Chemical and Morphological Analysis of *Enset (Ensete Ventricosum)* Fiber, Leaf, and Pseudo stem

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This work investigates the suitability of enset plant (Ensete Ventricosum) residues (fiber, leaf and inner part of pseudo stem) for use in paper pulp preparation through morphological, chemical, and FTIR analysis. The morphological analysis showed that the enset fiber have long fiber length (1.66 mm), tinny cell wall thickness (2.88 µm), large lumen diameter (25.87 µm) and thick fiber width (28.48 µm) compared to hard woods, agricultural residues, and bagasse. The runkel ratio of enset was found to be 0.223, indicating thin fiber walls, which are desirable for high quality paper production. The chemical analysis revealed that among the enset residues the fiber showed the highest cellulose (69.51%) and the smallest lignin (5.7%) contents while the leaf showed the smallest cellulose (37.96%) and the highest lignin (18.93%) contents. The leaf also showed highest extractive content (19.09%) compared to other enset residues. The difference in functional groups among enset residues was investigated using FTIR analysis. The high extractive and lignin content in enset leaf was associated with more intense band at 2920, 2850, 1734, and 1637 cm⁻¹. The results show that enset residue can be promising raw material for pulp and paper industry.

Keywords: Ensete ventricosum; Cellulose; lignin; Fiber dimensions; derived value; FTIR

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INTRODUCTION

Enset /Ensete ventricosum/ belongs to the order Scitamineae, the family Musaceae, and the genus *Ensete*. It is usually known as "false banana" due to its similarity to single-stemmed banana plant. However, *enset* is larger than banana plant. It is reported that the height of *enset* plants reach up to 10 meters but most of domesticated *enset* plants have heights of 4 meters to 6 meters and with the pseudo stem up to one meter diameter. In addition, the leaves are more erect than those of a banana plant and have the shape of a lance head (Brandt et al. 1997). In spite of the extensive distribution of wild *enset* in the tropical belt, it is only in Ethiopia that the plant has been domesticated. More than 20 percent of the populations in southern parts of Ethiopia depend on *enset* for food, fiber, fodder, construction materials and medicines (Ayele & Sahu 2014; Gabel & Karlsson 2013).

In Southern parts of Ethiopia, domestic *enset* is primarily grown to produce a starchy food from pseudo stem and corm (Gabel & Karlsson 2013). Different types of residues are disposed commonly during food preparation of *enset*. The fiber, the leaf and inner part of pseudo stem are the main solid residues which are not utilized for *enset*

based foods preparation. The *fiber*, with a hair like structure, locally called "kacha" is collected after scarping of the leaf sheath and leaf bases around the pseudo stem. The inner part of the pseudo stem is simply discarded as waste. These residues are abundant natural resources and can be potential source of cellulosic fiber (Ayele & Sahu 2014). Cellulosic fibers are widely used for many purposes, for example, in textile industries (Ayele & Sahu, 2014), papermaking and packaging industries (Johansson et al. 2012), pharmaceutical application (Kadajji & Betageri 2011), and preparation of innovative materials such as 'green' composites (KG 2015). In addition, plant fibers can also be used to produce fuel, chemicals (Taherzadeh & Karimi, 2007), enzymes (Cavka et al. 2013), and food (Lattimer & Haub 2010). Accordingly, with the increasing consumption and diversification of cellulose derivatives it is becoming difficult to satisfy the large demand from conventional resources. In this context, non-wood species can be viewed as alternative sources of cellulosic fibers, especially in regions that are poor in forest resources. Non-wood fibers are often obtained from agricultural wastes and industrial plants. There are many studies that have been carried out over many years to investigate the use of annual plants or/and agricultural wastes as alternative sources of fiber. Leucaena diversifolia (Feria et al. 2012), rice straw (Ho et al. 2012), vine stem (Mansouri et al. 2012), abaca fiber (Ramadevi et al. 2012), tobacco residue (Shakhas et al. 2011), banana fiber (Li et al. 2010; Bhatnagar et al. 2015), banana leaf and pseudo stem (Rahman et al. 2014), and giant reed (Arundo donax L.) (Shatalov & Pereira 2006) are some of the agricultural residues and industrial plants that have been investigated so far.

Suitability of cellulosic materials for paper pulp production is often assessed through analysis of fiber dimensions, determination of cellulose, lignin, and extractive contents. Therefore, in the present study, it has been attempted to investigate the suitability of *enset* residues, (in particular the fiber, the leaf and the inner part of the pseudo stem) for paper pulp production by conducting morphological and chemical characterizations of the residues. In addition, Fourier Transform Infrared (FTIR) Spectroscopy analysis was used to evaluate the main structural differences among the various *enset* residues.

EXPERIMENTAL

Raw Materials

The residues from the preparation of *enset* /*Ensete ventricosum*/ based food used in the present study were collected from *enset* plantation in Wolkite (Gurage zone, Ethiopia). The residues were collected randomly from different types of *enset* clones.

Morphological and Dimensional Analysis

For fiber length and fiber diameter determination, *enset* fiber were macerated with 67% nitric acid and boiled at 100°C for 10 min (Agnihotri et al. 2010). Then the samples were washed with distilled water and placed on a slide (standard 7.5cm×2.5cm) by using medical dropper. All fiber samples were viewed under microscope Motic model BA 210. A total of 75 randomly selected fibers were measured with $40 \times$ magnification.

For lumen diameter and cell wall thickness determination, *fresh enset* pseudo stem were taken at base, middle, and top of its length as tiny slices. To increase cell wall visibility, the slices were stained in 1% aqueous safranin solution for 5 minutes. Samples were then washed by different concentration (25%, 50%, and 75%) ethanol on petri dishes to remove excess safranin solution that may cause invisibility of cells. The slices were placed on a slide (standard of 7.5cm X 2.5cm) and covered by a cover clip after dropping hematoxylin as binder. Cell visualization was done at magnification of 100 X, by taking 25 random sampling each from the top, middle and bottom parts of the pseudo stem to make the measurement more descriptive.

Derived Values

Slenderness ratio, flexibility coefficient, Runkel ratio, and rigidity coefficient were calculated using measured fiber dimensions: as fiber length/fiber diameter, (fiber lumen diameter/ fiber diameter) \times 100, (2 \times fiber cell wall thickness)/lumen diameter and (2 \times fiber cell wall thickness)/ fiber diameter, respectively (Ibrahim & Abdelgadir 2015). The values were then compared to those of softwoods, hardwoods, agricultural residues and industrial plants.

Chemical Composition of Enset Residue

Prior to chemical analysis, the samples were air dried and ground, and the fraction between 40 and 60 mesh screen was used for further analysis. Hence, samples were extracted by Soxhlet using ethanol and toluene mixture with a ratio of 2:1 for 8 h. The amount of soluble products was then determined gravimetrically according to ASTEM E 1690.

Cellulose content was determined according to Kurschner-Hoffner approach. 2 g of extractive free sample were treated with 50 ml of alcoholic nitric acid solution under reflux with four cycles of 1 hour each. After each cycle, the solution was replaced by fresh solution. The alcoholic nitric acid solution was prepared by mixing one volume of 68% (w/w) solution of nitric acid with four volumes of 97% ethanol. At the end, the cellulose was washed, dried and weighted. The final content of cellulose was calculated by subtracting the ash content.

The lignin content was measured by using TAPPI standard method T222 om-06 by subjecting the extractive free sample to acid hydrolysis and filtering the obtained suspension for separating the insoluble lignin.

Ash, cold and hot water solubility and 1% NaOH solubility of enset residues were determined based on TAPPI standards TAPPI T211 0m-02, T207 cm-99, T212 os-58, respectively. All chemical analysis were done in triplicates.

Fourier Transform Infrared Spectroscopy Measurement

The difference in chemical compositions between the different enset parts was investigated using Fourier transform infrared spectroscopy. This method has been used as a simple technique for obtaining rapid information on the structure of different types of wood and among different parts (Poletto et al. 2012). In contrast to conventional chemical analysis, this method requires small sample sizes, short analysis time, without destroying the plant structure.

142

FTIR absorption spectra were obtained by mixing and grinding of the samples with dry KBr pellet with the ratio of 1:100. KBr does not show any absorption spectrum in mid infrared region. Then the mixtures were subjected to pressure to produce clear transparent discs. Finally, the samples were measured on spectrum 65 FTIR (Perkin Elmer) in the range of 4000 cm⁻¹to 400 cm⁻¹. Background spectra were corrected with pure KBr pellet.

RESULTS AND DISCUSSION

Morphological and Dimensional Analysis

The anatomical cell structure and dimensions are related to many structural, physical and chemical properties of plants. Parameters like fiber length, fiber width, lumen diameter and cell wall thickness are very good indicators to decide the material suitability for different end products. They affect many wood-product processing characteristics such as drying, resistance to cutting and machining and pulpwood quality (Ogunjobi et al., 2011, Ogunjobi 2014; Ibrahim & Abdelgadir 2015).

Enset fibers have an average length of 1.66 mm, width of 28.5 μ m, and have wider lumen (25.9 μ m) and thinner cell wall (2.9 μ m) as compared with sugarcane bagasse (Agnihotri et al. 2010), some agricultural residues (Kasmani & Samariha 2011) and hard woods such as eucalyptus tree (Miranda et al. 2012; Ogunjobi et al. 2014), olive and almond tree (Ververis et al. 2004) (Table 1). 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. Long fiber lengths are preferable for manufacture of paper. Long fibers give more open and less uniform sheet structure. 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).

Figure 1 (a) shows the image of the transverse section of *Ensete ventricosum* pseudo stem obtained by optical microscope.



Fig. 1. (a) Transverse section of *Ensete ventricosum* (1- Vascular bundle, 2- Fibers, 3-Parenchyma cell) and (b) macerated sample of *Ensete ventricosum*

The figure depicts scattered vascular bundles (1) with relatively large lumen diameter (2) and tinny cell wall. The vascular bundles of *enset* are collateral, which means xylem towards the inner side and phloem towards the outer side.

Fiber dimensions and derived values of *enset* and their comparison with other lignocellulosic material are summarized in table 1.

	Ensete Ventric osum stem *	Sugar- cane bagasse ^(a)	Wheat straw	Bam- boo ^(c)	Musa paradisi ca ^(d)	Euca- lyptus globulus tree ^(e)	Vitex Doniana tree ^(f)	Kenaf ^(g)
Fiber length (L), mm	1.66±0 .54	1.51±0.0 8	1.14	3.11	2.21±0. 03	0.98	1.48	2.9
Fiber width (D), µm	28.48± 6.79	21.4±1.6	19.32	8.03	22.2±1. 5	18.8	21.9	28.16
Lumen diameter (d), µm	25.87± 4.71	6.27±0.4	10.54	4.35	n.a.	n.a.	12.7	6.08
Cell wall thickness (w), µm	2.88±1 .01	7.74±0.2	4.39	6.98	n.a.	4.9	4.9	11.04
Slendernes s ratio (L/D)	58.41	70.56	59	387.29	99.54	52.12	67.57	105
Runkel ratio (2w/d)	0.223	2.46	0.83	3.20	n.a.	n.a.	0.77	0.76
Flexiblity coefficient (d×100/D)	90.83	29.29	54.55	54.17	n.a.	n.a.	57.99	57
Rigidity coefficient (2w/D)	0.2025	0.72	0.72	1.73	n.a.	0.52	0.45	n.a.
*Current study, a: (Agnihotri et al. 2010) b: (Kasmani & Samariha, 2011) c: (Kamthai & Puthson, 2005) d: (Rahman et al. 2014) e: (Miranda et al. 2012) f: (Ogunjobi et al. 2014a) g: (Udohitinah & Oluwadare, 2011)								

 Table 1. Morphological Characteristics of Ensete ventricosum

± refers to standard deviation,

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. 2014a).

In the present study, the Runkel ratio of *enset* was found to be 0.223, indicating thin fiber walls which are suitable for paper production favoring good sheet binding formation with high pulping quality (Kiaei et al. 2014; Ibrahim &Abdelgadir 2015).

Other calculated wood 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 (Ibrahim & Abdelgadir 2015). 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. The flexibility coefficient of *enset* was determined to be 90.8. Hence, *enset* fibers are flexible and with high strength properties and can be considered good for paper production (Ogunjobi et al. 2014a; Kiaei et al. 2014). 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.

The dimensional analysis shows that *enset* fiber exhibits fairly good properties for paper pulp production in all aspects except for its slenderness ratio. The slenderness ratio of *enset*, which was found to be 58.4 in the present study is below the recommended value of 70. Pulp tear resistance increases with increasing fiber slenderness (Ogunjobi et al. 2014a). This means paper made from *enset* residue will have low tear strength and therefore may not be suitable for wrapping and packaging purposes. Slenderness ratio also affects the tensile and busting strength of the fiber. However, fiber with thin cell wall thickness compromise low slenderness ratio regarding paper strength (Ogunjobi et al. 2014a).

Chemical Compositional Analysis

The main components of natural fiber include cellulose, hemicellulose and lignin. Cellulose is a structural component of plants and is often surrounded by matrices of other structural polymers, such as lignin and hemicellulose. Thus, during pulping reaction, the amorphous hemicellulose and lignin can be easily degraded and become soluble in cooking liquor (Ho et al. 2012). High cellulose content is considered desirable for the pulp and paper industry as it has been correlated with high pulp yield (Li et al. 2010).

The results of the chemical analysis made on *enset* residue in the present study are presented in Figure 2. Furthermore, the same results are compared with data obtained from literature on other lignocellulosic materials in Table 2.

As can be observed in Figure 2, the cellulose content is the highest in *enset* fiber (69.51%) and lowest in the leaf (37.96%). This content is also the highest among the other lignocellulosic materials presented in Table 2. It is also interesting to observe that the cellulose content of *enset* pseudo stem is comparable to those of bagasse and beech wood.



Fig. 2. Comparative analysis of chemical compositions of different parts of enset residue

venincosum) Residue with Other Paper making Raw Materials							
Chemical	Cel%	lig%	Ash%	Et%	Cw%	Hw%	1%
composition							NaOH
Fiber *	69.51±	5.7±	4.62±	5.29±	12.69±	6.67±	22.75±
	0.99	0.90	0.28	0.075	0.45	0.507	0.75
Leaf *	37.96±	18.93±	11.75±	19.09±	26.58±	16.66±	49±
	0.68	0.95	0.75	0.84	0.44	0.65	0.09
Pseudostem *	44.3±	6.82±	4.30±	7.95±	26.41±	14.96±	49.75±
	0.94	0.96	0.19	0.875	0.19	0.36	2.25
Bagasse fiber (a)	42.34±	21.7±	2.10±	1.85±	3.02±	7.42±	32.29±
	0.36	0.35	0.03	0.01	0.02	0.05	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-	39.12	8.88	8.20	3.05	n.a.	n.a.	n.a.
stem (d)							
Banana leaf	43.25±	16.02±	7.55±	1.96±	9.14±	n.a	32.44±
stalk/leaf (e)	1.8	1.2	0.7	0.1 ^(ac)	0.9		1.9
Beech wood ^(f)	45.8	21.9	0.4	n.a.	n.a.	n.a.	n.a.
Eucalyptus	56.9	17.8	1.0	1.4	1.6	n.a.	12.2
globulus ^(g)							
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.
(*) 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.							

Table 2. Comparison of the Chemical Composition of en	iset (Ensete
ventricosum) Residue with Other Paper making Raw	Materials

Et: solubility of ethanol-toluene, lig: lignin, Cw: cold water, Hw: Hot water, cel: cellulose, 'n.a.: not available, ± shows standard deviation, (ac): acetone extractive

2012): (h) (Kamthai & 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. Low lignin content in the raw material is related with low amount of energy and chemical requirement for delignification. As it is evident in both Figure 2 and Table 2, the lignin contents of *enset* fiber (5.7%) and pseudo stem (6.82%) are by far lower than that of *enset* leaf (18.93%). Furthermore, when compared with the lignin content of the other materials in Table 2, *enset* fiber and pseudo stem show lower lignin content. Even the materials like bagasse show higher lignin content (21.7%) than that of *enset* leaf (18.93%).

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. The ash content of *enset* pseudo stem and *enset* fiber is lower than the pseudo stem and peduncle of banana which is in the same family (Rahman et al. 2014; Li et al. 2010). The pseudo stem have high hot water solubility content because it has high amount of starch around 65% (Gabel et al. 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, 2006). In addition, 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).

FTIR Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is commonly used to study the functional groups of lignocellulosic biomass and the changes caused due to different treatments. The spectra offer qualitative and semi-quantitative information suggesting the presence and absence of functional groups and stretching bond in lignocellulosic biomass



Fig. 3. FTIR spectra of Enset / Ensete ventricosum/ samples: Fiber, leaf and pseudostem

and whether the intensity of an absorption band has changed after treatment or degraded by the cooking liquor (Fan et al. 2012; Poletto et al. 2012). The FTIR spectra for different *enset* residues are presented in Figure 3, showing the difference in the transmittance.

FTIR bands in plant can be classified into two regions due to their complexity. The first region is the OH and CH stretching vibrations in the range of 3800 to 2700 cm⁻¹. The second region is the "finger print" region which is assigned to stretching vibration of different groups of plant components in the range of 1800 to 400 cm⁻¹ (Poletto et al. 2012). In this region significant differences are revealed among *enset* residues.

In all three samples there is strong broad band around 3400 cm⁻¹ which is assigned to different OH stretching bands of the lignin and carbohydrate components. The other two bands around 2920 cm⁻¹ and 2850 cm⁻¹ are associated with asymmetric and symmetric methyl and methylene stretching groups. Since some compounds in organic extractives, like fatty acid methyl esters and phenolic acid methyl esters, contain methyl and methylene groups, the results are attributed to the higher content of extractive and lignin in leaf (Poletto et al. 2012; Ramadevi et al. 2012; Xu et al. 2013).

In the "finger print" region, the spectra contain several bands assigned to the main components of the plant. The band around 1637 cm⁻¹ is attributed to C=O and C=C stretching in carbonyl and alkene from fatty acid extractive components. In addition, the band at around 1734 cm⁻¹ is clearly shown in leaf while in other plant parts there is a shoulder at this band attributing to C=C and C=O stretching or bending in carbonyl and alkene of different group in lignin and fatty acid components of the extractive substances (Poletto et al. 2012; Ramadevi et al. 2012; Fan et al. 2012). This supports the chemical analysis in the leaf. It is the most lignified part of *enset* and high extractive content. Table 3 shows FTIR band assignments to *enset* residues.

Band Position (cm ⁻¹)	Functional Groups	Polymer
3400	O-H stretching	Lignin, cellulose and hemicellulose
2920, 2850	C-H stretching in methyl and methylene groups	Lignin
1734	C=O stretching in carbonyl group and ester groups	Lignin
1637	C=C and C=O alkene	Hemicellulose
1384	C-H bending	cellulose, hemicellulose
1317	Condensation of guaiacyl unit and syringyl unit, syringyl unit and CH ₂ bending stretching	Cellulose and hemicellulose
1240	O-H Phenolic hemicellulose and pectin	Hemicellulose
1040	1040 C-O-C symmetric glycosidic stretch C–O deformation in primary alcohols	
840	Glycosidic link	Hemicellulose
600	Lignin component	

Table 3. Assignments of FTIR Bands for *Enset* (Poletto et al. 2012; Chenet al. 2010; Xu et al. 2013)

The bands at 1384 cm⁻¹ are attributed to C-H in cellulose and hemicellulose. This band is seen to be more intense in the *fiber* than the other parts (Poletto et al. 2012), which might be due to the higher cellulose content observed in *enset* fiber.

The bands at 1317 cm⁻¹ and 1240 cm⁻¹ which attributed to the OH group in phenol, are shown clearly in pseudo steam and leaf. They are also attributed to hemicellulose and pectin (Ramadevi et al. 2012). The broad bands around 1040 cm⁻¹ and 600 cm⁻¹ are attributed to C-O-C symmetric glycosides stretch, C-H and C=O deformation, bending or stretching vibration of many groups in lignin and carbohydrate (Poletto et al. 2012; Ramadevi et al. 2012; Gabel & Karlsson 2013). It is related to starch found in pseudo stem which is revealed by high water solubility in pseudo stem and high lignin in leaf.

CONCLUSIONS

This study is focused on finding alternative source of cellulosic fiber for paper pulp production in Ethiopia. In this regard, residues of *enset*, an abundantly found plant in the country, have been investigated. In particular, *enset* fiber, leaf and pseudo stem were studied. The dimensional analysis and the derived values clearly show that these residues, but more importantly the fiber, possess the characteristics needed for pulp use. The study shows *enset* residue 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 are also comparable with other promising lignocellulosic fiber sources. In addition, the chemical analysis shows that the residue, mainly the fiber, is characterized by high amount of cellulose (69.5%) and low lignin (5.7%) 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* residue as paper pulp raw materials can bring about both environmental and economic benefits.

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