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Physico-chemical assays of oil palm (*Elais guineesis*) empty fruit bunch fractions for biosynthesis

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Graphical Abstract

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- ✓ *Spikelets;*
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Abstract: The oil palm empty fruit bunch (EFB) is a waste from oil palm processing. It is a lignocellulosic biomass identified as a potential feedstock for a number of industries including bioethanol, composites, as well as pulp and paper. Although EFB is an heterogenous mix of the stalk and spikelet, it is often treated as a single biomass. Physical, chemical and morphological assays were conducted on the EFB stalk and spikelet fractions to identify their unique characteristics. Percentage composition of the fractions, specific gravity and fiber characterization were some of the physical tests while the chemical tests include the proximate and solubility analyses. At 12% moisture content, the specific gravity of the EFB is 0.41. The percentage compositions by mass are comparable (54.1, 45.9%) for the stalk and spikelet, respectively, while the spikelet occupies considerably less volume than the stalk fraction (33.3, 66.7%). There are significantly more fibers present in the stalk than the spikelet fraction. The mean fiber lengths of 1.10±0.26mm and 1.52±0.56mm for the spikelet and stalk, respectively were in the short-medium range fiber length. Fiber morphology was comparable for both fractions. The photomicrograph shows widely different cell types in the spikelet than the stalk. The chemical compositions yielded 53.52± 0.01% and 54.12±0.04% for cellulose, 18.29±0.02% and 18.57±0.03% for lignin, and 13.42±0.04% and 18.57±0.03% ash content for stalk and spikelet, respectively. The lignin content is relatively low compared to other non-woody biomass types such as corn stover. Based on the morphology, the stalk is preferred for biosynthesis due to the presence of more cellulosic fibers.

1. Introduction

The oil palm plant, *Elaeis guineensis,* is typically known to have originated from West Africa. The oil palm tree produces bunches of fruits known as fresh fruit bunches. In West and Central Africa, where it originated, as well as in Malaysia, Indonesia, and Thailand, where it predominates and thrives

luxuriantly, oil palm is farmed on a large scale for oil and has become well-established over time (Noah 2022). About 8 million metric tons of the world's 30 million metric tons of oil palm production are EFB materials (Statista 2020). Nigeria is the world's fifth-largest producer of palm oil, after Thailand, Columbia, Indonesia, and Malaysia, accounting for about 1.5% of global consumption and more than half of all oil palm production in Africa (Zainal *et al*. 2017). Worldwide production of oil palm was estimated at 57 million tonnes in 2014 (FAOSTAT 2017).

The primary byproducts of the oil palm industry are crude palm oil and palm kernel, which produces palm kernel oil and a residue known as palm kernel cake. Among the solid wastes are the empty fruit bunches (EFB), mesocarp fiber, and palm kernel shell, which make up a sizeable portion of biomass in the oil palm industry (Kabir *et al*. 2017). An oil palm tree can produce up to 8–12 bunches per year, each weighing up to 13 kg and containing about 1000 fruits (Anyaoha *et al*. 2017). Fresh fruit bunch solid waste weighing 1.3 kilograms was produced for every kilogram of oil palm produced. As a result, 75 million tonnes of solid waste from fresh fruit bunches were reported in 2014 (Sulaiman *et al*. 2011). The fibrous material left over from the fruit bunch's spikelets and stalk after the fruits have been removed is known as EFB and is the primary biomass byproduct.

EFB is a beneficial organic element which is severely underutilized in agricultural production. They are highly useful as raw materials in fermentation due to their cellulose and hemicellulose contents (Noah 2022). Non-wood raw materials typically contain a similar amount of cellulose to other woody materials, but less lignin and more hemicelluloses. This offers some benefits for non-wood materials like EFB when used in pulp and paper processes, which has prompted several researchers to investigate this possibility (Harsono *et al*. 2016).

EFB is regarded as a superior feedstock for the production of biofuels due to its abundant supply, year-round availability, and the fact that, unlike palm oil, it is not used as food or animal feed (Kumar *et al.* 2013). Anaerobic digestion technology was used to produce biogas and methane from the juice obtained from crushed EFB mixed with oil mill effluent (Chiew and Shimada 2013). However, raw EFB have a significant potential to generate methane in the form of biogas because of their high moisture content and biodegradability. Composting is one of the conventional methods of recycling. Plantation-based palm oil mills have the option of using their EFB as mulch, fertilizer, or soil conditioning to keep the soil moist and cool. As a result, Malaysian agronomists proposed composting, which can reduce the volume of EFB by 50% to 75% and be used as fertilizer in oil palm plantations or for other purposes such as horticulture, landscaping, and golf courses (Chiew *et al*. 2013). EFB fibers can be processed into particleboards and used as the structural layer in wall panels (Aslam *et al*. 2016). According to research findings, the qualities of rubberwood and EFB medium density fiberboard (MDF) are comparable (Awang *et al*. 2023). EFB is broken down into fiber, mixed with formaldehyde, and heated to 413 K pressing temperature. Compared to MDF made from wood, which has a density of 400–800 kg/m3, MDF from EFB offers excellent absorption qualities (Ibrahim *et al.* 2016).

With the rapid expansion of the oil palm industry over the previous two decades, EFB has been consistently available as a feedstock for a variety of applications. However, the physical and chemical properties of the biomass material have a large influence on the end use. The breakdown of the bunch into fibers is the first step in all of the various uses of the EFB highlighted, followed by pretreatment in biofuel and fiberboard production, pulping in paper production, and even mulching. Physical examination of the EFB reveals a wide range of morphologies between the stalk and the spikelet (**Figure 1**). EFB has been identified as a biomass that is suitable for a variety of uses, but because the

biomass is a mixture of spikelet and stalk fractions, it has traditionally been treated as a single biomass. The properties and functionality of end-use applications are significantly influenced by the non-homogenous nature of EFB fibers (Li *et al*. 2020, Mishra *et al*. 2022). For instance, Almeida-Naranjo *et al*. (2022) and Valle *et al*. (2022) report that there is a wide range in the length of EFB fibers. The physical, chemical, and morphological characteristics of EFB may also change as a result of edaphoclimatic conditions (Aguilar *et al*. 2022). This has made it necessary to characterize EFB fibers in order to ascertain the end-use applications.

The antioxidant activity of Palm oil from seed of Phoenix dactylifera was discussed by El Ouadi *et al*. 2017, to serve as a new potential source of natural antioxidants, anticorrosion… The effect of the addition of palm oil and cinnamon on the physicochemical, mechanical, and biodegradation properties of cornstarch-based bioplastic, which can be proposed as environmentally friendly plastic and alternative to conventional plastic (Anggraeni *et al*. 2021).

The primary focus of this research is the separation of the EFB into homogenous fractions of stalk and spikelet, and the physical and chemical characterisation of these fractions, which determines their suitability or otherwise for various applications. The EFB consists of two distinct parts, the spikelet which holds the oil palm fruit, and the stalk which holds the spikelets.

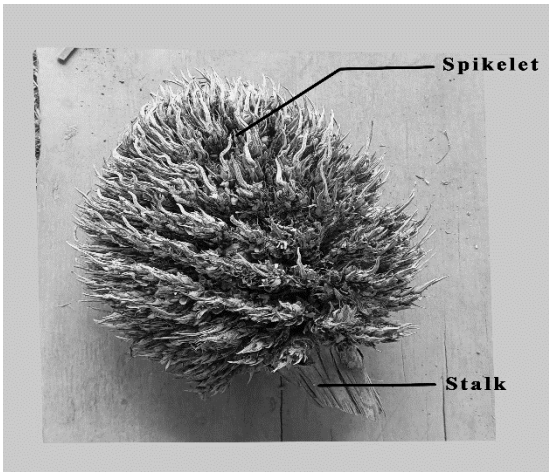


Figure 1: Oil palm EFB fractions

2. Materials and methods

2.1 Sample Collection and Preparation

The EFB were obtained from an oil palm processing unit, separated into stalk and spikelet fractions, and the average percentage of mass and volume of each fraction in the whole bunch was determined in replicates. Chips were obtained from the fractions, dried and milled using a laboratory mill. The grounded samples were sieved with a standard screen. The accepted dust-size was the amount that passed through 425µm diameter mesh and was used for the chemical assays.

2.2 Chemical assays

Alcohol-Benzene Soluble Matter

This is a measurement of the waxes, fats, resins, and oils, as well as tannins and other ether-insoluble components present in the fractions. A pre-extracted filter paper was used to contain two grams of air-dried, milled sample, which was then placed in a 250ml Soxhlet extractor. Using the alcohol-benzene mixture (1:2) and alcohol for seven hours separately, the samples were extracted in line with ASTM

standard D1107-56. The solvent was removed from the extraction flasks after the extraction. In an oven set at 105°C, the extraction flasks and their contents were dried to a consistent weight. The percentage of alcohol-benzene soluble matter was determined as in **Eqn. 1**:

$$Alcohol - benzene\ soluble\ matter\ (\%): (W_1 - W_2) \times W_1 \quad \textbf{Eqn. 1}$$

Where:
W_1 = *Weight of air-dried ground wood sample*
W_2 = *Weight of extractive free sample*

One Percent Caustic Soda Solubility

This test determines the solubility of the EFB in hot, diluted alkali solution. It is employed to assess the level of decay that may have occurred in the biomass fractions. Caustic soda with a 1% concentration solubility was prepared according to ASTM Standard D1109-56.

100ml of the prepared sodium hydroxide solution was added to two 200ml beakers containing two grams each of the oven-dried ground wood sample. The covered beakers were thoroughly stirred before being submerged in boiling water for nearly an hour, stirring the contents every 10, 15, and 25 minutes. Each beaker's contents were filtered after an hour, and the residue was thoroughly washed with hot water after each wash using 100 ml of hot water and 50 ml of 10% acetic acid. The leftover material was baked at 102°C until it reached a consistent weight. Another 2g sample was used after repeating the process. The weight percentage of dry matter soluble in one percent caustic soda solution, on a moisture free basis was calculated as in **Eqn. 2:**

$$Matter\ soluble\ in\ caustic\ soda\ (\%): (W_1 - W_2) \times W_1 \quad \textbf{Eqn. 2}$$

Where:
W_1 = *Weight of moisture-free sample prior to treatment with caustic soda*
W_2 = *Weight of oven-dried sample after treatment with the caustic soda solution*

Cold water solubility

The water-soluble matter of the samples was determined according to the ASTM standard designated D1110-56. The cold-water solubility is a measure of the organic and inorganic matter dissolvable in water at room temperature. A 400 ml beaker containing two grams of pulverized EFB that had been oven-dried was filled with 300 ml of distilled water. The mixture was continuously stirred for 48 hours at a temperature of 25°C. After the specified amount of time had passed, the sample was filtered, cleaned with ice-cold distilled water, and dried in the 102°C oven to a consistent weight. The percentage solubility in cold water on an oven dried basis was determined as in **Eqn. 3**:

$$Cold - water\ solubility\ (\%): (W_1 - W_2) \times W_1 \quad \textbf{Eqn. 3}$$

Where:
W_1 = *Weight of oven-dried sample prior to treatment with cold water*
W_2 = *Weight of oven-dried sample after extraction with cold – water.*

Hot water solubility

The hot water solubility is a measure of the tannins, gums, sugars coloring matter and starches in the sample. A 250ml quick fit flask was filled with 100ml of distilled water and 2g of ground wood sample.

In a water bath, the mixture was gently refluxed for three hours. The flask's contents were filtered and dried to a consistent weight in a 102°C oven after three hours. The following formula was used to determine the percentage of materials soluble in hot water on an oven-dried basis **Eqn. 4**:

$$Hot - water\ solubility\ (\%): (W_1 - W_2) \times W_1 \quad \textbf{Eqn. 4}$$

Where:
W_1 = *Weight of oven dried sample prior to treatment with hot-water.*
W_2 = *Weight of oven dried sample after treatment with hot-water.*

Ash Content Determination

The ASTM standard D1102-56 was used to calculate the ash content. This technique is used to calculate the amount of residue left over after dry oxidizing wood or related goods. A crucible was filled with two grams of the ground wood sample, whose moisture content had been measured. The crucible and its contents were heated to 550°C in a muffle furnace until they took on a grayish hue. Based on the weight of the moisture-free sample, the percentage of ash was estimated as follows **Eqn. 5**:

$$Ash\ percent: (W_1/W_2) \times 100 \quad \textbf{Eqn. 5}$$

Where:
W_1 = *Weight of ash in grams*
W_2 = *Weight of oven dry sample grams*

Lignin Content Determination

The milled sample was first extracted with ethanol-benzene mixture (1:2) and dried. After this, the determination of the lignin content was carried out in accordance with TAPPI standard designated T222 OM-98 for acid-insoluble (Klason) lignin **Eqn. 6**:

$$Lignin\ (\%): (100Y)\ /\ W \quad \textbf{Eqn. 6}$$

Where: *Y = weight of lignin (insoluble material) ; W = weight of oven dry test sample*

Cellulose Content Determination

This was done using the Kurschner-Hoffer cellulose process. A 250ml round bottom flask with a reflux condenser was filled with 1g of an air-dry sample. 1.5ml of concentrated nitric acid (HNO_3) and 15ml of 80% acetic acid were added. For exactly 20 minutes, the mixture was brought to a boil. A total of 20 cc of cold, 95% ethanol was added. The mixture that resulted was filtered and chilled. The residue was then cleaned with hot benzene, hot alcohol, and hot diethyl ether, in that order. The residue was dried overnight to a fixed weight before being heated for several hours at around 500°C in a muffle furnace. The weight loss after burning was used to calculate the cellulose content.

2.3 Fiber characterization

Slivers were extracted from the stalk and spikelet fractions separately. They were placed in test tubes and macerated with equal volumes (1:1) of 10% glacial acetic acid and 30% hydrogen peroxide (H_2O_2) at 100°C for about 120 minutes until soft and bleached white, as recommended by Franklin in 1945 and adopted by Oluwadare and Sotannde (2010) and Kolajo and Onilude (2019). The fiber bundles

were separated into individual fibers after thoroughly washing the slivers in distilled water and shaking them in 30 ml test tubes with 20 ml of distilled water. The macerated fiber suspension was pipetted onto a clear slide and magnified ten times with a Carl Zeiss photomicroscope. The resulting image on the microscope screen was used to calculate fiber length, diameter, lumen width, and cell wall thickness. These were used to calculate the Runkel ratio (**Eqn. 7**), slenderness ratio (**Eqn. 8**) and flexibility coefficient (**Eqn. 9**).

Derived morphological fiber characteristics:

$$\text{Runkel Ratio} = \frac{2 \times \text{Cell wall thickness}}{Lumen\ width} \quad \textbf{Eqn. 7}$$

$$\text{Slenderness Ratio} = \frac{\text{Fiber length}}{Fibre\ diameter} \quad \textbf{Eqn. 8}$$

$$\text{Flexibility Ratio/ Elasticity Coefficient} = \frac{\text{Lumen width} \times 100\%}{Fibre\ diameter} \quad \textbf{Eqn. 9}$$

3. Results and discussion

3.1 Mass-Volume Ratio of the EFB fractions

The specific gravity of the whole, spikelet and stalk fractions of the EFB are 0.74, 1.23 and 0.50, respectively as seen in **Table 1**. The average percentage mass of the spikelet and stalk of EFB is 54.1±0.954 and 45.9±0.954 respectively, while the average percentage volumes are 33.3±0.231 and 66.7±0.153. Therefore, the spikelet of EFB weighs more than the stalk, but the stalk has a higher volume than the spikelet.

Table 1: Specific gravity and percentage composition of the EFB fractions

EFB fractions	Specific gravity	% composition	
		by weight	by volume
Whole bunch	0.74	100	100
Spikelet	1.23	54.1±0.95	33.3±0.23
Stalk	0.50	45.9±0.95	66.7±0.15

Values are expressed as a mean of three measurements ± standard deviation

3.2 Chemical assays of the EFB fractions

More extractives from the fractions were dissolved in alcohol-benzene compared with alcohol, with the spikelet fraction having slightly higher dissolution than the stalk (**Figure 2**). This implies that more waxes, lipids and dissolvable organic compounds are present in the EFBs. This will influence the choice of chemicals to be used in biochemical conversion to ethanol and in pulping for paper production. Additionally, it makes pretreatment more necessary when the EFBs are considered for use in the production of composites such as fiber- and particle boards, as the presence of the dissolvable organic materials can hinder effective bonding between the EFB fibers and adhesives matrix.

More dissolution was expected in alcohol and alcohol-benzene than in cold and hot water, due to the ability of the earlier solvents to dissolve resins, waxes and oils the perceived to be present in the biomass matrix. However, more solubility was recorded in the reverse. This may be attributed to the escape of volatile organic compounds, dissolvable in alcohol-based solutions which may have been lost during the heating and milling of samples in preparation for assay. More substances were recorded to have been dissolved in hot water than cold water. This indicated that the pretreatment of the EFB in

either biochemical or composites production will be more effective at high temperatures. In cold and hot water, the quantity of extractives in stalks and spikelet are higher than those formed in woods. The 1% NaOH extractives are also found higher for stalk and spikelet fractions. Therefore, the stalk and spikelet are characterized by respectively high quantity of extractives. The implication of this is in lower crystallinity and smaller cellulose crystallite size (Blanco *et al.* 2018). It also suggests that the pretreatment liquor will contain a lot of dissolved extractives, which might lower the active alkali for delignification.

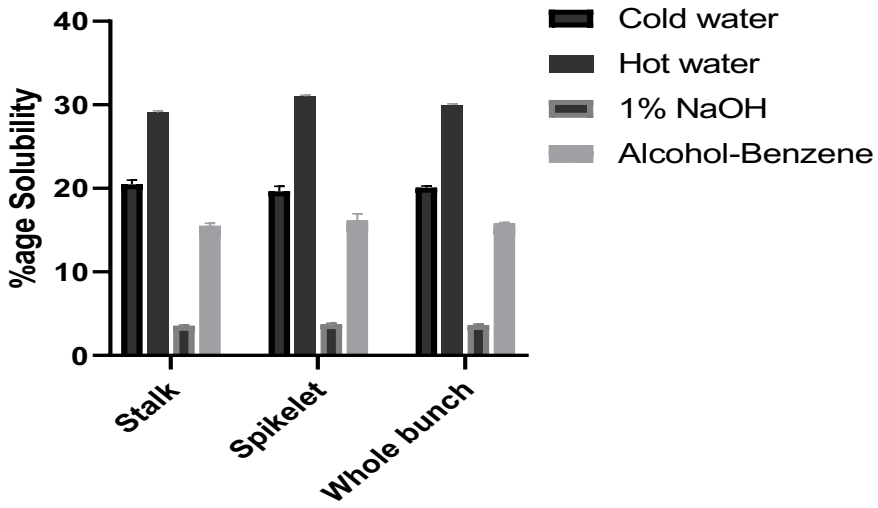


Figure 2: Dissolution of EFB extractives in solvents

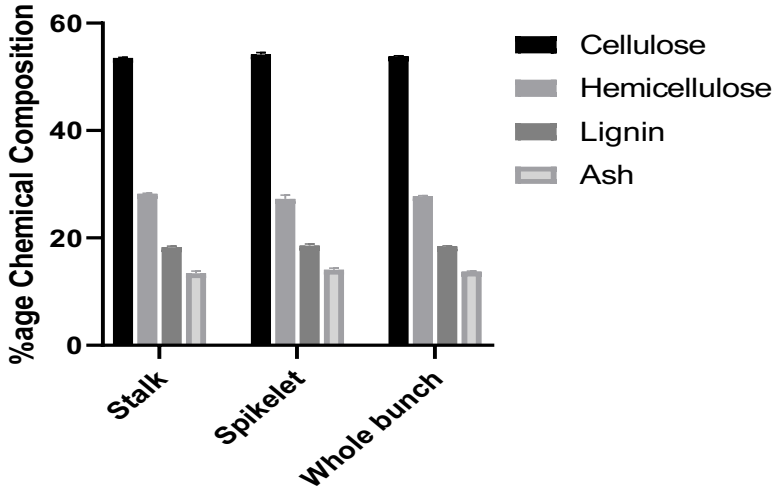


Figure 3: Chemical composition of EFB Fractions

The chemical compositions of the stalk and spikelet are comparable with very slight variations (**Figure 3**). However, there is a marked variation in the chemical composition of the EFB fibers obtained from this study and those recorded by Law *et al.* (2007) and Hill and Khalil (2000) in Malaysia. Hence, it is suspected that the chemical composition of the fibers is influenced by site indices of its growth. It is also reported that the percentage of cellulose can vary depending on the species and the age of the plant (Hassan *et al.* 2015). Typically, lignocellulose biomasses need to have between 40 and 50 percent cellulose in order to make bioethanol (Hori *et al.* 2000). EFB fibers have cellulose content within this range, and can therefore be suitable for biosynthesis.

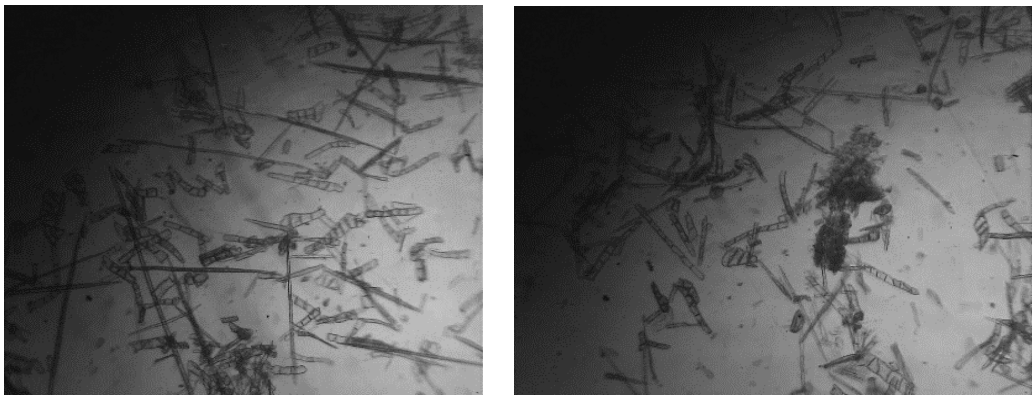
Compared with other non-woody biomass, EFB stalk and spikelet contain higher composition of cellulose compared to 46.3% for Maize stalk (Kolajo and Onilude 2019) and 37.16% Kenaf (Xu *et al.* 2004). Lignin, which contributes to tough and stiffness properties of the fibers, was lower than kenaf but higher than Maize stalk for both EFB stalk and spikelet fibers. This implies that the stalk and spikelet will be easier to pulp than kenaf, but when compared to Maize stalk, it will require a significant amount of energy and chemical to pulp. When compared to maize stalk, the ash content of both the stalk and spikelet is very high. This could be because its primary function was to house oil palm fruits. Because ash reacts with black liquor during pretreatment, a high ash content is important in the recovery of chemicals from black liquor.

3.3 Results of Fiber analysis

Table 3: Fiber characteristics of EFB fractions.

Fiber characteristics	Stalk fraction		Spikelet fraction	
	N	Mean	N	Mean
Fiber Length (mm)	30	1.52±0.56	10	1.10±0.26
Fiber Diameter (µm)	30	17.80±2.27	10	15.00±2.19
Lumen Width (µm)	30	14.60±2.71	10	11.50±1.88
Cell Wall Thickness (µm)	30	1.60±0.56	10	1.75±0.75
Runkel Ratio	30	0.24±0.12	10	0.31±0.14
Coefficient of Flexibility	30	81.59±7.25	10	77.04±8.72
Slenderness Ratio	30	0.09±0.03	10	0.08±0.02

Generally, the fiber characteristics of the stalk and spikelets fractions are comparable (**Table 3**), there is however the presence of more fibers in the stalk than the spikelet fraction (**Figures 4a and 4b**). The cell wall of cellulosic materials and the pore volume that is accessible to enzymes are crucial factors in determining how well these materials will hydrolyze (Ohgren *et al.* 2007, Yu *et al.* 2011). The effects of fiber length primarily relate to the pretreatment step and subsequent enzymatic hydrolysis, which convert the complex carbohydrates in the biomass into fermentable sugars. It is also important to note that the amount of cellulose present increases with fiber length. Since the EFB stalk has considerably more fibers than the spikelets, it is more suitable for use in bioethanol production.



(a) (b)

Figure 4: Photomicrograph of (a) Stalk (b) Spikelet fractions of EFB (x10 magnification)

The mean fiber lengths of 1.10±0.26mm and 1.52±0.56mm observed in the spikelets and stalk respectively are quite lower than 2.7-4.6mm reported by (Egbewole *et al.* 2015) on fiber lengths of

selected softwoods. It is very close to the minimum 0.7mm-1.6mm values for hardwood fiber sources and to 1.7mm values reported by Noah (2009) for bagasse fibers (another non-woody biomass). Fiber length is an important consideration when selecting a species for the production of high-quality pulp for paper production. The mean fiber diameter for the spikelets was 15.00±2.29µm ranging between 20µm and 12.50µm, while the mean fiber diameter for the stalk was 17.8±2.27µm ranging between 22.50µm and 12.50µm. The mean diameters 15.00±2.29µm and 17.8±2.27µm of the spikelets and stalk respectively fall below the range of 20- 40µm fiber diameter for hardwood fibers as reported by (Egbewole *et al.* 2015), it is also at a distance range to the 20.0µm fiber diameter in bagasse as reported by Noah (2009). Mean fiber diameter for both the spikelets and stalk falls within the range recorded for coniferous and commercial pulp woods (Hammet *et al*. 2001, Ververis *et al.* 2004).

The average Runkel Ratio of the EFB spikelet was 0.31±0.14, while that of the EFB stalk was 0.24±0.12. Bektas *et al*. (1999) stated that a higher Runkel ratio results in lower strength properties, specifically lower burst, tear, and tensile indexes. Oluwadare and Egbewole (2008) stated that Runkel ratio is closely related to cell wall thickness and influences fiber strength properties.

Conclusion

The stalk and spikelet fractions are both suitable for the production of bio-ethanol due to their high cellulose and low lignin content; however, the stalk is preferred for biosynthesis due to its higher fiber concentration and more homogeneous composition when compared to the spikelet fraction. The stalk fraction of the empty fruit bunch can also be utilized to make pulp and paper, as well as bio-composites. Dissolution of other cell components as well as higher ash content in the spikelet makes it advantageous to separate the empty fruit bunches into stalk and spikelet fractions for biosynthesis.

Competing Interests: The authors declare that this is original research conducted by the authors and is not under consideration for publication in any other journal or publication outlet. There is therefore no conflict of interest to declare.

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