaf[(e)]	43.25± 1.8	16.02± 1.2	7.55± 0.7	1.96± 0.1 (ac)	9.14± 0.9	n.a	32.44± 1.9
Beech wood [(f)]	45.8	21.9	0.4	n.a.	n.a.	n.a.	n.a.
Eucalyptus globulus [(g)]	56.9	17.8	1.0	1.4	1.6	n.a.	12.2
Sweet bamboo [(h)]	68.11	28.70	1.46	5.91	7.03	8.03	24.91
Cotton stalk [(i)]	43.8	17.6	3.5	n.a.	n.a.	n.a.	n.a.

n.a.: not available, ± shows standard deviation, (ac): acetone extractive. (*) Current study: (a) (Agnihotri et al., 2010) (b) (Ramadevi et al., 2012): (c) (Mansouri et al., 2012): (d) (Li et al., 2010): (e) (Rahman et al., 2014): (f) (Demirbas, 1998): (g) (Miranda et al., 2012): (h) (Kamthai and Puthson, 2005): (i) (Ververis et al., 2004)

The main objective of pulping is to remove lignin since it is undesirable component in paper making. It affects the quality and properties of pulp such as color, hardness, bleaching ability and paper durability. As it is evident Table 3.3, the lignin contents of Enset fiber (8.22%) and pseudo stem (3.62%) are by far lower than that of Enset leaf (18.93%). Furthermore, when compared with the lignin content of the other materials in Table 3.3, Enset fiber and pseudo stem show lower lignin content.

The comparative analysis has also revealed other significant compositional differences among the Enset residues. The leaf and the pseudo stem have higher extractive content and lower cellulose content compared to the fiber. Alkaline solubility indicates the degree of fungus decay or degradation by heat, light and oxidation. The results show that alkaline solubility is higher in Enset pseudo stem (49.75) and leaf (49.09) compared to Enset fiber (22.75) and baggase fiber (32.29) (Agnihotri et al., 2010). The pseudo stem have high hot water solubility content because it has high amount of starch around 65% (Gabel and Karlsson, 2013). In the leaf there is coloring matter which might be responsible to the observed high amount of extractive content. High ash, solubility and extractive content is related to large amount of inorganic compounds, sugars, coloring material, starch, tannins and gums which are common in grasses (Shatalov and Pereira, 2007).

In complement, the high hemicelluloses content in Enset fiber bundle may require the use of pre-treatments for their removal, for instance by water thermal treatments (auto-hydrolysis) as proposed for various straws and other crop residues (Moniz et al., 2013, 2014). Auto hydrolysis of Enset fiber can be used under a bio refinery frame work for production of oligosaccharides in

general, and of xylose oligosaccharides in particular due to their high xylan content (Carvalheiro and Silva-fernandes, 2009; Moniz et al., 2014).

3.3.3. Ash content and mineral composition

The ash content of the stalk was 12.3% in the main fraction and 24.6% in the fines while in the fibers and leaf it was 3.8% and 11.75% respectively (Table 3.2 and Table 3.3). The ash content of Enset stalk and Enset fiber is lower than the pseudo stem and peduncle of banana which is in the same family as shown in Figure 3.3 (Li et al., 2010; Rahman et al., 2014). Advantages of Enset fibers in relation to other herbaceous plants are the low ash and extractives content (Table 3.2).

The inflorescence stalk had high mineral content and the fines obtained upon grinding were enriched in minerals showing double ash content. It is known that ash tends to accumulate in the finest sized fraction during biomass processing due to the small size and brittleness of the inorganic materials (Bridgeman et al., 2007; Liu and Bi, 2011).

There are a few reports on the ash content in different parts of Enset, which are similar to the results obtained here, mainly related to its use as food and fodder: leaf sheaths 6.4%, stalk 15.3% (Fekadu and Ledin, 1997), pseudo stem 8.8% and 7.5% (Mohammed et al., 2013; Nurfeta et al., 2008a). The reported higher ash content value in the leaf sheath than in the fibers may be explained by removal of leaf sheath components during the scraping procedure (Table 3.2.). High ash content was also found for stalk and leaf sheath of banana plant (Oliveira et al., 2007) while lower ash content was reported for fiber bundles isolated from the banana pseudo stem, similar to what was found in the present work (Bhatnagar et al., 2015). C. cardunculus hairs and pappis had even lower ash content than Enset fiber bundles (Gominho et al., 2009).

The ash composition of the fiber and the stalk is summarized in Table 3.4. It is dominated by the presence of potassium in both materials but especially in the inflorescence stalk, while calcium, magnesium and sodium were present in similar and low amounts. While, among the micronutrients iron was present in considerable amount (Table 3.4). In relation to micronutrients, the content of iron was very high in both materials [139-177 mg/kg].

Table 3.4: *Elemental composition of the mineral component of the Enset fibers and inflorescence stalk (stalk main and stalk fines).*

Minerals	Units	Fiber	Stalk main	Stalk fines
N	**g/kg**	1.5	8.6	7.3
Na	**g/kg**	0.7	0.8	0.9
K	**g/kg**	5.7	60.9	57.7
Ca	**g/kg**	2.8	1.4	1.3
Mg	**g/kg**	0.6	1.5	1.4
Fe	**mg/kg**	158.31	139.81	176.84
Cu	**mg/kg**	1.79	7.18	8.62
Zn	**mg/kg**	8.79	6.94	8.44
Mn	**mg/kg**	13.93	18.79	18.51

This has been reported in Enset pseudo stem as well as in other plant parts (K 21-52 g/kg) (Nurfeta et al., 2008b). The mineral composition is similar to that given for floral stalks and leaf sheaths of the banana plant with values for the different elements in the same order of magnitude (Cordeiro et al., 2004; Oliveira et al., 2007). It is interesting to notice that the mineral

composition of Enset and banana biomass components is very specific in the high K and Fe contents. In contrast, the floral stalk of *Cynara cardunculus* has only 9 g/kg K and 0.6 g/kg Fe (Gominho et al., 2009).

The inflorescence stalk has a high mineral content that may have nutritional value (Table 3.4), protein, a large proportion of extractives, a high content in glucan polymers, including starch, and little lignin (Table 3.2). Therefore, its use for food and feed can be an option, and valorization routes may include fermentation processes e.g. for ethanol. Moreover, the inflorescence stalk can produce enzymes such as amylolytic and lignocelluloytic enzymes by using microorganisms, as already tried for banana waste (Panda et al., 2016). The Enset inflorescence stalk may therefore integrate a valorization flow sheet as proposed by Panda et al. (Panda et al., 2017) for producing bio commodities from agricultural wastes.

The Enset stalk contains 3% protein, as shown by the determination of organic nitrogen (Table 3.2) as well as by pyrolysis data (Table 3.5). Crude protein content of various parts of Enset plant has been reported from 3.7% to 13.2 % (Mohammed et al., 2013) which is therefore considered of nutritional value and used for human food and for fodder. Similar amounts of protein (range of 2-8%) were also reported for the banana plant (Bilba et al., 2007; Chen et al., 2010; Liu and Bi, 2011).

Overall chemical composition of the inflorescence stalk of Enset is to some extent similar to that of stalk of banana plant with lower lignin content as shown in Figure 3.3 (Cordeiro et al., 2004; Oliveira et al., 2007).

3.3.4. Lignin monomeric composition

The pyrograms of the Enset fibers and inflorescence stalks are shown in Figure 3.4 and the list of the lignin peaks with their quantitative proportion (% of total peak area) as well as the H:G:S and S: G ratios are given in Table 3.5.

The peaks corresponding to carbohydrate-derived compounds were dominant in all the pyrograms in comparison with the lignin-derived compounds. Levoglucosan (peak 72) was the major carbohydrate-derived compound representing 17.1% of the pyrolysis products of fibers, 5.4% of main stalks and 11.4% of stalk fines. Other carbohydrate-derived compounds were also found: 2-hydroxy-2-cyclopenten-1-one (peak 21) represented 1.4%, 3.1% and 2.8% respectively, 2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4*H)*-pyran-4-one (peak 59) represented respectively 1.1%, 0.7%, 1.5%; and 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (peak 28) was higher in fibers (1.2%) and lower in stalks (0.3% and 0.6%). The low molecular weight compounds - hydroxyacetaldehyde (peak 4), acetic acid (peak 6), 1-hydroxy-propan-2-one (peak 7), 2-oxo-propanal (peak 1), 3-hydroxypropanal (peak 11) - represented 24.8%, 27.1% and 26.6% of total area for fibers, stalk and fines, respectively (Appendix E).

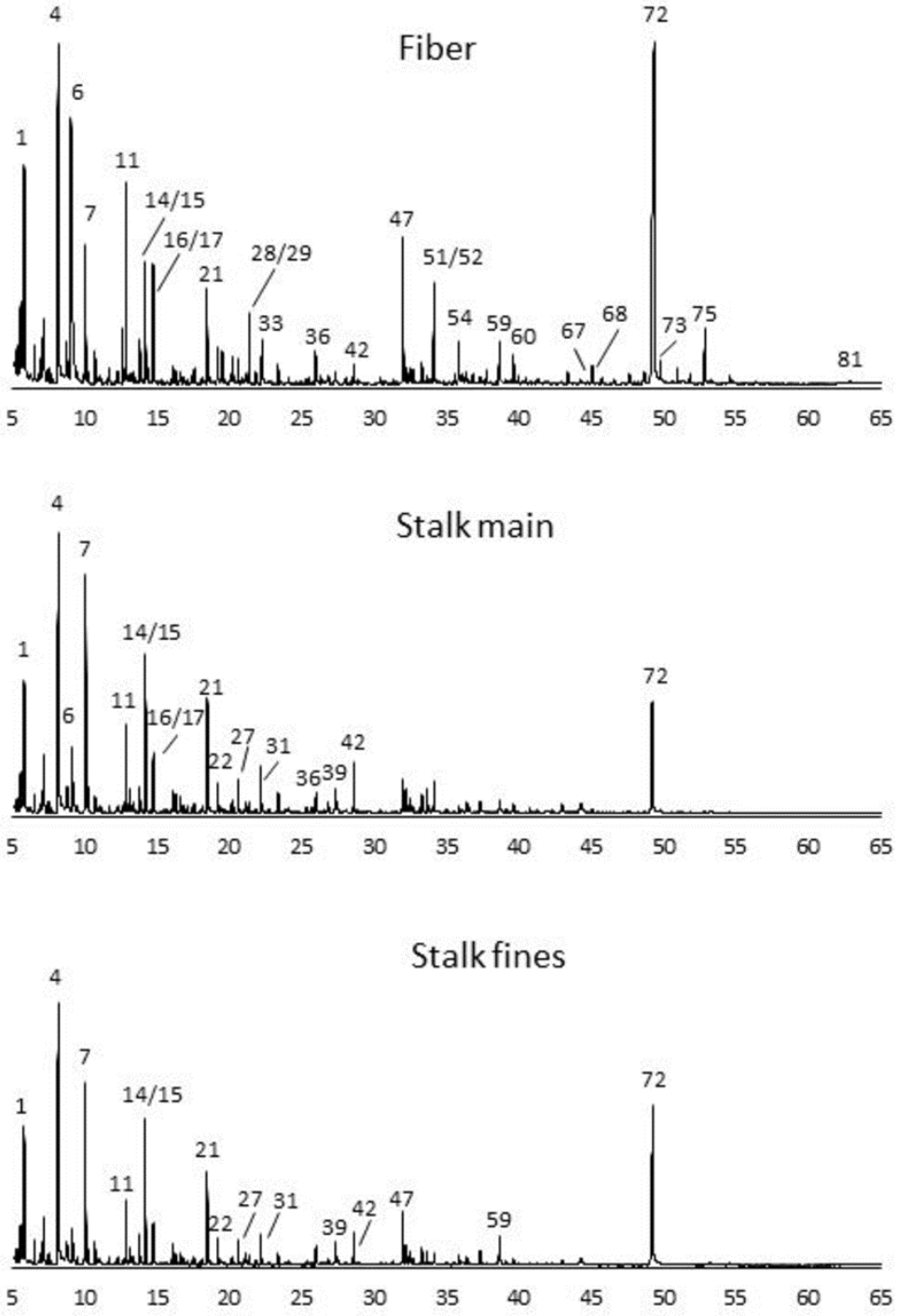

Figure 3. 3: *Pyrograms of the Enset fiber bundles and inflorescence stalk (main fraction and fines).*

Where, 1 – 2-oxo-propanal; 4 – hydroxyacetaldehyde; 6 – acetic acid; 7 - 1-hydroxy-propan-2-one; 11 – 3-hydroxypropanal; 14 – CH_3-CO-CHOH-CHO; 15 – CHO-CH_2-CH_2-CHO; 16 – furfural; 17 – 2-cyclopenten-1-one; 21 – 2-hydroxy-2-cyclopenten-1-one; 22 – dihydro-methyl furanone isomer; 27 – 5H-furan-2-one; 28 – 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one; 29 – 3-methyl-furan-2,5-dione; 31 – 2-hydroxy-3-methyl-2-cyclopenten-1-one; 33 – 2-hydroxy-1-methyl-1-cyclopentene-3-one; 36 – sugar derivative; 39 – 5-hydroxymethyldihydrofuran-2-one; 42 – sugar derivative; 47 – sugar derivative; 51 – 2,3-dihydrobenzofuran; 52 – 4-vinylguaiacol; 54 – similar to 4-allylphenol; 59 – 2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4H)-pyran-4-one; 60 – methylindole; 67 – 4-vinylsyringol; 68 – 4-allylsyringol; 72 – 1,6-anhydro-β-D-glucopyranose; 73 – trans-4-propenylsyringol; 75 – Not identified; 81 – trans-sinapaldehyde.

Table 3.5: *Peak identification, retention time and quantification (% of total area of the chromatogram) of the lignin pyrolysis products from Enset fiber bundle and inflorescence stalk*

Peak	RT	Compound	Origin	Fiber	Stalk main	Stalk fines
34	23.36	Phenol	H	0.36	0.67	0.42
35	24.16	Guaiacol	G	0.15	0.24	0.10
37	26.79	4-methylphenol (p-cresol)	H	0.18	0.36	0.21
38	26.88	3-methylphenol (m-cresol)	H	0.09	0.14	0.16
41	28.32	4-methylguaiacol	G	0.05	-	-
43	28.72	2,4-dimethylphenol (2,4-Xylenol)	H	0.09	0.21	0.12
45	30.46	3-ethylphenol	H	0.17	0.18	0.09
51	34.15	2,3-dihydrobenzofuran (coumaran)	H	1.0	0.61	0.34
52	34.15	4-vinylguaiacol	G	1.0	0.61	0.34
54	35.84	4-allylphenol	H	0.21	-	-
55	36.38	Syringol	S	0.24	0.11	0.09
62	40.02	4-methylsyringol	S	0.12	0.04	0.07
63	40.47	Vanillin	G	0.17	0.10	0.06
64	40.95	1-(4-hydroxy-3-methoxyphenyl)propyne	G	0.07	-	-
65	41.25	4-hydroxybenzaldehyde	H	0.04	0.18	0.07
66	41.36	1-(4-hydroxy-3-methoxyphenyl)propyne	G	0.08	-	-
67	45.11	4-vinylsyringol	S	0.34	0.18	-

68	45.78	4-allylsyringol	S	0.15	0.03	-
69	46.54	P-coumaric alcohol	H	0.11	-	-
70	47.69	*Cis*-4-propenylsyringol	S	0.25	-	-
71	49.02	4-propinylsyringol	S	0.09	-	-
73	49.82	*Trans*-4-propenylsyringol	S	-	0.10	-
74	51.01	Syringaldehyde	S	0.31	0.03	-
76	53.34	Acetosyringone	S	0.09	-	-
77	54.65	*Trans*-coniferaldehyde	G	0.09	-	-
78	54.78	Syringylacetone	S	0.08	-	-
79	55.84	Propiosyringone	S	0.02	-	-
80	56.33	Syringyl vinyl ketone	S	0.01	-	-
81	62.89	*ztrans*-sinapaldehyde	S	0.11	-	-
Total lignin				6.1	4.6	2.7
H				2.3	2.3	1.4
G				1.6	0.9	0.5
S				1.8	0.5	0.2
S/G				1.1	0.5	0.3
H:G:S				1:0.7:0.8	1:0.4:0.2	1:0.4:0.1

S-syringyl, G-guaiacyl, H-hydroxyphenyl,

The pyrograms show clearly a lower abundance of lignin-derived peaks, which is in accordance with the lignin content determined by wet chemical analysis (Table 3.2). The peaks identified as lignin-derived were mostly from H-lignin units such as phenol (peak 34), p-cresol (peak 37), m-

cresol (peak 38), 2,4-dimethyl-phenol (peak 43), 3-ethylphenol (peak 45), 2,3-dihydrobenzofuran (peak 51), a compound similar to 4-allyphenol (peak 54), 4-hydroxybenzaldehyde (peak 65) and *p*-coumaric alcohol (peak 69). The main compound derived from G-units was 4-vinylguaiacol (peak 52) with 1.0%, 0.6% and 0.3% of total area for fiber, stalk and stalk fines respectively. Several peaks were derived from S-units in lower proportion; they summed 1.8% and less than 0.5% in fibers and stalks respectively. The compounds indole (peak 57) and methylindole (peak 60) were also identified in the pyrograms of the stalks where they represented a small proportion of the total peak area (0.31% and 0.18% in stalks and fines respectively).

The monomeric composition of Enset lignin, therefore, showed a predominance of H units: the H:G:S ratio was 1:0.7:0.8 for the fibers, 1:0.4:0.2 for the main stalk and a similar value of 1:0.4:0.1 for the stalk fines (Table 3.5). The S/G ratio was higher in the fibers (1.1), than in stalks (0.5 and 0.3 respectively). This is the first time that Enset lignin composition is reported. The proportion of H-units in Enset lignin is overwhelming in the macromolecular structure and above values reported on lignin in other grasses. The presence of H-units in lignin is characteristic of herbaceous plants although the composition varies with species: the H:G:S ratio of corn, wheat and rice straws being 1:8.8:15.3, and 1:9.8:9.2, 1:3.0:2.6, respectively (Buranov and Mazza, 2008). Higher proportion of H-units is found in *Musa* species: in abaca fibers [*Musa textilis*] the lignin has a H:G:S ratio of 1:0.7:2.1 (Rio et al., 2007) while in the banana plant [*Musa acuminata* Colla var. *cavendish*] the H:G:S ratio of 1:1.0:0.2 in leaf sheaths nears the values obtained here for the Enset lignin (Oliveira et al., 2007).

The Enset stalk lignin is mostly H-lignin (Table 3.5) with a predominance of H units much above that of the Enset fibers. The only available comparison can be made with the banana plant floral stalk and that shows a H:G:S ratio of 1: 1.9:0.4 (Oliveira et al., 2007).

The pyrogram of extractive-free Enset fibers and stalk were dominated by carbohydrate-derived peaks. Levoglucosan was the compound representing the major proportion of the pyrolysis products of fibers, stalks and stalk fines, as shown in Table 3. This is the common feature in the pyrolysis of carbohydrates and lignocellulosic materials (Lourenc et al., 2013). Other carbohydrate-derived compounds were 2-hydroxy-2-cyclopenten-1-one; 2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4*H*)-pyran-4-one; and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one. The presence of the pyran structure in the pyrolysis products reflects the higher content of xylans in the fibers (Table 2.1) since this is an indicator for xylans (Ralph and Hatfield, 1991). The compounds indole and methylindole are protein-derived peaks (Ralph and Hatfield, 1991) and their presence in stalks and absence in fibers is in accordance with the protein content in these plant parts.

The fact that fiber bundles have higher cellulose content and long cellular elements, i.e. the fibers, makes them a candidate for pulping. However, although present in moderate amounts, the lignin has a monomeric composition with low syringyl content that will not be removed under mild reactive conditions and therefore the need for strong delignification conditions is to be expected.

3.3.5. Lipophilic and ethanol-water extracts

The compositional profile of the lipophilic extracts from the fiber bundles and inflorescence stalk is given in Table 3.6 and shows little differences between the materials. The major fractions correspond to fatty acids, mainly saturated alkanoic acids (32-40% of the total compounds) that included mainly hexadecanoic acid, and substituted alkanoic acids (16-29%) including mainly 9-octadecenoic acid in the stalks and 9,12-octadecadienoic acid in the fibers. In addition, significant amount of glycerol derivatives were also present (16% in fiber and 25% in stalk), mainly β-sitosterol was the most important followed by stigmasterol and campesterol.

Table 3. 6: *Composition (% of total peak area) of TMS-derivatized dichloromethane extracts from Enset fibers and inflorescence stalk (stalk main and stalk fines)*

Compounds	**Fiber**	**Stalk main**	**Stalk fines**
Phenolics	**1.97**	**-**	**-**
4-Hydroxybenzyl alcohol	0.30	-	-
4-Hydroxy-3-methoxybenzaldehyde	0.47	-	-
3,5-Dimethoxy-4-hydroxybenzaldehyde	0.41	-	-
4-Hydroxy-3-methoxybenzoic acid	0.42	-	-
3,5-Dimethoxy-4-hydroxybenzoic acid	0.37	-	-
Alkanols	**3.85**	**0.86**	**0.76**
1-Dodecanol	0.20	-	-
1-Tetradecanol	-	0.11	-
1-Hexadecanol	1.35	0.29	0.37

1-Heptadecanol	-	0.18	-
1-Octadecanol	1.22	0.28	0.39
1-Eicosanol	0.41	-	-
1-Tetracosnaol	0.67	-	-
Saturated alkanoic acids	**31.71**	**34.64**	**39.53**
Decanoic acid	0.24	0.10	-
Dodecanoic acid	0.34	-	-
Tetradecanoic acid	1.56	0.58	0.51
Pentadecanoic acid	1.97	1.18	0.87
Hexadecanoic acid	10.12	17.67	24.11
Heptadecanoic acid	1.67	1.09	0.87
Octadecanoic acid	3.98	6.23	10.98
Nonadecanoic acid	0.46	0.21	-
Eicosanoic acid	2.42	1.78	1.30
Heneicosanoic acid	1.88	1.01	-
Docosanoic acid	2.30	1.63	0.89
Tricosanoic acid	1.45	1.08	-
Tetracosanoic acid	2.22	1.81	-
Pentacosanoic acid	0.55	0.27	-
Hexacosanoic acid	0.55	-	-
ω-Hydroxy alkanoic acid	**-**	**0.2**	**-**
ω-Hydroxyoctadecanoic acid, methyl ester	-	0.2	-

Substituted alkanoic acids	**15.79**	**25.62**	**28.96**
cis-Eicosenoic acid	-	0.10	-
9-Tetradecenoic acid	0.70	-	-
13-Methyl-9-tetradecenoic acid	-	0.07	-
9-Hexadecenoic acid	2.40	1.61	1.15
10-Heptadecenoic acid	0.87	0.68	0.56
9-Octadecenoic acid	-	21.54	21.87
10-Nonadecenoic acid	-	0.10	-
9,12-Octadecadienoic acid	11.73	1.03	5.38
13-Docosenoic acid	-	0.49	-
4-Pentenoic acid	0.09	-	-
Saturated alkanoic di acids	**0.41**	**0.08**	**-**
Nonanedioic acid	0.41	0.08	-
Glycerol derivatives	**15.68**	**25.05**	**24.94**
Campesterol	2.09	3.51	3.98
Stigmasterol	3.73	5.68	4.79
β-Sitosterol	9.05	12.79	15.54
Fucosterol	0.70	0.82	-
Cycloartenol	-	0.80	-
ni sterol1	0.11	0.75	0.63
ni sterol2	-	0.19	-
ni sterol3	-	0.51	-

Triterpenes/Triterpenoids	**0.90**	**0.06**	**-**
Isochiapin β	-	0.06	-
Dehydroabietic acid	0.90	-	-
Sugars	**0.15**	**0.58**	**0.61**
ni sugar 1	0.15	-	-
ni sugar 2	-	0.15	-
ni sugar 3	-	0.10	-
ni sugar 4	-	0.33	0.61
Other	**2.71**	**0.35**	**-**
Sitosteryl-3β-D-Glucopyranoside	2.22	0.35	-
Campesteryl.3β-D-glucopyranoside	0.24	-	-
Stigmasteryl-3β-D-glucopyranoside	0.25	-	-
TOTAL ID	**82.49**	**94.8**	**98.59**
TOTAL NI	**17.51**	**5.2**	**1.41**
TOTAL	**100**	**100**	**100**

NI: Not Identified, ID: Identified

The characterization of the ethanol-water extracts (Table 3.7) shows that the content in phenolic compounds is very low and that they have no relevant antioxidant properties.

Table 3.7: *Ethanol-water extraction yield, total phenolics, tannins, flavonoids and monosaccharides contents, and antioxidant activity of Enset fiber and inflorescence stalk (stalk main and stalk fibers)*

Ethanol-Water extracts	**Fibers**	**Stalk main**	**Stalk fines**
Extraction yield (%)	1.85	17.64	13.73
Total phenolics (mg GAE/g of extract)	38.22	17.61	23.01
Tannins (mg catechin/g of extract)	0	5.88	5.79
Hydrolysable tannins (mg of tannic acid/g of extract)	0	24.85	0
Flavonoids (mg catechin /g of extract)	19.2	5.34	44.42
Antioxidant capacity TEAC (mg Trolox/ g of extract)	142.11	22.255	23.41
IC_{50} value (µg extract /ml)	67.51	214.62	154.55
Total monosaccharides (mg/g of extract)	63.76	30.63	5.79

3.3.6. Extractives content and composition

The Enset inflorescence stalk contained substantially more extractives than the fiber bundles that were mostly solubilized by polar solvents (Table 3.2). Floral stalk of banana plant also showed high extractives content similar to Enset inflorescence stalk, as shown in Figure 3.3 (Oliveira et al., 2007). Herbaceous plants, like rice straw and *C. cardunculus* often have a large content of extractives (Figure 2.3)(Oliveira et al., 2005; Moniz et al., 2014).

The ethanol-water extracts yielded low content of phenols compounds (Table 3.7) and the antioxidant capacity was low; this indicates that these extracts are mainly composed of

carbohydrates, as observed in water-soluble extracts from different morphologic regions of the banana plant (Oliveira et al., 2007).

The lipophilic extracts included mainly fatty acids and their derivatives; sterols were present but triterpenes appeared in very low amounts. This is the first time that the composition of Enset lipophilic extracts is reported. The composition is very similar to that obtained for the banana plant which showed a predominance of saturated and unsaturated acids, mostly of hexanoic acid and 9,12- octadecadienoic acid, accompanied by a large content of sterols and little presence of aromatics (Oliveira et al., 2005). Steryl glucosides that were present in the Enset extracts from the fibers bundles (Table 3.6) were also found in the banana plant (Oliveira et al., 2005).

Although lipophilic extractives in fibrous materials may cause problems if pulp production is envisaged, in the Enset fiber bundles they were present in very low contents (0.4%), therefore without significance for the formation of stickies in the pulp (Table 3.2).

In the case of the Enset floral stalk, the lipophilic compounds are present in higher amounts (1.1%) as shown in Table 3.2 and they will add to the nutritional value of the material.

3.3.7. FTIR-ATR spectra of Enset fibers and inflorescence stalk

The FTIR-ATR spectra for the extractive-free fiber bundles and inflorescence stalk are given in Figure 3.5.

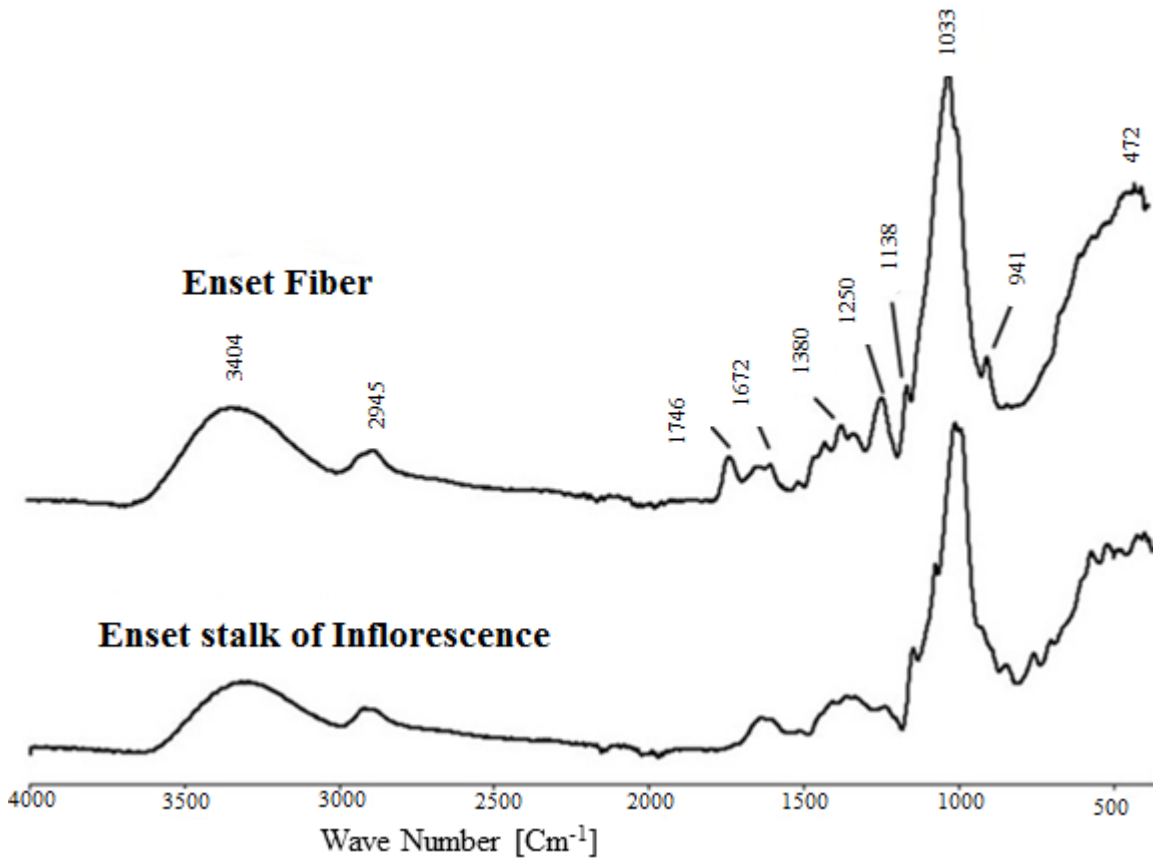

Figure 3.4: *FTIR/ATR spectra of Enset fiber bundles and Enset inflorescence stalk*

The FTIR spectral features of the extractive free Enset fiber and stalk (Figure 3.5) show similar peaks around 3400 cm^{-1}, 2945 cm^{-1}, 1746 cm^{-1}, 1380 cm^{-1} and 1033 cm^{-1} which correspond to different functional groups of cellulose, hemicelluloses and lignin (Chen et al., 2010; Ramadevi et al., 2012). These bands were also found in different parts of the banana plant (Oliveira et al., 2007, 2008). The peaks around 1670 cm^{-1} and 1250 cm^{-1} are originated from S and G units of lignin respectively, and were noticeable in the fibers. This result supports the data obtained from lignin pyrolysis since the predominance of H unit is more pronounced in the stalk.

3.4.Conclusions

The biomass residues remaining from the exploitation of the Enset food crop as fiber bundles and inflorescence stalk have different structural and chemical characteristics. Accordingly, the two residual materials have different potential applications and should have different valorization routes.

The fiber bundles are aggregates mainly of long and slender fibers with low ash, extractives and lignin contents, and high holocellulose and α-cellulose contents. These features show the possibility of delignification to produce paper pulps but taking into account that the high content of H-units in the lignin structure may need strong pulping conditions. The hemicelluloses, mostly highly acetylated xylans, may be removed prior to delignification for xylan-derived added-value products.

The Enset pseudo stem residual components remaining after plant harvest and processing for food that were here chemically characterized for the first time, show potential for valorization thereby allowing a more complete resource use and an improvement of the crop economy. The inflorescence stalk has a nutritional value for food and fodder, since it contains high amounts of potassium and iron, protein and non-cellulosic carbohydrates associated to low lignin content. It may be used also for sugar fermentation products such as ethanol.

CHAPTER FOUR

4. Optimization of Ethanol-Alkali Delignification of Enset Fibers

4.1.Introduction

The industrial fiber pulps used for paper production have relied mostly on wood as feedstock material although other fibrous raw-materials such as straw and bamboo are also used commercially. Under the present concepts of smart full natural resource use and integrated multiproduct valorization, there is a renewed interest in the study of less-valued resources, including by-products and residues from existing crops and production lines.

The availability of large quantities of fibrous residues from Enset plant has triggered the interest in their valorization as a natural fiber reinforcement in composites (Mizera et al., 2016, 2017). The chemical features of the Enset fiber bundles that are rich in polysaccharides and have a low lignin content (10.5%) together with the predominance of long and slender fibers have suggested that these Enset fibers could be a raw material for paper pulp production (Chapter 3).

Kraft pulping is the dominant chemical pulping process because it produces pulps with high quality and allows recovery of chemicals, although environmental effects are a continuing concern due to the presence of sulfur in the reactive liquor (Gierer, 1980). To overcome such drawbacks, sulfur-free processes have been investigated including solvent-based (organosolv) processes aiming at a low environmental impact. One such process involves the utilization of ethanol in the alkaline pulping system. This process, also called ethanol-alkali or ethanol-

reinforced soda pulping, seems very effective in improving the rate and selectivity of delignification and in preventing polysaccharide degradation and lignin condensation, allowing higher pulp yields and maintaining good resistance properties of the pulps (Shatalov and Pereira, 2002, 2004).

Ethanol-alkali pulping has been applied to various types of lignocellulosic materials: *Eucalyptus globulus* wood (Gilarranz et al., 1998), giant reed (*Arundo donax*) (Shatalov and Pereira, 2013), wheat straw (Jiménez et al., 2004), cardoon (*Cynara cardunculus*) stalks (Shatalov and Pereira, 2014), cotton stalks (Akgül and Tozluo, 2010) and jute (Sahin, 2003). From such studies, it is well established that the reaction conditions depend on the chemical and structural features of the specific raw-material, therefore requiring a targeted study on the operation variables and the corresponding process optimization (Gilarranz et al., 1998; Ligero et al., 2007).

Response surface methodology is one optimization method that allows analysis of the simultaneous effects of multiple independent variables while requiring a small number of experiments (Bas and Boyaci, 2007). Therefore, it is frequently applied in chemical and biochemical processes including delignification studies (Jiménez et al. 1999; Neiva et al. 2014).

The present study assesses the possibility of using ethanol-alkali pulping on Enset fibers for paper pulp making with the aim of strengthening the overall valorization of the Enset crop and of enlarging the pulp raw-material sources. In this part of the work, the delignification performance of the material was studied using an ethanol-reinforced alkali reactive system and results on pulp yield, kappa number, brightness, and color are analyzed. The effects of the process parameters such as temperature, time, ethanol and alkaline concentration were investigated and the process

conditions were optimized by means of response surface methodology with central composite design.

4.2.Materials and Methods

4.2.1. Ethanol-alkali pulping of Enset fiber

The collected fibrous materials were washed and sun-dried. Prior to pulping, the fiber threads were cut to average size of 5 mm.

Ethanol-alkali delignification of the Enset fibers was carried out under an isothermal condition in stainless steel digesters of 100 mL capacity each which were rotated in a temperature-controlled mineral oil bath. Each digester was loaded with 5 g of the fiber sample (oven dry basis) and an ethanol-alkali aqueous solution with a solid-to-liquid ratio of 1:12 (g of Enset fibers/g of liquor) for all type of pulping. The solid to liquid ratio was selected based on the results of exploratory experiments. The process was performed under different experimental conditions of reaction temperature, reaction time, ethanol-to-water ratio and alkali charge, as detailed below. After the targeted reaction time, the reactors were cooled in an ice bath and the pulps were disintegrated in a laboratory blender, vacuum filtrated, and washed thoroughly with distilled water until neutral pH was obtained; subsequently, the total pulps were oven dried at 50^{o}C.

The total pulp yield was calculated based on the oven-dry mass of the obtained solid as a percentage of the original oven-dry sample.

4.2.2. Experimental design

Response surface methodology (RSM) was used to analyze the effect of four independent process variables - reaction temperature (130 °C < T < 170 °C), reaction time (30 min < t < 150

min), ethanol-to-water ratio (20 % < eth < 60%) and alkali concentration (0% < alk < 20%) on the dependent variables of pulp yield, kappa number, brightness, whiteness and yellowness. Central composite design (CCD) was used to formulate model equations for each response.

A total of 30 experiments were done to formulate model equations and to see linear, quadratic and interaction effects of each factor on each response. The experimental points and corresponding responses are listed in table 4.1.

Table 4. 1: *Experimental design data for the ethanol-alkali pulping of Enset fibers*

Run	Coded variable				Decoded variable				Dependent variable				
	X_T	**X_t**	**X_{eth}**	**X_{alk}**	**Temp (^OC)**	**Time (min)**	**Eth %**	**Alk %**	**Y_Y**	**Y_K**	**Y_{Bt}**	**Y_W**	**Y_{Yellow}**
1	-1	1	-1	-1	140	120	30	5	73.8	7.9	54.9	12	27.1
2	0	0	2	0	150	90	60	10	68.5	4.0	60.5	14	25.8
3	-1	-1	-1	-1	140	60	30	5	75.3	12.2	57.7	16	26.0
4	0	0	0	0	150	90	40	10	67.1	5.0	61.9	18	24.8
5	1	-1	-1	1	160	60	30	15	62.0	5.5	67.1	41	17.0
6	1	1	1	1	160	120	50	15	66.8	5.2	65.8	36	18.9
7	-1	1	-1	1	140	120	30	15	66.9	4.8	70.9	42	16.9
8	0	0	-2	0	150	90	20	10	63.8	7.9	64.4	27	21.8
9	0	0	0	-2	150	90	40	0	86.6	-	-	-	-
10	1	-1	1	1	160	60	50	15	60.3	4.8	64.6	35	19.2
11	1	1	1	-1	160	120	50	5	71.9	12.3	46.9	-1.3	30.9
12	-1	-1	1	1	140	60	50	15	70.0	4.2	64.2	26	21.8
13	0	0	0	0	150	90	40	10	67.4	5.76	59.3	13.1	26
14	-2	0	0	0	130	90	40	10	69.0	8.8	66.7	24	22.9
15	1	-1	-1	-1	160	60	30	5	69.8	10.2	53.9	13	26.2
16	0	0	0	0	150	90	40	10	67.8	4.6	61.8	20	23.8
17	2	0	0	0	170	90	40	10	66.7	3.6	57.4	22	23.5

18	-1	-1	1	-1	140	60	50	5	73.8	9.2	56.6	5.7	28.9
19	1	1	-1	-1	160	120	30	5	74.4	9.2	48.1	9.8	27.9
20	0	0	0	0	150	90	40	10	66.6	6.3	55.5	4.6	28.8
21	-1	1	1	-1	140	120	50	5	75.6	7.9	58.5	11	27.2
22	-1	-1	-1	1	140	60	30	15	65.9	6.1	69.8	35	19.2
23	1	-1	1	-1	160	60	50	5	74.2	9.3	53.6	5.6	28.9
24	-1	1	1	1	140	120	50	15	71.3	6.4	68.0	34	19.7
25	0	0	0	0	150	90	40	10	69.5	7.1	57.5	8.2	27.4
26	0	2	0	0	150	150	40	10	67.5	6.7	64.0	32	20.7
27	0	-2	0	0	150	30	40	10	62.4	7.0	65.4	23	22.5
28	0	0	0	2	150	90	40	20	60.7	9.1	68.3	43	16.5
29	1	1	-1	1	160	120	30	15	63.8	4.8	74.6	62	10.7
30	0	0	0	0	150	90	40	10	64.7	5.7	59.8	15	25.4

Experimental data for each of the responses were modeled by fitting to a second order polynomial equation using multiple regression analysis through the least square method:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j \qquad (1)$$

where Y is the predicted response (i.e. pulp yield or Kappa number or brightness or whiteness or yellowness), k is the number of independent variables, x_i and x_j are the coded independent variables, β_o, β_i, β_{ii}, and β_{ij} are the regressions coefficients for intercept, linear, quadratic and interaction terms, respectively. The model coefficients were obtained using Design Expert (version 7.0) software and multivariant analysis was done by using JMP software.

The analysis of variance (ANOVA) for experimental results was used to identify significant effects and to check the statistical significance of the model.

4.2.3. Kraft pulping of Enset fiber

Kraft pulping of the Enset fibers was carried out in stainless steel micro digester as stated in section 4.2.1. The process was performed under a temperature of 160^{o}C for 60 min with different active alkali (10% and 15 %) and sulfidity (10% and 20%) as Na_2O based on previous experiments (Colodette et al., 2002). Four different active alkali concentration and sulfidity arrangement was done in duplicate. After the targeted reaction time, the reactors were cooled in an ice bath and the step in section 4.2.1 was followed.

The total pulp yield was calculated based on the oven-dry mass of the obtained solid as a percentage of the original oven-dry sample. The cooking conditions for Kraft pulping are presented in Table 4.2.

Table 4.2: *Cooking conditions for Kraft pulping of Enset fiber*

Pulping Conditions	**Values**
Active Alkali concentration	10% and 15%
Sulfidity	10% and 20%
Solid-to-liquid concentration	1:12
Time at maximum temperature	60 min
Temperature	160^{o}C
Raw material weight (oven dry base)	About 5g

4.2.4. Pulp characterization

The obtained pulps were characterized in relation to the delignification degree by determination of kappa number, viscosity and color parameters (brightness, whiteness, and yellowness).

The kappa number was determined following TAPPI UM 246, including a prior milling step in a centrifugal mill to dimensions below 0.15 mm to ensure the complete oxidation reaction of lignin during the kappa number test (*TAPPI useful methods*, 1991). The intrinsic viscosity of the pulps was determined by dissolving the pulp in cupriethylenediamine (CED), following the SCAN-CM 15:88 test method.

Hand sheets with 60 g/m^2 were prepared according to TAPPI standard method and the physical properties were measured following the appropriate TAPPI standard (*TAPPI standard test methods*, 1994-1995).

The color and brightness of the hand sheet papers were analyzed using a Minolta CM 3630 (d/0º) spectrophotometer using CIE1976 Lab color space coordinates (ISO 11664-42008 1976) and

brightness was measured following *TAPPI standard test methods*, T 525 om-92 1995). Measurements were conducted in triplicate, and the results are expressed as mean.

4.3.Results and Discussion

4.3.1. Ethanol-alkaline pulping

In the central composite design presented in Table 4.1, the effects of four independent variables i.e. cooking temperature (130 °C < X_T < 170 °C), cooking time (30 min < X_t < 150), ethanol concentration (20 % < X_{eth} < 60 %) and alkali concentration (0% < X_{alk} < 20%) on pulp yield and various pulp quality parameters were evaluated. Statistical significance of the main, interaction and quadratic effect of all the variables were predicted by analysis of variance (ANOVA). Table 4.3 summarizes the statistical significance of each variable (P-value) and the coefficient of determination (R^2) and the adjusted coefficients of determination (R^2 adj).

Table 4.3: *ANOVA table for the response surface quadratic model of the ethanol-alkali pulping of Enset fibers*

	P – value				
	Yield	**Kappa number**	**Brightness**	**Whiteness**	**Yellowness**
A- Temperature	0.0003	<0.0001	0.0010	0.5789	0.4656
B- Time	0.0049	0.0074	0.8205	0.1075	0.1658
C- Ethanol/water	0.0523	0.0003	0.0269	0.0014	0.0010
D- NaOH	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AB	0.7143	0.1531	0.3788	0.7984	0.7485
AC	0.3620	0.1422	0.4490	0.2808	0.2504
AD	0.0536	0.5553	0.0149	0.0218	0.0223
BC	0.0949	0.0005	0.9772	0.5635	0.5737
BD	0.2029	0.0055	0.0081	0.0500	0.0455
CD	0.3604	0.2890	0.0324	0.3669	0.2876
A^2	0.9147	0.4344	0.1167	0.0146	0.0191
B^2	0.8275	0.5763	0.0057	0.0014	0.0015
C^2	0.6340	0.3808	0.0780	0.0452	0.0482
D^2	<0.0001	<0.0001	0.0353	0.4905	0.6622
Lack of fit	0.5844	0.9127	0.7414	0.6755	0.7204
R-Squared	0.9578	0.9607	0.9448	0.9334	0.9416
Adj R-Squared	0.9185	0.9214	0.8896	0.8667	0.8832

The models for each response are as follows for pulp yield (Y_Y), kappa number (Y_K), brightness (Y_{Bt}), whiteness (Y_W) and yellowness (Y_{Yellow}) as a function of temperature (X_T), time (X_t), ethanol concentration (X_{eth}) and alkali concentration (X_{alk}):

$$Y_Y = 67.18 - 1.43X_T - 1.02X_t + 0.65X_{eth} - 4.65X_{alk} + 0.14X_TX_t - 0.35X_TX_{eth} - 0.79X_TX_{alk} - 0.67X_tX_{eth} - 0.50X_tX_{alk} + 0.36X_{eth}X_{alk} + 0.031X_T^2 + 0.064X_t^2 - 0.14X_{eth}^2 + 2.25X_{alk}^2$$ ……(2)

$$Y_K = 5.93 - 0.64X_T - 0.36X_t - 0.55X_{eth} - 1.87X_{alk} - 0.21X_TX_t + 0.22X_TX_{eth} + 0.085X_TX_{alk} + 0.64X_tX_{eth} + 0.46X_tX_{alk} - 0.15X_{eth}X_{alk} - 0.088X_T^2 + 0.062X_t^2 + 0.099X_{eth}^2 + 1.56X_{alk}^2$$ ……(3)

$$Y_{Bt} = 59.28 - 1.86X_T - 0.10X_t - 1.11X_{eth} + 7.14X_{alk} - 0.50X_TX_t - 0.43X_TX_{eth} + 1.52X_TX_{alk} + 0.016X_tX_{eth} + 1.69X_tX_{alk} - 1.30X_{eth}X_{alk} + 0.71X_T^2 + 1.39X_t^2 + 0.81X_{eth}^2 - 1.28X_{alk}^2$$ ……(4)

$$Y_W = 13.06 + 0.61X_T + 1.85X_t - 4.30X_{eth} + 14.50X_{alk} - 0.34X_TX_t - 1.48X_TX_{eth} + 3.41X_TX_{alk} - 0.78X_tX_{eth} + 2.83X_tX_{alk} - 1.23X_{eth}X_{alk} + 2.85X_T^2 + 4.06X_t^2 + 2.25X_{eth}^2 + 0.94X_{alk}^2$$ ……(5)

$$Y_{Yellow} = 26.03 - 0.25X_T - 0.48X_t + 1.35X_{eth} - 4.87X_{alk} + 0.13X_TX_t + 0.48X_TX_{eth} - 1.03X_TX_{alk} + 0.23X_tX_{eth} - 0.88X_tX_{alk} + 0.44X_{eth}X_{alk} - 0.82X_T^2 - 1.22X_t^2 - 0.67X_{eth}^2 - 0.18X_{alk}^2$$ ……(6)

The above models include all the linear, quadratic and interaction terms, although several terms did not have statistical significance (Table 4.3).

The actual vs. predicted responses of ethanol-alkali pulping of Enset fibers are shown in Figure 4.1 A-B and Figure 4.2A-C. The responses exhibit almost linear relationships and the coefficients of determination were above 93% for all models.

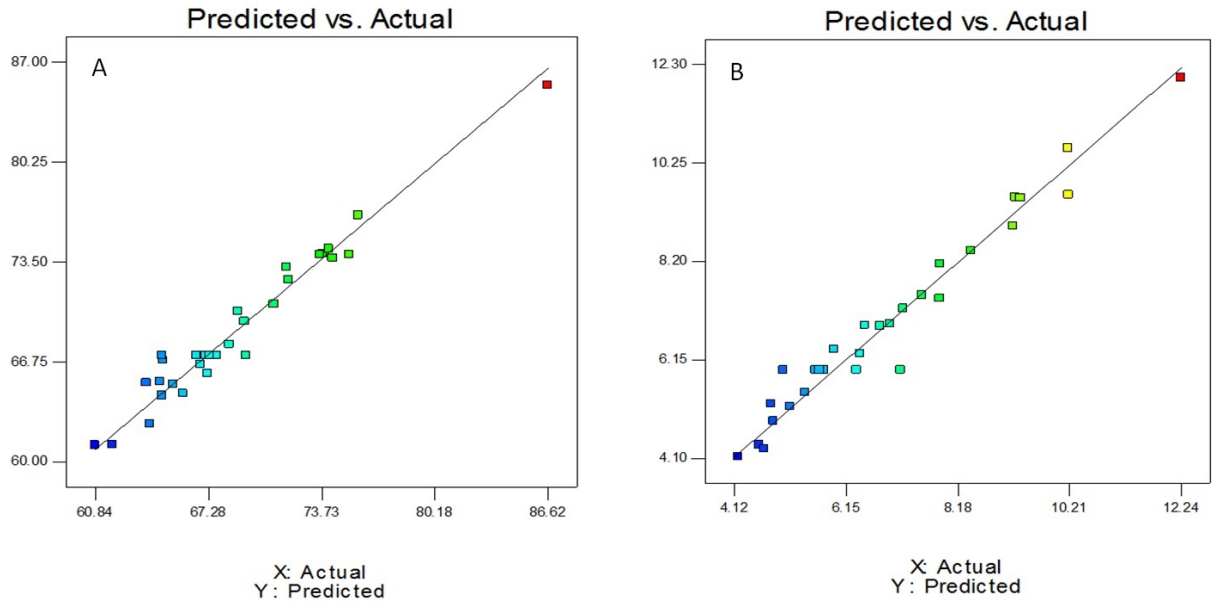

Figure 4. 1: *Actual vs. predicted responses of ethanol-alkali pulping of Enset fibers: (A) Yield; (B) Kappa Number*

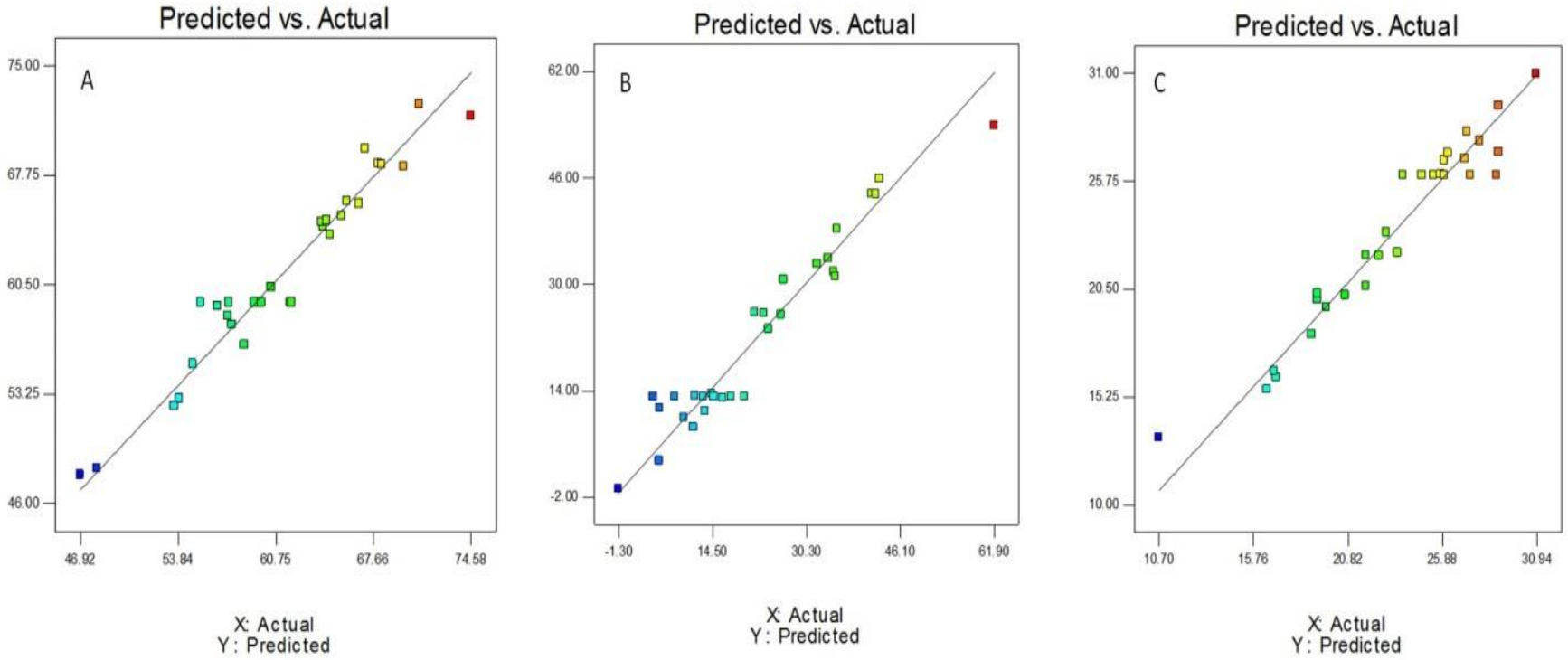

Figure 4. 2: *Actual vs. predicted responses of ethanol-alkali pulping of Enset fibers: (A) Brightness; (B) Whiteness; (C) Yellowness*

As it is clear from the results shown in Table 4.3, alkali concentration is the principal factor for all responses ($P<0.0001$). This is because the main active species in the ethanol-alkali reaction solution are hydroxide ions like in conventional soda pulping (Shatalov and Pereira 2007, 2013; Neiva et al. 2014). Nevertheless, the other factors and their interactions also have an effect on the responses, as discussed below.

4.3.2. Response surfaces

The individual and interaction effect of the factors on the responses is demonstrated in Figure 4.3- Figure 4.9 through three-dimensional response surface plots.

4.3.2.1.Pulp yield

The pulp yields obtained in the experiment are found to be in the range of 60.3 - 86.6% with the mean value of 68.9 % (Table 4.1). These values are high and certainly related to the higher alpha-cellulose content and low lignin content of the Enset fibers (Chapter 3). The lowest pup yield was obtained at 50% and 15% of ethanol and alkali concentration respectively at a temperature of 160^{o}C for 120 min. The highest pulp yield was obtained at 0% alkali concentration and 40% ethanol concentration at a temperature of 140^{o}C for 150 min. At this pulping condition the highest portion of the pulp was un-delignified.

Pulp yields obtained by ethanol-alkali pulping of other lignocellulosic materials with higher lignin content are lower. For instance, yields of 44.0-47.6% were reported in the 20-40 % ethanol-alkali pulping of giant reed (Shatalov and Pereira, 2002, 2013) and yields of 44.5-62.0% were obtained in the 40-60% ethanol-alkali pulping of cardoon stalks (Shatalov and Pereira, 2014).

With regard to the regression coefficients shown by the response models (Table 4.3), pulp yield is significantly influenced by the alkali concentration ($P<0.0001$), temperature ($P= 0.0003$), reaction time ($P=0.0049$) and ethanol concentration ($P=0.052$) as well as by the quadratic effect of alkali concentration ($P<0.0001$). No significant interaction effects on pulp yield were observed.

Temperature and time had reverse effect on the total yield of ethanol alkali pulping as shown in figure 4.3 A and figure 4.3 B. It shows that an increase in temperature and higher ethanol concentration a higher yield of pulp than at lower ethanol concentration, which could be attributed to the unwanted side reaction of either the lignin or the carbohydrate compounds and figure 4.4 A and figure 4.4 B the interaction effects of temperature vs. alkali concentration and time vs. ethanol concentration respectively. An increase of the factors resulted in decline of the pulp yield. This is due to degradation of lignin and carbohydrate at higher temperature and extended time. Extended time may further degrade desirable carbohydrate (Neiva et al., 2014; Shatalov and Pereira, 2014).

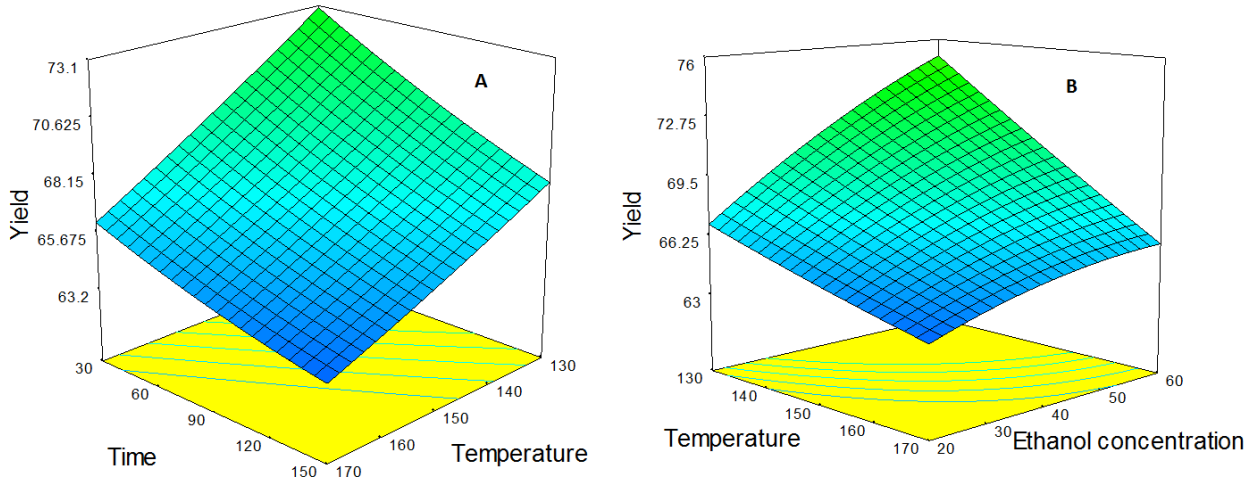

Figure 4. 3: *Response surface plot of pulp yield (%) due to (A) Interaction of time (min) and temperature (^oC) (B) Interaction of temperature (^oC) and ethanol concentration (%)*

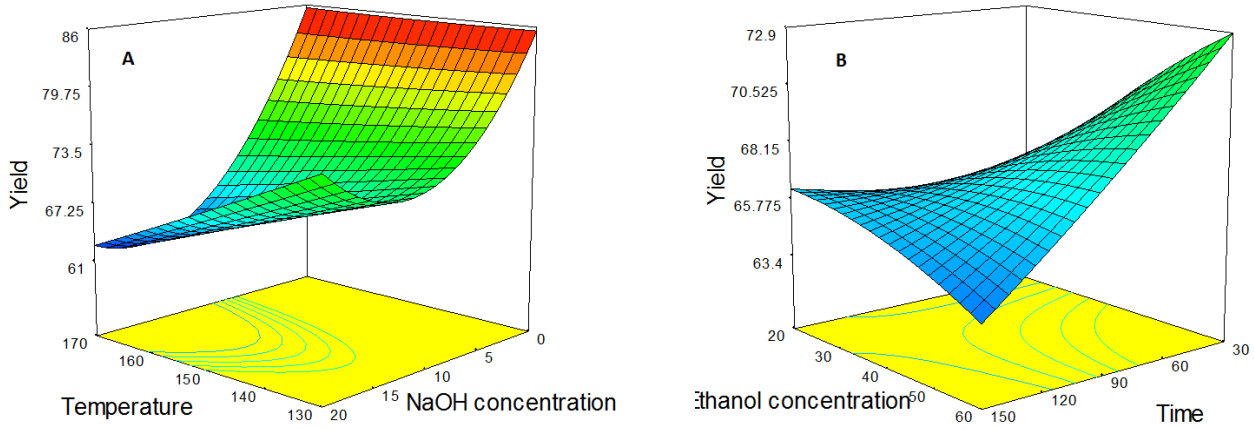

Figure 4.4: *Response surface plot of pulp yield (%) due to (A) Interaction of temperature (^oC) and alkali concentration (%) (B) Interaction of ethanol concentration (%) and time (min)*

Ethanol concentration positively affect total and screened pulp yield as ethanol hinder carbohydrate degradation during alkali pulping as shown in figure 4.3 B and figure 4.4 B (Akgül and Tozluo, 2010; Gilarranz et al., 1998; Shatalov and Pereira, 2004, 2002). Alkali concentration

is the main factor which affect pulp yield (figure 4.4 A and figure 4.5 A and B). It positively affect screened pulp yield but the total yield was decreased by increment in alkali concentration.

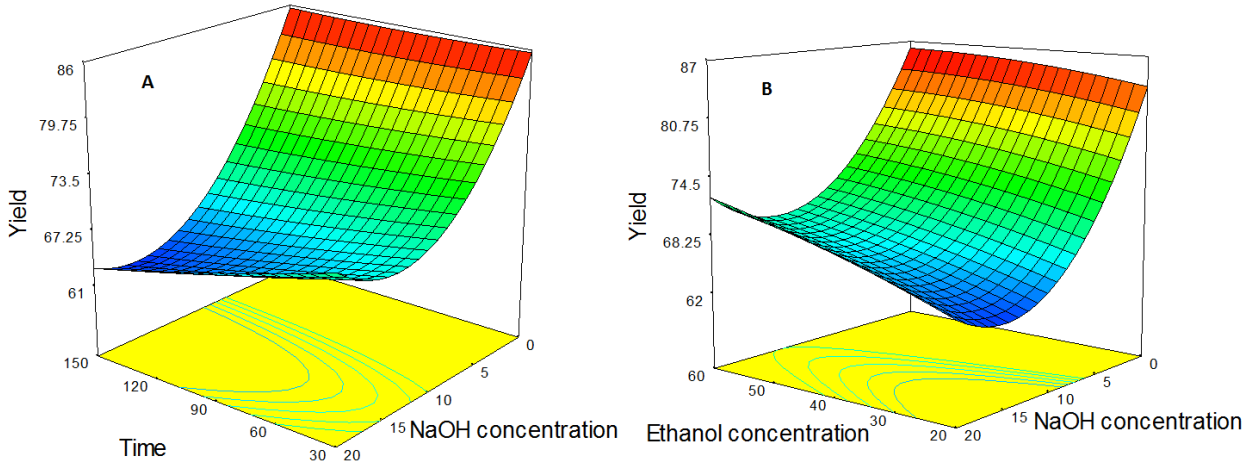

Figure 4. 5: *Response surface plot of pulp yield (%) due to (A) Interaction of time (min) and alkali concentration (%) (B) Interaction of ethanol concentration (%) and alkali concentration (%)*

4.3.2.2.Pulp kappa number

The pulp kappa numbers obtained in the experiment were in the range of 4.0 - 12.3 (Table 4.1). They are comparable with the kappa number of 4.1 achieved using pre-hydrolysis followed by ethanol-alkali pulping of giant reed (Shatalov and Pereira, 2013).

In the present study, pulp kappa number is found to be significantly affected by all linear process variables (Equation 3). In addition, the interaction between cooking time and ethanol concentration as well as between cooking time and alkali concentration are seen to have considerable influence on the kappa number. The quadratic effect of alkali concentration was also observed.

Increment in temperature and time has desirable effect on kappa number as shown in figure 4.6 A and B and Figure 4.7 A and B. The increment of ethanol concentration has a desirable effect on the kappa number by improving lignin degradation and preventing lignin condensation as shown figure 4.6.B and figure 4.7 B. Ethanol reduces the surface tension of the pulping liquor and increases dissolution of extractives, penetration of the active pulping chemicals into the material and diffusion of the degradation product into the liquor. Therefore, higher alcohol contents increase the effective alkali concentration in the material and contribute to increasing delignification (Shatalov and Pereira, 2004).

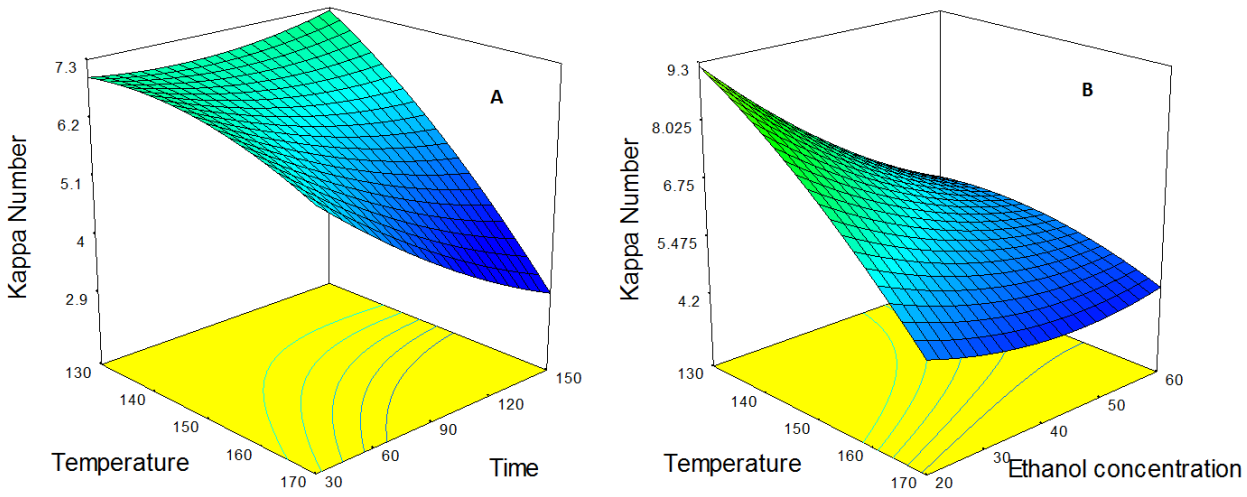

Figure 4.6: *Response surface plot (A) interaction of temperature (^oC) and time (min) on pulp kappa number (B) interaction of temperature (^oC) and ethanol concentration (%) on pulp kappa number.*

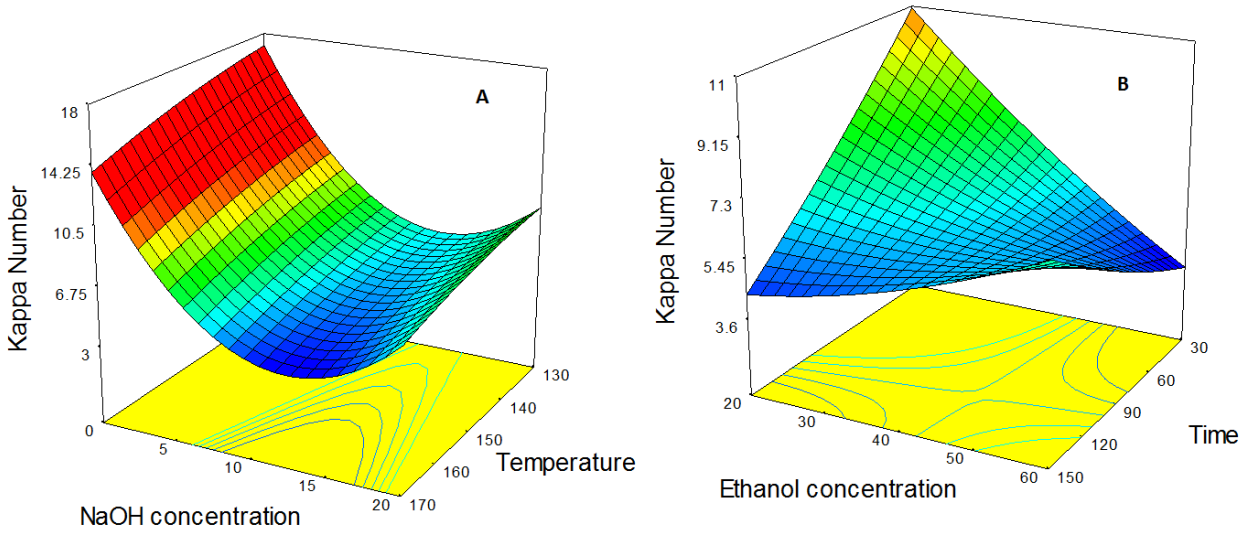

Figure 4. 7: *Response surface plot (A) interaction of alkali concentration (%) and temperature (^oC) on pulp kappa number (B) interaction of ethanol concentration (%) and time (min) on pulp kappa number.*

The interaction effects between pulping time and ethanol concentration on kappa number at 150^{o}C and 10% alkali concentration are shown in Fig. 4.7 B. In this surface plot, kappa number decreases with the increase in pulping time and ethanol concentration till the center point. However, kappa number slightly increases at high ethanol concentration with extended time, which could be explained with the reverse action of lignin monomers to condensation reaction.

Pulp kappa number is highly influenced by alkali concentration as shown in figure 4.7 A and figure 4.8 A and B. Figure 4.8 A shows the response surface of Enset pulp kappa number as a function of cooking time and alkali concentration at 150^{o}C and 40% ethanol concentration. The pulp kappa number decreased with the increase of both pulping time and alkali concentration although the influence of alkali concentration is seen to be higher (Table 4.3).

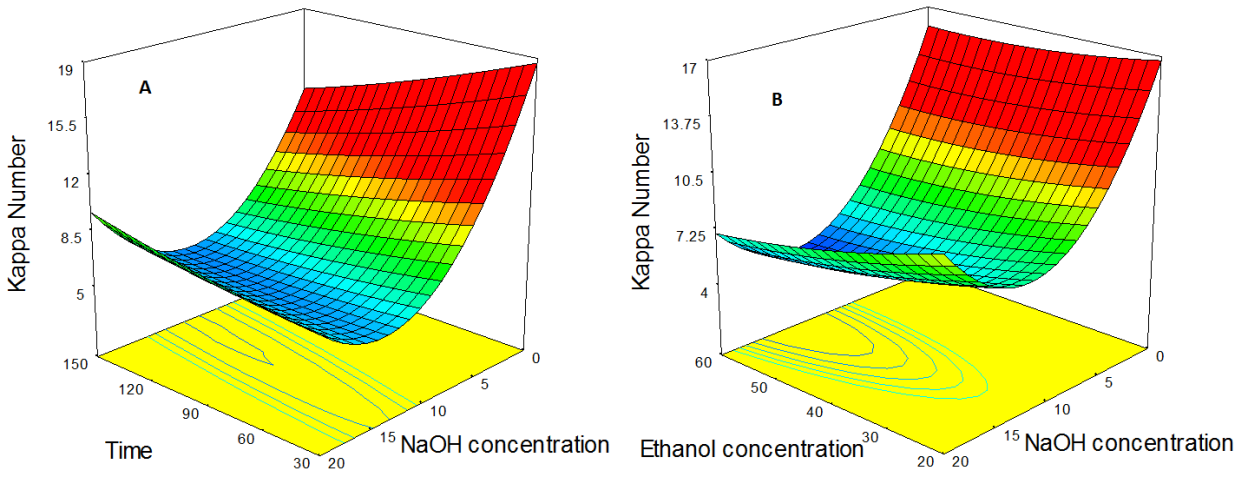

Figure 4. 8: *Response surface plot (A) interaction of time (min) and alkali concentration (%) on pulp Kappa number (B) interaction of ethanol concentration (%) and alkali concentration (%) on pulp Kappa number*

Furthermore, kappa number is observed to be positively correlated with total yield and yellowness and negatively correlated with the whiteness of the paper (Figure 4.9) since the presence of lignin increases the yellowness of pulp and paper.

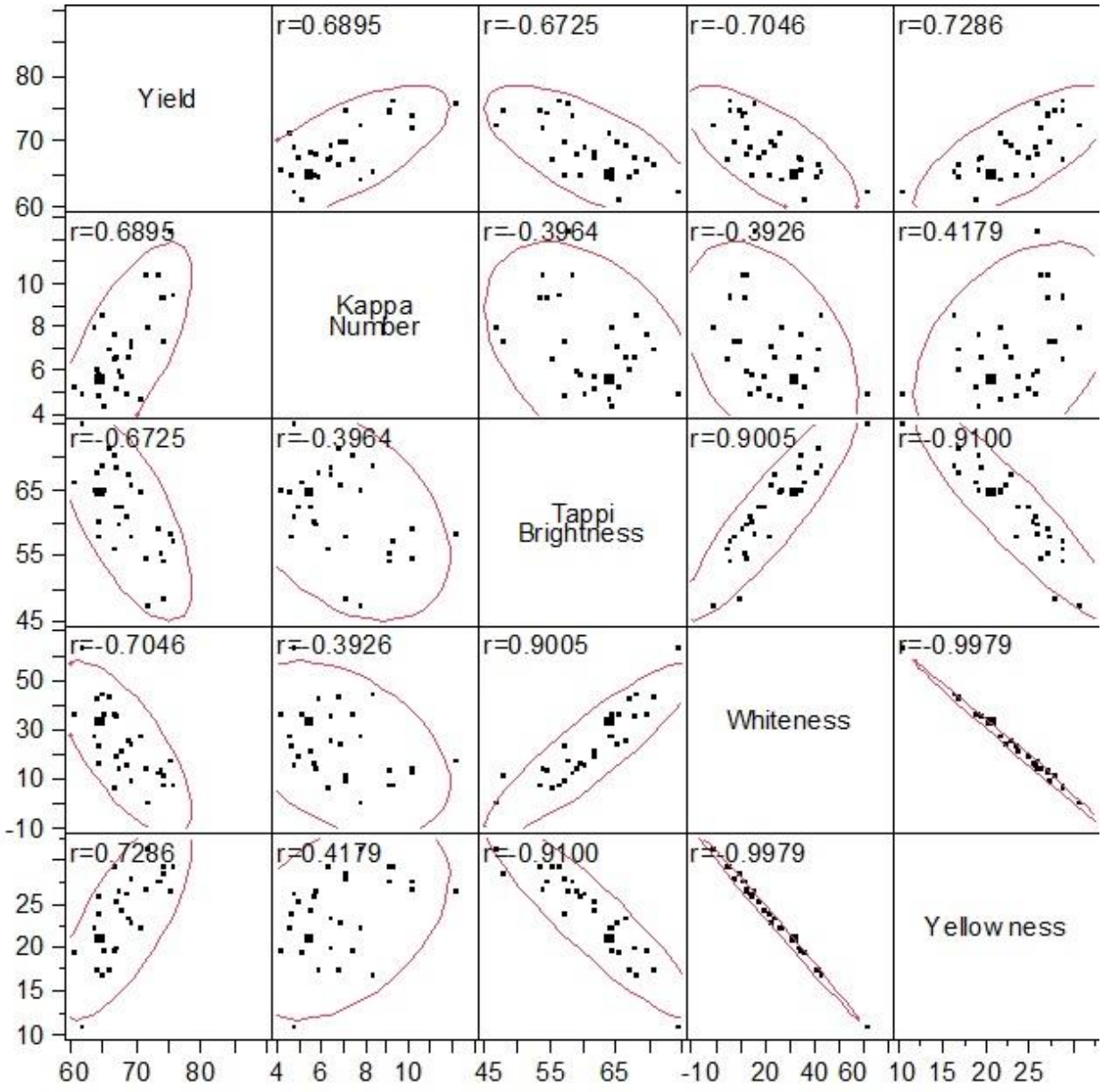

Figure 4. 9: *Scatter plot matrix of multivariate correlations between responses*

4.3.2.3.Brightness

Brightness was significantly affected by pulping temperature, ethanol concentration, and alkali concentration, as evidenced by the value of the coefficients concerning variables Y_{Bt} (equation 4). Pulping temperature negatively affects brightness of the paper (Figure 4.10 A and B), probably due to condensation of hemicellulose monomers, caramels like formation (De Melo et al., 2017). The interaction of alkali concentration with temperature, time and ethanol concentration has significant effect on brightness. Moreover, time and alkali concentration have a significant quadratic effect on brightness (Table 4.3).

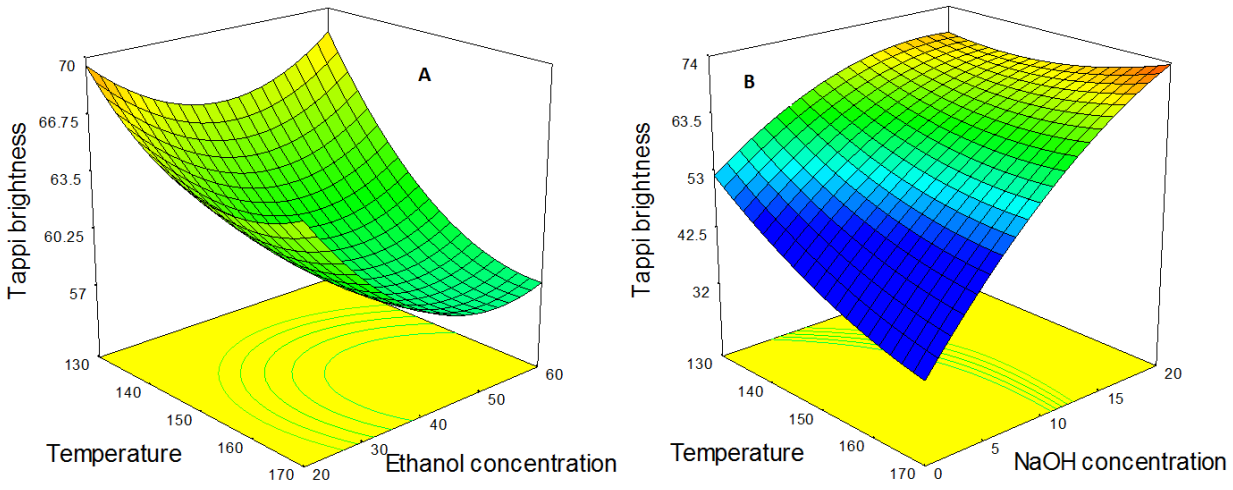

Figure 4.10: *Response surface plot (A) interaction of temperature (^oC) and ethanol concentration (%) on pulp brightness (B) interaction of Temperature (^oC) and alkali concentration (%) on pulp brightness*

Interaction of alkali concentration with temperature and time shows positive and prevailing effect on the pulp brightness (Figure 4.10 B and Figure 4.11 A). Cooking time has a slight and positive effect on pulp brightness at higher alkali concentration.

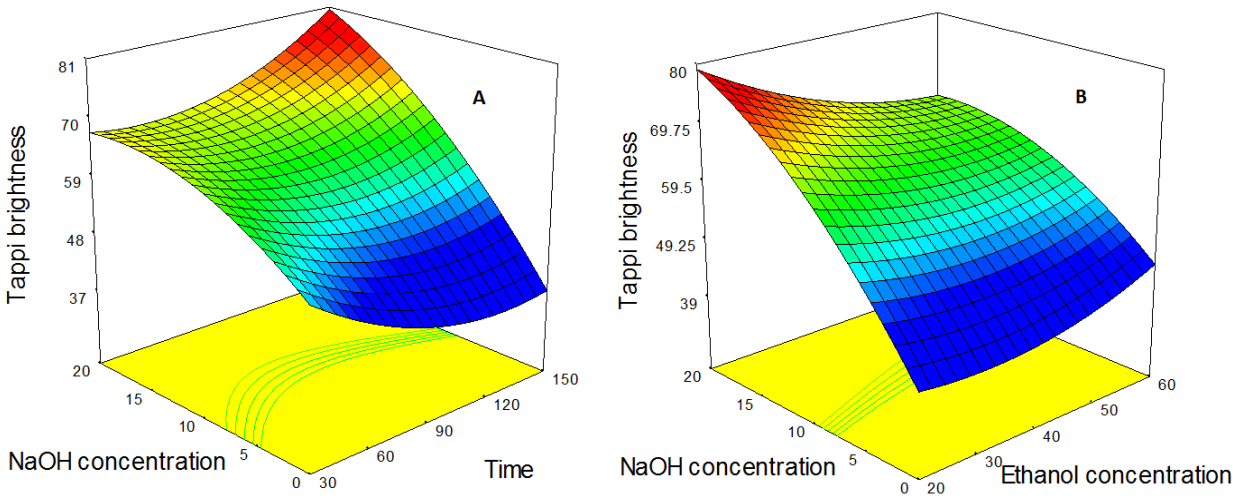

Figure 4. 11*: *Response surface plot (A) interaction effect of alkali concentration (%) and time (min) on pulp brightness B) interaction effect of alkali concentration (%) and ethanol concentration (%) on pulp brightness

The interaction effect of ethanol and alkali concentrations is shown in Figure 4.11 B. The figure reveals that the effect of alkali concentration is dominant compared to that of ethanol concentration, as previously reported (Shatalov and Pereira, 2013).

4.3.2.4.Whiteness and Yellowness

As it is clear from Figure 4.12 (A-F) - 4.13 (A-F), the pulping variables are seen to have opposite effects on whiteness and yellowness. They are inversely correlated responses (99.8%).

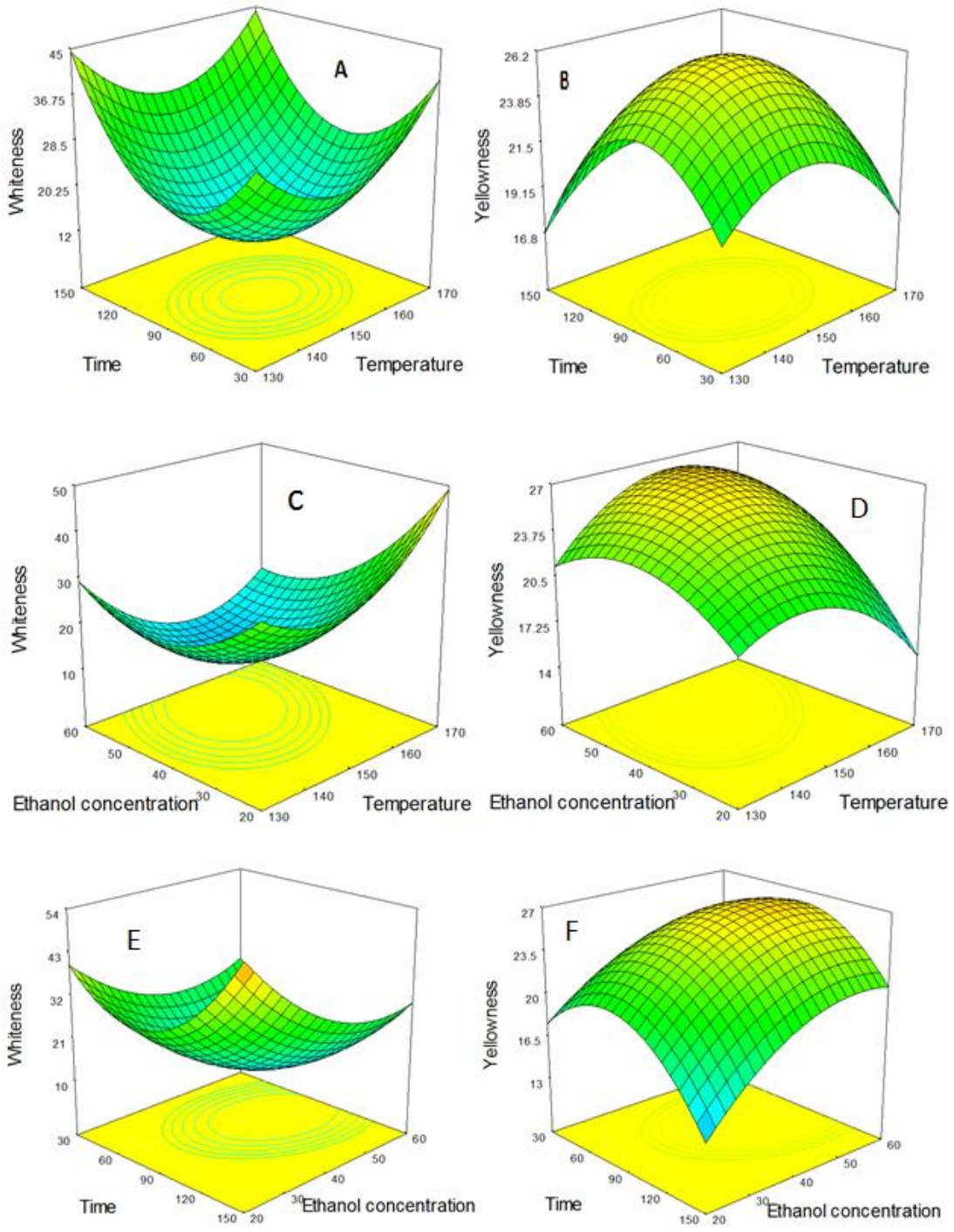

Figure 4. 12: *Response surface plot on interaction effect of time, temperature and ethanol concentration*

Where, Interaction effect of time and temperature on pulp whiteness (A) pulp yellowness(B) interaction effect of ethanol concentration and temperature on pulp whiteness(C) on Pulp yellowness (D) interaction effect of time and ethanol concentration on pulp whiteness (E) on pulp yellowness (F)

Yellowness is observed to be influenced by linear variables (ethanol concentration and alkali concentration) and three quadratic variables such as temperature, time and ethanol concentration (Table 4.3). Increment in alkali concentration has positive effect on whiteness and it has opposite effect on the yellowness of the paper as shown in figure 4.13 (A-F). This is due to degradation of lignin at higher alkali concentration. Yellowness is affected by the interaction effect of alkali concentration with temperature and time (Figure 4.13 B and D).

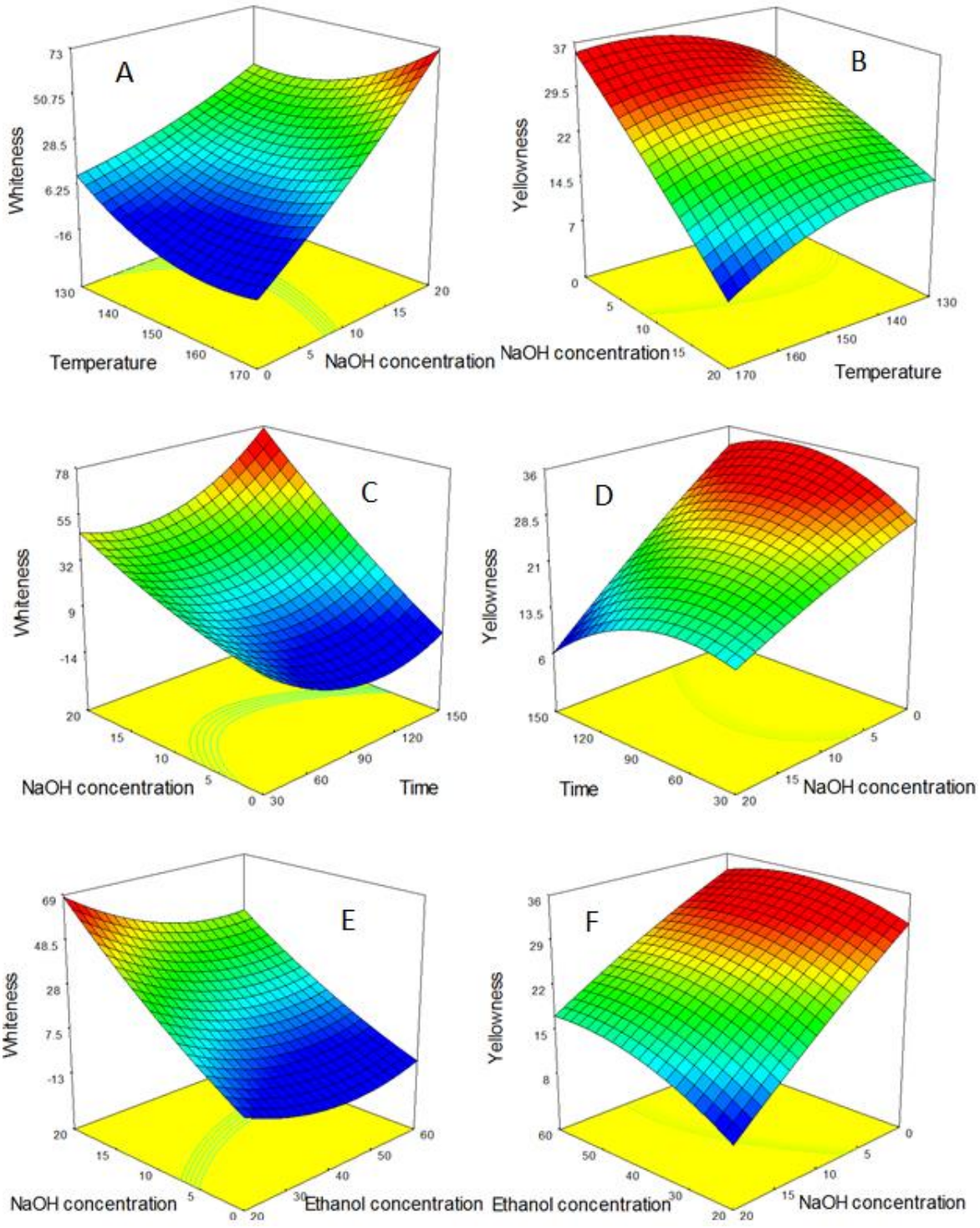

Figure 4. 13: *Response surface plot on interaction effect of alkali concentration, ethanol concentration, time and temperature*

Interaction effect of temperature and alkali concentration on pulp whiteness (A), on pulp yellowness (B), interaction effect of alkali concentration and time on pulp whiteness(C), pulp yellowness (D) interaction of alkali concentration and ethanol concentration on pulp whiteness (E) on pulp yellowness (F)

4.3.3. Optimization of ethanol-alkali pulping

The numerical optimization of ethanol-alkali pulping was performed by Design expert software by setting the desired goal for each factor and responses. The optimum combinations of process variables were selected in order to obtain the highest amount of pulp with low kappa number and acceptable color and brightness.

Accordingly, the maximum pulp yield with lowest kappa number and yellowness was obtained at ethanol concentration and alkaline concentration of 46.4% and 15% respectively, at 140ºC and for a pulping time of 61 min. At these conditions, the pulp yield was predicted to be 69.9% with a kappa number of 4.9, 64.5% brightness, and whiteness and yellowness of 25 and 22.2, respectively. The pulp brightness and whiteness were highest at this optimum point.

To validate this data, duplicated experiments were performed at the optimized condition. The results were found to be consistent with the model.

Table 4. 4: *Comparison of experimental results and model results at the selected optimum point (46.5% ethanol concentration, 15% alkali concentration, 140^{o}C and 61 min)*

	Yield (%)	**Kappa No**	**Brightness**	**Whiteness**	**Yellowness**
Model	69.9	4.9	64.5	25	22.2
Experimental	67.5	5.2	65.2	21.1	21.5

4.3.4. Kraft pulping

Kraft pulping of Enset fiber was done at 160 ^{0}C for 60 min at four different sodium hydroxide and sodium sulfide concentration combination. Maximum pulp yield was obtained at 10% alkali concentration and 10% sulfidity. The yield was 66% with a kappa number of 6.8. Higher brightness and whiteness was obtained at higher alkali concentration (Table 4. 5). The total solid content and relative density of black liquors were found to be 0.04-0.05 g/ml and 1.02-1.03 respectively.

Table 4. 5: *Kraft pulping of Enset fiber at different white liquor composition*

Alkali concentration	**Sulfidity**	**Yields**	**Kappa Number**	**WRV**	**Brightness**	**Whiteness**	**Yellowness**
10%	10%	66.0	6.85	7.0	61.5	17.5	24.1
10%	20%	63.43	10.36	6.2	53.8	16.9	29.9
15%	10%	62.21	4.92	6.7	64.8	26.6	21.1
15%	20%	62.16	3.66	7.4	64.3	25.3	21.6

WRV: *Water retention value of the pulp*

The comparison between Kraft pulping and ethanol-alkali pulping is presented in Table.4.6. The comparative study was done at optimum point of ethanol alkali and at 160ºC, 60 min, 10% active alkali and 10% sulfidity for Kraft pulping. The results show that ethanol-alkali pulping of Enset fibers allowed higher pulp yield and lower kappa number as compared with the kraft pulping (67.5 % vs. 66% yield and 5.2 vs. 6.9, respectively).

Table 4. 6: *Comparative results of Kraft pulping (160 ºC, 60 min, 10% active alkali and 10% sulfidity) and ethanol-alkali pulping at optimum point (140ºC, 61 min, 46.5% ethanol, 15% alkali) of Enset fibers*

	Kraft pulping	**Ethanol-alkali pulping**
Yield (% o.d. fiber)	66	67.5
Kappa number	6.9	5.2
Brightness (%)	64.5	65.2
Whiteness	17.5	21.1
Yellowness	24.1	21.5
Viscosity (cm^3/g)	1392	1158
DP	4992	4021
Burst index ($kPa.m^2g^{-1}$)	18.1	13.6
Tensile index ($N.\ m\ g^{-1}$)	166.7	167.0
Tear index ($mN.\ m^2\ g^{-1}$)	11.8	11.1

DP is degree of polymerization

The brightness, whiteness, and yellowness of the Kraft and ethanol-alkali pulps were almost similar. The viscosity of the ethanol-alkali pulp was slightly lower than that of the Kraft pulp (1158 vs. 1392 cm^3/g respectively). Regarding strength properties, the ethanol-alkali pulps showed comparable burst index, tear index and tensile index to the Kraft pulp. Compared to conventional paper pulps, the Enset fibers produced pulps with high viscosity and strength properties already as unrefined pulp. For instance, refined pulps from eucalypt wood showed a tensile index of 78.2 Nm/g and burst index of 6.2 kPa m^2/g (Miranda et al., 2012) while

unrefined eucalypt pulps showed 780-871 cm^3/g viscosity and 20-30 Nm/g tensile index (Gominho et al., 2016).

4.4.Conclusions

The Enset fibers were delignified by the ethanol-alkali process to produce well delignified, high yield and resistant pulps. The major parameter influencing the ethanol-alkali pulping as regards pulp yield and kappa number was found to be the alkali concentration followed by temperature. The optimum delignification conditions predicted for maximum pulp yield and lowest kappa number were 15% alkaline concentration and 46% ethanol concentration at a temperature of 140^oC and cooking time of 61 min. Under these conditions, the pulp yield was 67.5 (69.9% predicted) with a kappa number of 5.2 (4.9 predicted). The results suggest that valorization of the Enset fibers for production of high-grade pulps is a promising approach deserving further studies.

CHAPTER FIVE

5. Fractionating Enset fiber bundles using hydrothermal pretreatment prior to ethanol-alkali pulping

5.1.Introduction

The structural and chemical features of Enset fiber suggested their use as a fiber source for paper pulp production but the high hemicelluloses content necessitates the application of pre-treatments for their previous removal, mainly by using low chemical input processes such as hydrothermal treatments. The hemicellulose extraction with chemical pulping processes arrangement is one method to generate a sugar feedstock amenable to biochemical transformation to fuels and chemicals (Helmerius et al., 2010; Ruiz et al., 2013).

Hydrothermal treatments at temperatures ranging from 150ºC to 240ºC, also called auto-hydrolysis, are suitable to solubilize hemicelluloses from lignocellulosic materials. Depending on the process severity, it may solubilize oligosaccharides or monosaccharides to the aqueous phase, while the solid phase retains most of the cellulose and lignin (Carvalheiro and Silva-fernandes, 2009; Moniz et al., 2013). The low by-product generation, the limited equipment corrosion due to the neutral pH of the reaction media and the low operational costs are some of the advantages associated with auto-hydrolysis processes (Carvalheiro et al., 2005; Moniz et al., 2015).

Auto-hydrolysis can be considered as self-catalytic process due to the cleavage of acetyl and uronic acid ether substitutions that result in the formation of acetic and other organic acids which further hydrolyze of polysaccharides to oligomers and monomers, it will results in hemicellulose solubilized as a mixture of oligomers and monomers (Helmerius et al., 2010).

Auto-hydrolysis was tested on several residual materials e.g. corncobs (Ruzene et al., 2008), corn straw (Moniz et al., 2013), rice straw (Moniz et al., 2015), eucalypt wood (Ma et al., 2017), brewery's spent grain (Carvalheiro et al., 2005), cistus industrial residues (Alves-Ferreira et al., 2017), spent coffee grounds (Ballesteros et al., 2017) or various lignocellulosic mixtures (Silva-Fernandes et al., 2015). Overall it was considered that auto-hydrolysis could be envisaged as a pre-treatment process for the valorization of those residues and integrated into a bio-refinery approach.

Treatment with alkali at moderate temperatures is a well-established laboratory method for extracting hemicelluloses (Helmerius et al., 2010). Alkali catalyzed hydrothermal processes may be used when a stronger impact on the chemical fractionation is intended (Mcintosh and Vancov, 2011). For instance, alkali treatments may cause a reduction in the polymerization and crystallinity of cellulose, disruption of the lignin structure, and swelling of fibers due to cleavage of hydrolyzable linkages between lignin and polysaccharides (Chen et al., 2013).

In this work, alkali catalyzed and un -catalyzed hydrothermal processes were applied to the Enset fiber bundles obtained as a residue from the scraping of the Enset leaf sheaths. These processes are intended to constitute a pretreatment phase that will be integrated into a stepwise fractionation leading to the full valorization of this Enset residual material. Auto-hydrolysis was performed under isothermal conditions and the effect of temperatures in the range 130ºC-200ºC

on the chemical composition of the liquid and solid phases was assessed. Further, an alkali-catalyzed hydrothermal treatment was also tested using 0.1% NaOH concentration. The structure of the pretreated Enset fiber bundles was observed in view of their subsequent potential as a fiber source for pulp production. The solid residue from alkali catalyzed and un-catalyzed extractions were subjected to subsequent ethanol alkali pulping at optimum point obtained in chapter four and the refined pulps were made into hand sheets. Several strength properties for hand sheet were performed and compared with a reference pulp made without an extraction step.

5.2.Materials and methods

5.2.1. Sampling

Enset fiber bundles were collected from private Enset plantation located around wolkite, south-western Ethiopia at harvest age of the plants for food production. The fibers were obtained as long threads that remained after the manual scraping of the leaf sheaths from the pseudostem by pressing out and collecting the starchy pulp used for food. The fibers were washed with cold water, sun dried and stored in polyethylene bag. Before laboratory processing, the fiber bundle were cut to an approximate length of 5cm.

5.2.2. Pretreatments

Hydrothermal pretreatments of false banana fiber bundles were done with and without alkali catalyst. Each treatment was conducted under isothermal conditions in 100 mL stainless steel digesters, rotated in a mineral oil bath. Un-catalyzed hydrothermal treatment using only distilled water and an alkali-catalyzed hydrothermal treatment using 0.1% reagent grade NaOH (98%) aqueous solution. The treatments were carried out with 5 g fibers (oven-dry basis) with a solid-to-liquid ratio of 1:12 (g of Enset fibers/g of liquor). Factorial experimental design with two factors (Temperature × alkali concentration) under four temperatures level (130 ^{o}C, 150 ^{o}C, 180

^{o}C and 200 ^{o}C) and two alkali concentration level (0% and 0.1%) for 90 min was used as shown in Table 5.1. Upon completion, the reactors were cooled in an ice bath and the contents filtrated to separate the liquid and the solid phases.

Table 5.1: *Experimental design data for alkali catalyzed and un-catalyzed hydrothermal treatment*

Run no	**Temperature ^{o}C**	**Alkali Concentration (%)**
Run-1	130	0
Run-2	150	0
Run-3	180	0
Run-4	200	0
Run-5	130	0.1
Run-6	150	0.1
Run-7	180	0.1
Run-8	200	0.1

The mass percent loss during the pretreatment was calculated as the mass difference between the original and the treated sample reported as a percentage of the original oven-dry sample.

To allow comparison with literature data on hydrothermal treatment under different conditions, the effects of time and temperature of hydrothermal treatment were also interpreted based on the severity factor, log R_0 (Overend and Chornet, 1987):

$$R_0 = \int_0^t \exp\left(\frac{T(t)-100}{14.75}\right)dt$$

where the temperature T (^{o}C) is a function of time t (min), and 14.75 is an empirical parameter related to activation energy and temperature. The severity factors for the tested autohydrolysis

conditions were 2.8, 3.4, 4.3 and 4.9, respectively for the treatments at 130ºC, 150ºC, 180ºC, and 200ºC.

5.2.3. Spent liquor characterization

The spent liquors were characterized by its pH and the monosaccharide composition including neutral sugars, uronic acids, and acetates that were quantitatively determined by High-Performance Anion Exchange Chromatography. The determination was made in the liquors as obtained and after an acid hydrolysis. Acid hydrolysis of the liquor is used to convert soluble hemicelluloses to their constituent sugar monomers. For the hydrolysis, 84 mL of the solution was added to 3 mL of a 72% H_2SO_4 solution and reacted 60 min at 120ºC. The increase in the concentration of sugar monomers after acid hydrolysis as analyzed by High-Performance Anion-Exchange Chromatography corresponds to the amount of oligosaccharides. It is named as oligosaccharides concentration and the hemicellulose-derived oligosaccharides containing xylose and arabinose units were named XOS.

5.2.4. Characterization of the treated solids

The solid samples obtained after the hydrothermal treatments were chemically analyzed after grinding to 40-60 mesh fraction size. The Klason lignin and acid soluble lignin were determined following TAPPI T222 om 98 and TAPPI UM 250 methods respectively. The polysaccharides of the treated solids were calculated as the sum of neutral sugars and uronic acids as well as acetates that were quantitatively determined in the hydrolysis liquor by High-Performance Anion Exchange Chromatography.

5.2.5. FTIR/ATR of treated fibers

A 1 mg of untreated and treated Enset fiber samples were ground with a Retsch MM200 mixer mill for 10 min and dried at 105°C for 24 h. The samples were analyzed in ATR mode FTIR in the range 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans accumulation.

5.2.6. Color measurement of treated fibers

A color spectrophotometer Minolta cm-3630 was used to determine the color parameters L*, a* and b* for the treated Enset fiber by the CIELAB method. The corresponding variations with various treatments were analyzed. Each value corresponds to the average of three replicates.

5.2.7. Scanning electron microscopic image of treated fibers

The treated samples were observed by scanning electron microscopy using a Hitachi-TM3030 Plus tabletop microscope.

5.2.8. Ethanol alkali pulping of treated Enset fiber

Among the pretreated Enset fibers produced by various conditions some of them were selected for further delignification based on the residual yield of solid fraction, the recovery of desirable oligosaccharides and fibrous nature of residual solid fraction. Ethanol alkali delignification of selected pre-treated Enset fibers were carried near optimum point (40% ethanol conc., 15% alkali conc., 140^0C, 60 min) obtained in chapter 4. The untreated sample was also delignified at this point as a reference. All delignification were done using 1:12 solid-to-liquid ratio. The cooking procedure was the same as the procedure stated in chapter 4, section 4.2.2.

The resulting pulps were evaluated for its yield, kappa number, viscosity, several strength properties of hand sheet and color and compared with a reference pulp made without an extraction step. The pulp yield of the ethanol alkali delignified pulp is reported as the oven-dry

mass of the obtained pulp as a percentage of the original oven-dry sample and/or the treated oven-dry sample.

5.3.Results and discussion

5.3.1. Un-catalyzed and alkali catalyzed hydrothermal treatments

The un-catalyzed and the 0.1% NaOH catalyzed hydrothermal treatments of Enset fiber showed a temperature-dependent mass loss. At the lower temperatures of 130^{o}C and 150^{o}C, the mass loss was 9.2% and 19.5% for the un-catalyzed and 12.3 % and 15% for the catalyzed treatment respectively. A substantial mass loss of more than 30% of the initial material occurred upon increasing the temperature to 180^{o}C while a further temperature increase did not induce significant increment of mass loss in all hydrothermal treatments (Table 5.2). The increase in the mass loss is mainly due to the hydrolysis of xylan and other hemicellulose sugars which leads to a significant increase in the glucan content of the treated solids (Table 5.2).

Table 5. 2: *Characterization of Enset fiber bundles after pretreatment (un-catalyzed and the 0.1% NaOH catalyzed hydrothermal treatment)*

% of the initial material	**Run-1**	**Run-2**	**Run-3**	**Run-4**	**Run-5**	**Run-6**	**Run-7**	**Run-8**
Yield	91.8[a]	80.5[b]	64.2[c]	64.2[c]	87.7[a,b]	85.0[a,b]	69.6[c]	66.7[c]
% of treated solid								
Klason lignin	11.8[a,b]	12.5[a,b]	14.4[a]	14.7[a]	7.7[b]	10.9[b]	14.7[a]	14.6[a]
Acid soluble lignin	1.8[a]	1.6 [a]	1.0 [a]	0.9 [a]	1.8 [a]	1.5 [a]	0.9 [a]	1.1 [a]
Arabinose	1.3	0.5	0.0	0.0	0.8	0.9	0.0	0.0
Glucose	50.3	61.9	87.5	88.8	65.4	61.3	67.6	79.3
Xylose	22.9	16.9	2.4	-	8.7	11.8	5.4	2.57
Galacturonic acid	0.6	0.2	-	-	0.4	0.4	-	-
Glucuronic acid	1.4	0.6	-	-	1.0	1.6	0.2	-
Acetyl	-	-	-	-	-	-	-	-

Values in the same raw with different superscripts for each type of analysis are significantly different

The alkali-catalyzed treatment showed a lower mass loss at 150ºC, 180ºC, and 200ºC compared to the un-catalyzed hydrothermal treatment. This should be due to the pH buffering effect of alkali in the reaction medium. In fact, the hydrothermal spent liquor showed an acid pH that decreased with temperature (4.8 at 130ºC and 3.8 at 200ºC) resulting from the hydrolysis of the acetyl groups of hemicelluloses; the alkali-catalyzed treatment partially neutralized the liquor (5.8 at 130ºC and 4.2 at 200ºC), thereby protecting the polysaccharides from a more drastic acid

depolymerization (Table 5.3). The acidification of the hydrothermal spent liquors with increasing reaction severity due to hydrolysis of the hemicellulose acetyl groups was previously reported (Moniz et al., 2013).

Table 5. 3: *The pH and polysaccharide composition (% of initial dry fiber) of spent liquor from un-catalyzed and 0.1% NaOH catalyzed hydrothermal treatment*

Run	pH	Ara	Gal	Glu	Xyl	Gal U	Glu U	Ace acid	XOS	GOS
Run-1	4.8	0.08	0.01	0.04	0.16	0.01	0.00	0.23	0.16	6.92
Run-2	4.3	0.28	0.03	0.02	0.64	0.01	0.00	0.65	0.96	5.41
Run-3	4.0	0.29	0.12	1.00	1.29	0.01	0.04	3.11	1.64	4.70
Run-4	3.8	0.00	0.01	0.81	0.10	0.00	0.01	4.23	0.28	0.29
Run-5	5.8	0.00	0.00	0.00	0.00	0.00	0.00	1.39	0.09	12.4
Run-6	5.3	0.11	0.01	0.01	0.18	0.01	0.00	1.67	0.75	5.41
Run-7	4.5	0.11	0.07	0.12	0.27	0.01	0.05	3.01	3.35	5.48
Run-8	4.2	0.00	0.01	0.20	0.03	0.00	0.01	4.65	0.12	0.62

The increase of mass loss with the severity of the treatment was also reported for the auto-hydrolysis of wheat straw and corn straw, corresponding to the enhancement of hydrolysis and solubilization of the hemicelluloses into the liquid, while the remaining solid retained most of the cellulose and lignin (Carvalheiro and Silva-fernandes, 2009; Moniz et al., 2013, 2014, 2015).

5.3.2. Characterization of the treated solids

The treated Enset fiber was rich in cellulose and lignin as shown by the composition of the solids after the hydrothermal treatments (Table 5.2). This is due to the predominant effect of the hydrothermal treatment on the hemicellulose components and the resistance of cellulose and lignin to depolymyerization under these process conditions.

The treated Enset fibers showed higher Klason lignin content (11.8%-14.7%) than the untreated Enset fibers (8%) (Chapter 3). There is an insignificant lignin solubilization by the hydrothermal treatment and therefore the proportion of Klason lignin increased with severity of the hydrothermal pretreatment, as also described in Moniz et al. 2013, 2014. In addition, the formation of insoluble compounds which quantify as Klason lignin may also occur e.g. lignin condensation with sugar-derived compounds (Moniz et al., 2013, 2014; Lourenco et al., 2017).

The glucan proportion in the treated Enset fibers increased with treatment severity: 50.3 to 88.8% for the un-catalyzed hydrothermal treatment and 65.4 to 79.3% for the catalyzed hydrothermal treatment. Moreover, the acetyl groups were not recognized in both un-catalyzed and catalyzed hydrothermal treated fibers.

FTIR/ATR Spectra of treated and un treated Enset fiber

Figure 5.1 shows the FTIR/ATR spectra from 2000 cm^{-1} to 800 cm^{-1} of the pretreated and the untreated Enset fibers. Spectral changes occurred with the treatment in the line with the chemical changes shown in Table 5.2. The band at 1735 cm^{-1}, assigned to C=O stretching vibrations of the carboxyl and acetyl groups in hemicelluloses (Poletto et al., 2012) decreases with the severity of the treatment and disappears in the material treated at 180ºC.

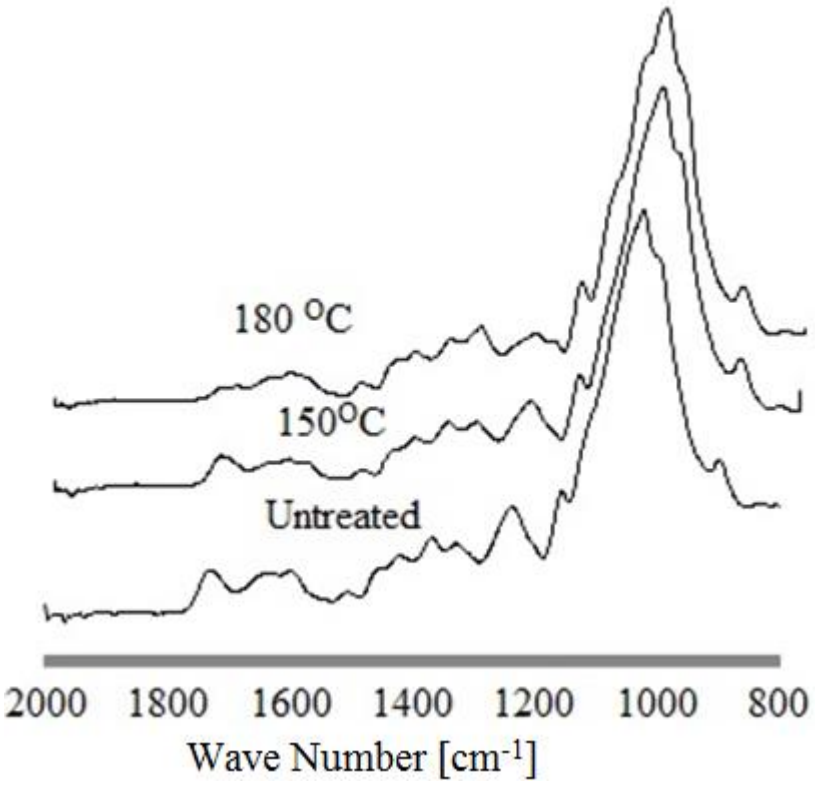

Figure 5. 1: *FTIR-ATR spectra of the untreated Enset fibers and hydrothermal treatment at 130ºC and 180ºC in the range of 2000-800 cm^{-1}*

There is also a decrease in the peak around 1246 cm^{-1} due to the cleavage of acetyl groups of hemicelluloses and solubilization into the liquid phase. On the contrary, the absorption peaks related to lignin and cellulose are present in the treated samples e.g. at 1510 cm^{-1} arising from the aromatic skeletal vibration C=C of the benzene ring, the band at 1420–1430 cm^{-1} related to aromatic skeletal vibrations associated with C-H in-plane deformation of cellulose and at 1327 cm^{-1} related to C=C in lignin (Chen et al., 2010).

Macroscopic Appearance of treated fiber

The macroscopic appearance of the treated solids is shown in Figure 5.2. It is clear that the fibrous bundle nature of the material is retained in all the cases. Nevertheless, at the higher

treatment intensity, temperature of 200ºC, the formation of smaller dimensional material could be observed that upon filtration built an underlying cake.

Figure 5. 2: *Macroscopic appearances of the Enset fiber bundles after the un-catalyzed hydrothermal treatment and alkali-catalyzed (0.1% NaOH) hydrothermal treatment at 130ºC, 150ºC, and 200ºC*

SEM image of treated fiber

The observation of the treated solids by scanning electron microscopy (Figure 5.3) showed that the fibrous bundles of the material were preserved. In the more severe treatment conditions that led to the formation of material with smaller dimensions that sedimented first upon filtration

(Figure 5.2), it could be observed that cellular dissociation occurred to some extent and the long fibers that compose the Enset fibrous bundles making a loose network.

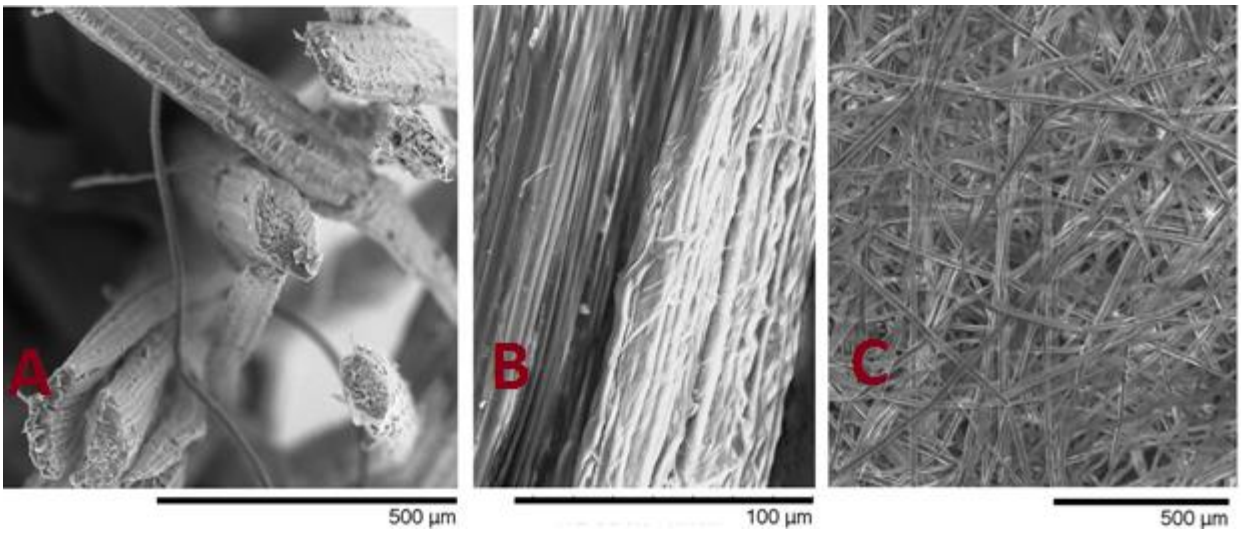

Figure 5.3: *SEM observations of the Enset fiber bundles after the un-catalyzed treatment at 130ºC (A) and at 150ºC (B) and the filter cake after the alkali-catalyzed (0.1% NaOH) hydrothermal treatment at 200ºC (C)*

Color change

The color changes of the solids after both un-catalyzed and catalyzed hydrothermal treatments could be macroscopically observed Figure 5.2 and are shown in Figure 5.4 regarding the reflectance spectra and in Figure 5.5 regarding the L*, a*, and b* parameters. Increasing the temperature from 130 ^{o}C to 200 ^{o}C in both treatment methods negatively affected the brightness of the treated solid. When the severity increased from log R_o = 2.8 to log R_o = 4.9, L* decreased from 65.1 to 32.3 in un-catalyzed and from 64.0 to 34.1 in the catalyzed hydrothermally treated materials. There was an increase in a* from 5.6 to 7.6 for the un-catalyzed treatment at 130ºC and 200^{o}C respectively and a small decrease in b* from 18.7 to 15.4 for the un-catalyzed

treatment at 130^{o}C and 200^{o} C respectively. The darkening of lignocellulosic samples with higher temperature of hydrothermal treatment is resulting from cleaved carbohydrate condensation reaction and products of the Maillard reaction formed by the degradation of sugars (De Melo et al., 2017).

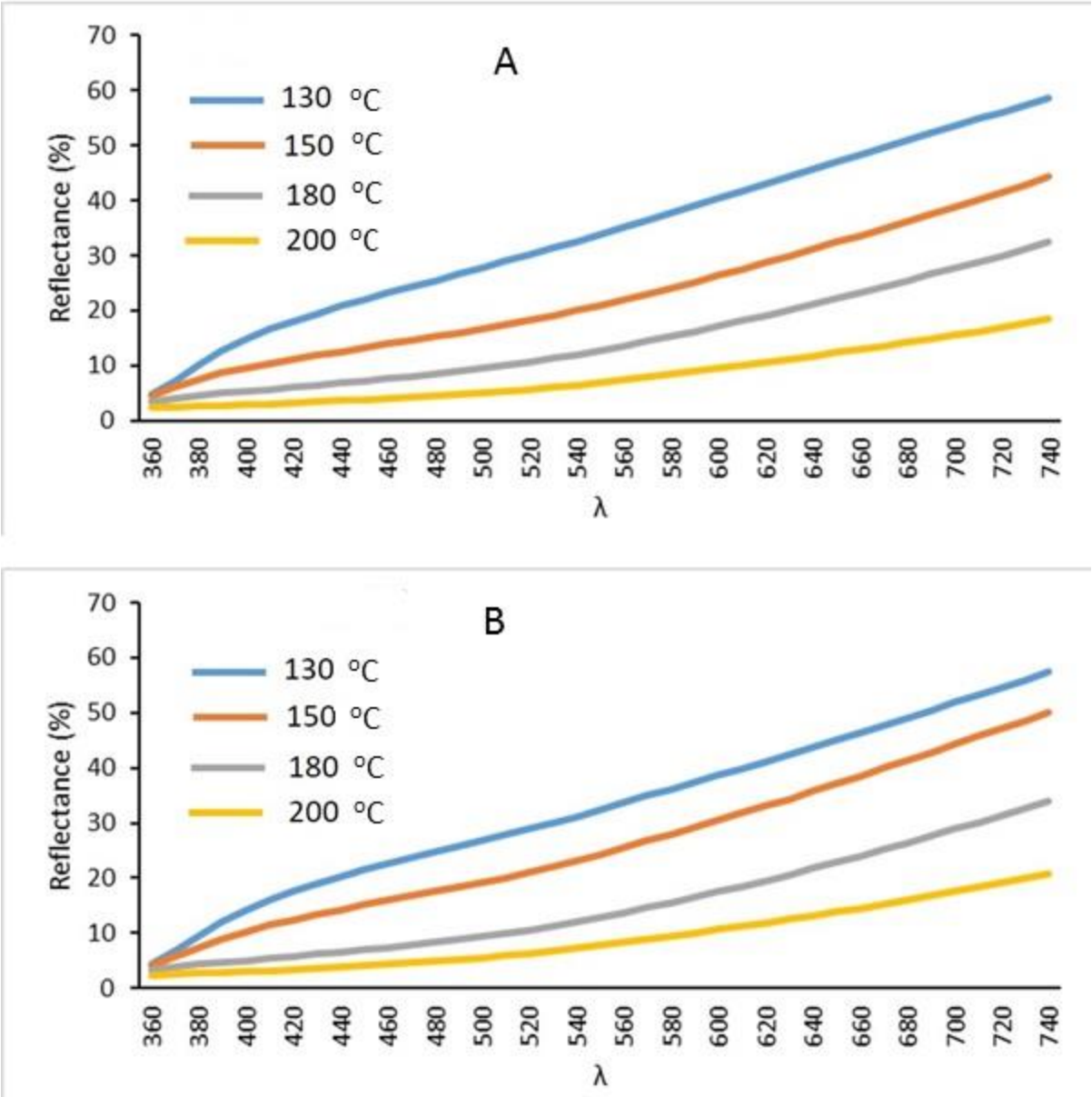

Figure 5.4: *Reflectance spectra of the Enset fibers obtained after un-catalyzed (A) and alkali-catalyzed(B) hydrothermal treatment at 130ºC, 150ºC, 180ºC, and 200ºC*

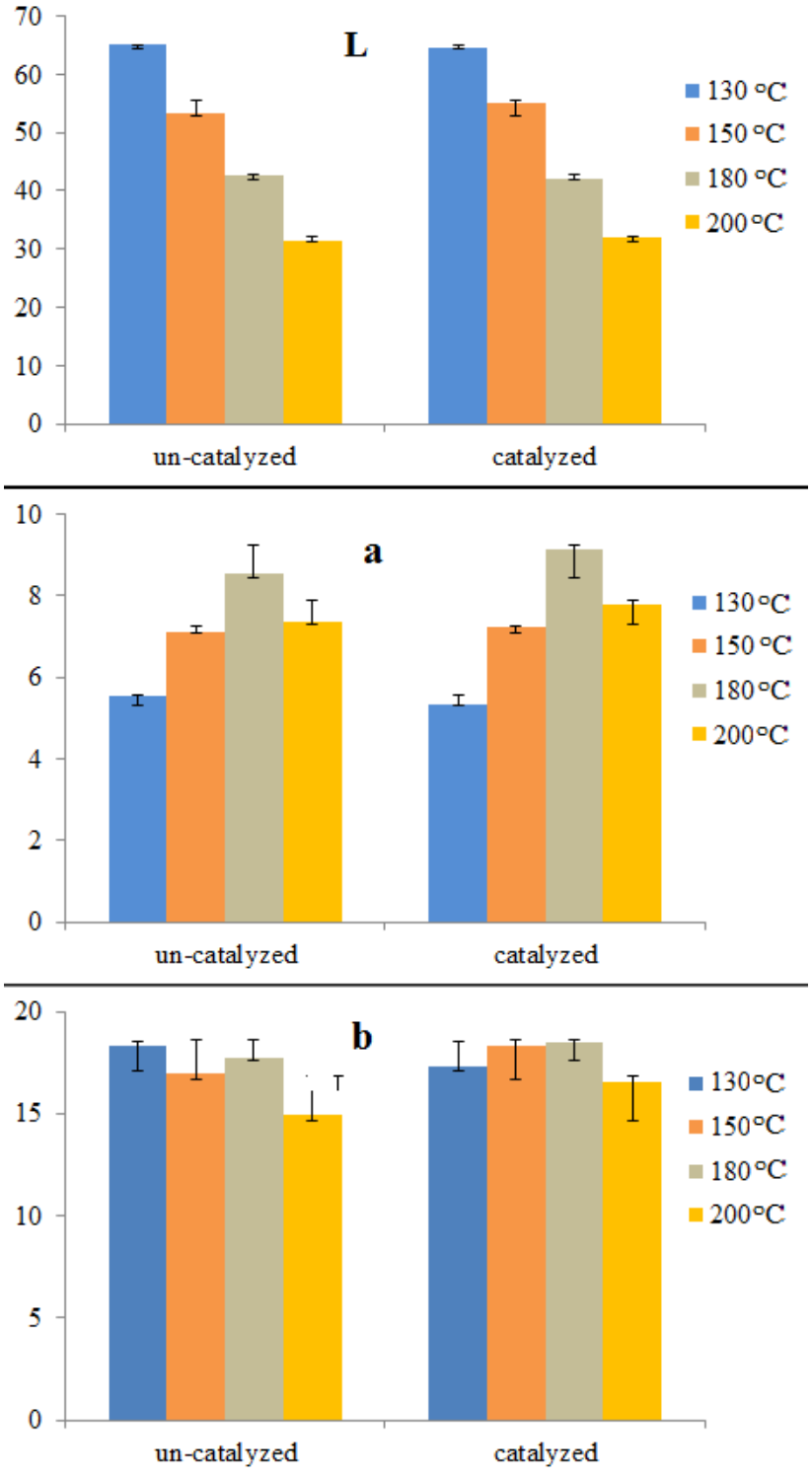

Figure 5. 5: *CIE LAB color parameters (L*, a*, b*) of the Enset fibers obtained after un-catalyzed and alkali-catalyzed hydrothermal treatment at 130ºC, 150ºC, 180ºC, and 200ºC*

5.3.3. Characterization of the spent liquors

The sugar compositions of the liquors obtained after un-catalyzed and alkali-catalyzed hydrothermal treatment are summarized in Table 5.3 that includes the determinations made directly on the liquor and after an acid hydrolysis. The comparison of the results obtained before and after hydrolysis allows the estimation of the monosaccharides and oligosaccharides present in the liquid.

The liquor pH decreased with the severity of the treatment from 4.8 to 3.8 for un-catalyzed and 5.8 to 4.2 for alkali-catalyzed hydrothermal treatments. The decrement in pH value is due to the hydrolysis of the hemicellulose acetyl groups which is higher under the more severe conditions, as seen by the concentrations of acetic acid in the liquor (Table 5.2).

In both un-catalyzed and catalyzed hydrothermal treatments, the liquor contained small amounts of monosaccharides and the main released unit was acetic acid. The concentration of acetic acid increased with the severity factor and corresponded to 4.2% and 4.7% of the Enset fibers in the liquid of the un-catalyzed and catalyzed treatments respectively. Xylose amounts was increased with temperature until 180^{o}C to 1.3% for the un-catalyzed treatment; after that, the xylose concentration declined sharply due to its degradation to furfural at higher temperatures (Carvalheiro and Silva-Fernandes 2009; Moniz et al. 2014). The composition of the liquor after hydrolysis shows that all monosaccharides increased, therefore meaning that carbohydrates were present in the liquor as oligomers. The concentration of monomeric compounds was always low which makes auto-hydrolysis an adequate method for oligosaccharides production (Moniz et al., 2013).

Oligosaccharide concentration sharply decreased upon severity increment above 180^{o}C in both treatment methods (Table 5.3). The highest oligomer concentration was of glucose (GOS), this may be due to cleavage of glucomannan hemicellulose and starch residue. In the un-catalyzed hydrothermal treatment, GOS decreased with the temperature from 6.92 g/100 g of the initial sample at 130ºC to 4.70 g/100 g of the initial sample at 180ºC, and then sharply decrease to 0.29 g/100 g at 200ºC (Table 5.3). A similar trend occurred in the catalyzed treatment where GOS decreased from 12.44 g/100 g at 130ºC to 5.48 g/100 g at 180ºC and drastically decrease to 0.62g/100g at 200ºC (Table 5.3).

The highest xylose oligomer (XOS) content was obtained by the alkali-catalyzed hydrothermal treatment performed at 180 ^{o}C, corresponding to a severity factor of log R_o = 4.3, yielding 3.35 g/100 g of the original sample. The maximum XOS recovery in the un-catalyzed hydrothermal treatment was 1.7 g/100 g of the original sample also at log R_o = 4.3. When the severity increased to 4.9, there was a sharp decrease in the recovery of XOS and other oligomers due to their depolymerization and the monosaccharides degradation reactions.

The recovery of the glucose oligosaccharide was higher at lower severity. The highest GOS amount was recovered in the alkali-catalyzed hydrothermal treatment done at 130^{o}C (log R_o = 2.8) yielding 12.44 g/ g of original dry sample. The maximum GOS recovery in the un-catalyzed hydrothermal treatments was 6.9 g/g of original dry sample performed at log R_o = 2.8.

5.3.4. Ethanol-alkali pulping of treated Enset fiber

Subsequent ethanol alkali pulping was done on the treated sample using un-catalyzed and alkali catalyzed hydrothermal treatment at a temperature of 130^{o}C, 150^{o}C and 180^{o}C as shown in table 5.4. The treated sample at 200^{o}C was rejected because the resulting solid residue loses its fibrous nature (Figure 5.2).

Table 5.4 shows the effect of catalyzed and un-catalyzed pretreatment on the ethanol alkali pulp yield and kappa number. It is observed that the pre-extracted Enset fiber found to have lower yield and kappa number than the control sample in all cases.

Table 5. 4: *Yield and Klason lignin content of ethanol-alkali pulp of treated Enset fiber near optimum point (40% ethanol conc., 15% alkali conc., 140^{0}C, 60 min)*

Treatment condition	**Yield per treated sample (%)**	**Yield per original sample (%)**	**Klason lignin**
130^{0}C/un-catalyzed	64.1	58.8	4.8
150^{0}C/un-catalyzed	62.1	50.0	4.3
180^{0}C/un-catalyzed	76.6	49.4	3.8
130^{0}C/Catalyzed	69.6	61.03	4.4
150^{0}C/Catalyzed	70.6	60.0	4.1
180^{0}C/Catalyzed	66.9	46.1	4.2
Original sample	-	69.1	5.0

The viscosity of treated Enset fiber pulp was higher in both catalyzed and un-catalyzed treatment (1290 cm^{3}/g-1318 cm^{3}/g) than the original sample ethanol alkali pulp (1158 cm^{3}/g). This is due

to the removal of low viscosity hemicellulose and enhances the ratio of cellulose in the material (Hamzeh et al., 2013; Walton et al., 2010).

5.4.Conclusions

The potential towards a further chemical fractionation of the material e.g. targeted to a cellulose-based utilization is also discussed and integrated into a bio-refinery approach. The overall aim is to strengthen the Enset crop value chain by contributing to a full resource use and valorization of an endogenous crop relevant to the local economy.

Un-catalyzed and alkali-catalyzed hydrothermal processing of Enset fibers mainly cause solubilization of oligosaccharides that may be recovered from the liquors as the major products. The maximum recovery of oligosaccharides was achieved with an alkali-catalyzed hydrothermal treatment at 130ºC for GOS (12.4 g/100 g of original dry sample) and at 180^{O}C for XOS (3.4 g/100 g of initial material). Hence, hydrothermal treatments possibly will be applied as a first fractionation step in the valorization of Enset fibers followed by subsequent use of the solid as a fiber source for pulping.

This study demonstrated that alkali catalyzed and un-catalysed hydrothermal treatment can be utilized to extract hemicellulose sugar in the form of oligosaccharide and monosaccharide from Enset fiber prior to ethanol alkali pulping without significant decrement of the pulp yield and paper strength properties.

CHAPTER SIX

6. Conclusions and Recommendations

6.1.Conclusions

The objective of this dissertation was to assess the possible ways for valorization of Enset /*Ensete ventricosum/* residues as a raw material for pulp and paper industry. In this regard, chemical and morphological characteristics of Enset residue, an abundantly existing plant Ethiopia, have been investigated. The result showed that, the residues (the fiber bundle and inflorescence stalk) have different valorization potential.

The structural and chemical characteristics of Enset fiber bundle clearly show that fiber bundle, possess the characteristics needed for paper pulp production. The study shows Enset fiber bundle have long fiber length, thin wall thickness and large lumen diameter which are desirable to produce good quality paper. The derived values of Enset fiber bundle are also comparable with other promising lignocellulosic fiber sources. In addition, the chemical characteristics of fiber bundle were found to have high polysaccharide content with low lignin content compared to other wood and non-wood fiber sources. Thus, this biomass could be viewed as a potential source of cellulose for the production of cellulose derivatives. It is therefore, evident that use of Enset fiber bundle as paper pulp raw materials can bring about both environmental and economic benefits. In other case, the inflorescence stalk can be used as food and fodder since it has nutritional value.

Optimization of process conditions of ethanol alkali pulping was done using response surface methodology, central composite design. The individual and interaction effect of process conditions such as temperature, time, alkali concentration and ethanol concentration on the responses such as yield, kappa number, brightness and color parameter were assessed. The optimum process condition was cooking temperature of 140^{o}C for 61 min with 46% ethanol concentration and 15% alkali concentration. At this point the pulp yield was found to be 67.5% with a kappa number of 5.2. Comparison was made with conventional kraft pulping. Kraft pulping was performed with different liquor composition. The highest yield with desirable kappa number was obtained at 10% alkalinity and 10% sulfidity at temperature of 160^{0}C for 60 min. At this point the pulp yield was found to be 66% with kappa number of 6.9. Various pulp quality parameters were also evaluated at this point and the comparison shows that pulp obtained from ethanol alkali method is comparable with kraft pulp regarding pulp yield, kappa number, brightness, strength and viscosity. Based on CSA report on pulp and paper import and annual Enset plant production in integration with the obtained pulp yield, it can be estimated that paper production from Enset fiber can cover more than 50% of the country demand.

The hydrothermal treatments can be applied as a first fractionation step in the valorization of Enset crop residue, allowing the subsequent use of the solid as a fiber source for pulping. The hemicellulose of the fiber bundle is mostly highly acetylated xylans. The recovery of this monomeric sugar was evaluated using un-catalyzed and alkali-catalysed hydrothermal processing. Temperature-dependent mass loss was observed in both un-catalyzed and alkali catalyzed hydrothermal treatment due to solubilization of oligosaccharides. The maximum recovery of oligosaccharides was achieved with an alkali-catalyzed hydrothermal treatment. The

fibrous nature is preserved after the pretreatment. Hence, hydrothermal treatments possibly will be applied as a first fractionation step in the valorization of Enset fibers followed by subsequent use of the solid as a fiber source for pulping.

In this regard, the use of Enset agricultural residues in pulping and papermaking is highly desirable because it adds value on the residual material and become additional income for the farmers. Moreover, this natural fiber has excellent physical and mechanical properties and can be utilized more effectively in the production of quality pulp for paper.

6.2.Recommendations for further study

This research work has met its objectives stated in chapter one. However, further work regarding economic analysis and technical issues might be necessary to utilize Enset fiber for paper production in industrial scale. The following points are recommended for future work.

a) Economic evaluation

Huge amount of Enset fiber are dispersed throughout southern and south western part of Ethiopia. Collection of this huge residue will be the crucial issue when large scale production is planned. By taking the aforementioned point in to consideration, economical evaluation has to done prior to implementing this study.

b) Recovery Technique

Due to environmental concerns of commercial delignification technologies, the environmental friendly ethanol-alkai pulping was employed. In this research work, ethanol alkali pulp was found to be comparable to kraft pulp regarding its yield and pulp quality parameters. However, the chemical recovery issues of this process need to be solved.

c) Purification of hemicellulose sugar

Fractionation of Enset fiber prior to ethanol alkali pulping enables to obtain cellulose and lignin in solid phase and hemicellulose sugars in liquid phase. There are several strategies followed to purify hemicellulose sugar from the liquid phase. These strategies should be evaluated for their technological and economic feasibility prior to implementation of bio-refinery frame work.

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Appendices

Appendix-A: List of Publications

The following original papers have been published and/or submitted to different journals as part of this dissertation.

Berhanu, H., Kiflie, Z., Miranda, I., Lourenco, A., Ferreira, J., Feleke, S., Yimam, A., Pereira, H., 2018. Characterization of crop residues from Enset */Ensete ventricosum/* in Ethiopia in view of a full resource valorization. PLoSONE 13(7).

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Berhanu, H., Kiflie, Z., Feleke, S., Yimam, A., 2016. Chemical and Morphological Analysis of Enset */Ensete ventricosum/* fiber, leaf, and Pseudo stem. Lignocellulose. 5(2), 139-151.

Berhanu. H., Kiflie, Z., Feleke, S., Yimam, A., Pereira, H., 2018. Un-catalyzed and alkali catalyzed hydrothermal pretreatments of Enset *(Ensete ventricosum)* fiber. Biomass and biorefinery (Under Review)

Appendix B: Photographic Image of Enset */Ensete ventricosum/* Plant

A: Private Enset plantation located in Gurage zone, Wolkite, Ethiopia

B: Manual scraping of Enset leaf sheaths for separation of Enset fiber (decortication)

C: Enset fiber after manual separation

Appendix C: Photographic image of Laboratory equipment used

Extractives content determination

Hand sheet paper making

Minolta CM 3630 (d/0º) spectrophotometer

Tearing Strength Measuring Device

Burst Streangth Measuring Device

Appendix D: Photographic image of pulp (A) and paper (B) produced from Enset fiber

Appendix E: Peak identification, retention time, origin (C-carbohydrate, S-syringyl, G-guaiacyl, H-hydroxyphenyl, NPS-undetermined phenolic, P-protein) and quantification (% of total area of the chromatogram) of the pyrolysis products from Enset fiber bundle and inflorescence stalk (stalk main and stalk fines).

Peak	RT	Compound	Origin	Fiber	Stalk main	Stalk fines
1	5.82	2-oxo-propanal	C	4.6	4.6	5.6
2	7.11	Methyl vinyl ketone	C	0.67	0.50	0.61
3	7.18	Butane-2,3-dione	C	1.4	1.9	1.8
4	8.14	Hydroxyacetaldehyde	C	7.3	11.0	11.9
5	8.73	But-(E)-2-enal	C	0.70	0.86	0.90
6	9.08	Acetic acid	C	8.4	3.3	2.2
7	10.06	1-hydroxy-propan-2-one (acetol)	C	2.0	6.2	5.3
8	10.25	Toluene	NPS	0.23	0.79	0.58
9	10.72	Ethendiol	C	0.53	0.49	0.70
10	12.64	Ethane-1,2-diol	C	0.89	-	0.18
11	12.86	3-hydroxypropanal	C	2.5	1.9	1.6
12	13.82	2-oxo-3-en-butanal	C	0.44	0.45	0.56
13	13.82	3-furaldehyde	C	0.44	0.45	0.56
14	14.19	3-oxo-2-ol-butanal	C	1.0	2.4	2.3
15	14.19	Butandial	C	1.0	2.4	2.3

16	14.76	Furfural	C	1.0	0.88	0.76
17	14.76	2-cyclopenten-1-one	C	1.0	0.88	0.76
18	16.01	Furfuryl alcohol	C	0.31	0.82	0.79
19	17.49	4-cyclopentene-1,3-dione	C	0.21	0.28	0.24
20	17.64	Similar to 4-cyclopentene-1,3-dione	C	0.29	0.31	0.27
21	18.46	2-hydroxy-2-cyclopenten-1-one	C	1.4	3.1	2.8
22	19.2	Dihydro-methyl furanone isomer	C	0.67	0.82	1.05
23	19.45	Unidentified sugar-derived	C	0.12	0.12	0.11
24	19.45	5-methyl-2-furaldehyde	C	0.12	0.12	0.11
25	19.53	Sugar derived (m/z 55, 86, 114)	C	0.50	0.15	0.15
26	20.24	Dihydro-2(3*H*)-furanone (butyrolactone)	C	0.54	0.45	0.37
27	20.62	(5*H*)-furan-2-one	C	0.54	1.1	1.0
28	21.39	4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one	C	1.2	0.25	0.55
29	21.37	3-methyl-furan-2,5-dione	C	-	0.25	-
30	21.84	(2*H*)-pyran-2-one	C	0.26	0.19	0.22
31	22.15	2-hydroxy-3-methyl-2-	C	-	1.3	1.0

		cyclopenten-1-one				
32	22.21	Methyl-dihydro-(2*H*)-pyran-2-one	C	0.46	-	-
33	22.28	2-hydroxy-1-methyl-1-cyclopentene-3-one	C	1.1	0.22	0.20
34	23.36	Phenol	H	0.36	0.67	0.42
35	24.16	Guaiacol	G	0.15	0.24	0.10
36	25.96	Not identified sugar (overlapped spectra)	C	1.0	1.1	1.2
37	26.79	4-methylphenol (p-cresol)	H	0.18	0.36	0.21
38	26.88	3-methylphenol (m-cresol)	H	0.09	0.14	0.16
39	27.35	5-hydroxymethyl dihydrofuran-2-one	C	0.36	1.2	1.3
40	28.07	Levoglucosenone	C	0.10	-	-
41	28.32	4-methylguaiacol	G	0.05	-	-
42	28.59	Unidentified sugar-derived	C	0.40	1.7	1.3
43	28.72	2,4-dimethylphenol (2,4-Xylenol)	H	0.09	0.21	0.12
44	28.91	3,5-dihydroxy-2-methyl-(4*H*)-pyran-4-one	C	0.09	-	-
45	30.46	3-ethylphenol	H	0.17	0.18	0.09
46	30.74	Benzoic acid	NPS	0.09	-	-

47	32.01	Sugar derived (m/z 43, 57, 69, 82, 85, 96, 116)	C	2.9	-	2.0
48	32.20	Similar to dihydro-6-methyl-2H-pyran-3(4*H*)-one	C	0.35	0.81	0.78
49	33.33	1,4:3,6-dianhydro-α-D-glucopyranose	C	0.43	0.62	0.62
50	33.66	Sugar-derived (m/z 43, 57, 60, 68, 73, 86)	C	0.18	0.99	0.65
51	34.15	2,3-dihydrobenzofuran (coumaran)	H	1.0	0.61	0.34
52	34.15	4-vinylguaiacol	G	1.0	0.61	0.34
53	35.84	5-hydroxymethylfurfural	C	0.85	0.22	0.61
54	35.84	Similar to 4-allylphenol	H	0.21	-	-
55	36.38	Syringol	S	0.24	0.11	0.09
56	36.38	Unidentified sugar-derived	C	0.24	0.46	0.35
57	36.63	Indole	P	-	0.22	0.11
58	37.32	Dihydro-4-hydroxy-2-(3*H*)-furanone	C	-	0.47	0.61
59	38.68	2-hydroxymethyl-5-hydroxy-2,3-dihydro-(4*H*)-pyran-4-one	C	1.1	0.66	1.5
60	39.64	Methylindole	P	-	0.09	0.07

61	39.66	1,5-anhydro-arabinofuranose	C	0.71	0.37	0.30
62	40.02	4-methylsyringol	S	0.12	0.04	0.07
63	40.47	Vanillin	G	0.17	0.10	0.06
64	40.95	1-(4-hydroxy-3-methoxyphenyl)propyne	G	0.07	-	-
65	41.25	4-hydroxybenzaldehyde	H	0.04	0.18	0.07
66	41.36	1-(4-hydroxy-3-methoxyphenyl)propyne	G	0.08	-	-
67	45.11	4-vinylsyringol	S	0.34	0.18	-
68	45.78	4-allylsyringol	S	0.15	0.03	-
69	46.54	P-coumaric alcohol	H	0.11	-	-
70	47.69	*Cis*-4-propenylsyringol	S	0.25	-	-
71	49.02	4-propinylsyringol	S	0.09	-	-
72	49.31	1,6-anhydro-β-D-glucopyranose	C	17.1	5.4	11.4
73	49.82	*Trans*-4-propenylsyringol	S	-	0.10	-
74	51.01	Syringaldehyde	S	0.31	0.03	-
75	52.86	Unidentified	-	1.2	0.1	-
76	53.34	Acetosyringone	S	0.09	-	-
77	54.65	*Trans*-coniferaldehyde	G	0.09	-	-
78	54.78	Syringylacetone	S	0.08	-	-
79	55.84	Propiosyringone	S	0.02	-	-

80	56.33	Syringyl vinyl ketone	S	0.01	-	-
81	62.89	*ztrans*-sinapaldehyde	S	0.11	-	-
Total carbohydrates				67.4	61.8	69.5
Total lignin				6.1	4.6	2.7
H				2.3	2.3	1.4
G				1.6	0.9	0.5
S				1.8	0.5	0.2
S/G				1.1	0.5	0.3
H:G:S				1:0.7:0.8	1:0.4:0.2	1:0.4:0.1