# COMPARATIVE ASSAY OF WAXES FROM CASSAVA AND SUGARCANE BAGASSE

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**ABSTRACT**

Extraction of products of high value from agricultural wastes is an essential component for sustainable techno economic development. In this study, extraction of wax from cassava and sugarcane bagasse was carried out using a mixture of benzene and methanol (mass ratio of 2:1) as the extraction reagent. About 9.86% (w/w) of crude cassava wax and 4.10% (w/w) of sugarcane bagasse wax. The physicochemical properties and characterisation was compared for both waxes. Results of fourier transform-infrared spectroscopy (FT-IR) showed prominent peaks at CH, $CH_2$, C-O and OH indicating the presence of alkanes, carbonyls and alcohol, respectively for both waxes. Ultraviolet (UV) qualitative analysis revealed that the waxes absorbed UV light at 245 nm and 215 nm for cassava and sugarcane bagasse wax, respectively, indicating the presence of conjugated dienes. In addition, the physicochemical properties of the cassava and sugarcane bagasse wax evaluated were; melting point (62.5$^o$C and 76$^o$C), saponification value (275 mg/KOH/g and 202.6 mg/KOH/g), acid value (29.15mg/KOH and 6.7 mg/KOH/g), ester value (245.85 and 180.06), free fatty acid (14.66 mg/KOH/g and 3.45 mg/KOH/g) and iodine value (22.54 mg/KOH/g), respectively. Thus, cassava and sugarcane bagasse wax have potential for various applications in the industry such as food preservative, medicinal and has several biological and industrial importance.

**Keywords:** Cassava bagasse, sugarcane bagasse, extraction, wax, agricultural wastes.

## 1.0 INTRODUCTION

Renewable organic matters are of enormous concern as a result of its sustainability and biodegradability (Travalini, Pretes, Pinheiro & Demiate, 2017). Waxes are a distinct class of organic compounds that are lipophilic, malleable solids near ambient temperatures. They include higher alkanes and lipids, typically with melting points above 40°C (Aro & Aletor, 2012). Waxes are insoluble in water but soluble in organic, non-polar solvents. Natural waxes of different types are produced by plants and animals and occur in petroleum. Waxes are organic compounds that characteristically consist of long alkyl chains. They may also include various functional groups such as fatty acids, primary and secondary long chain alcohols, unsaturated bonds, aromatics, amides, ketones, and aldehydes (Bhosale, Chonde & Raut, 2012). They frequently contain fatty acid esters as well. Wax is synthesized by many plants and animals. Synthetic waxes are often long-chain hydrocarbons (alkanes or paraffins) that lack functional groups. Those of animal origin consist of wax esters derived from a variety of carboxylic acids and fatty alcohol. In waxes of plants origin, characteristic mixture of unesterified hydrocarbon may predominate over esters (Anam & Gathuni, 2014).

Crop residues such as cassava bagasse are annually renewable sources of energy. Though they are rich in carbohydrate, their utilization for any direct application is very less due to the low content of protein and poor digestibility (Pallar, Elakkiya, Tennety & Devip, 2012). However, the utilization of such agro-industrial residues provides alternative substrate for bioprocesses and will solve the problem of environmental pollution to an extent. Several processes have been developed to utilize cassava bagasse, the fibrous residue of the tropical tuber for the production of value added products such as organic acids, ethanol, aroma, mushroom among others (Bekele, Desalegn & Mitiku, 2015).

Sugarcane can produce epicuticular wax on the stems and leaves. Sugarcane bagasse is one of the major lignocellulosic biomass in tropical countries. In general, approximately 270 kg of bagasse (with 50% moisture) is

produced from one metric ton of sugarcane (Gaoxiang, Fen, Lian, Xiaoqing, Chao, Hailong, Xuefang & Xinde, 2016). Sugarcane bagasse, the major by-product of the sugarcane industry, is a very promising raw material for the production of glucose, xylose, ethanol and methane (Hofsetz & Silva, 2012). There are increasing indiscriminate disposal of these agro-waste on land and water bodies causing changes in the physical, chemical and biological integrity of the environment, thereby creating nuisance in the environment such as producing foul smell and breeding various types of infectious organisms which affects human and its environment, therefore this study was aimed at comparing the properties of waxes obtained from cassava and sugarcane bagasse.

## 2.0 MATERIALS AND METHODS

### Sample Collection

Cassava bagasse samples were amassed from the cassava processing factory in Ilaro. The bagasse was oven-dried at 60°C for 24 hours. Sugarcane bagasse was collected from sugarcane farm at Papalanto in Ogun State, the agro-waste was oven dried at 60°C for 24 hours. The dried samples were crushed in a mill, packed in polyethylene bags and stored at room temperature for further use. All reagents used were of analytical grade.

### Extraction of Wax

Wax was extracted from the bagasses as reported by Mangesh & Lele (2012). The process comprises of soxhlet extraction of bagasse powder with methanol and benzene using ratio 1:2 for 8–10 hours. The solvent was then recouped using rotary evaporator RE300. The residue was dissolved in isopropanol and was refluxed with charcoal for 1-2 hours to remove any undesirable colour or pigments present. The extract was then filtered to remove charcoal and evaporated at 60°C to obtain crude wax.

### Percentage Yield (%)

The dried bagasses were weighed before dewaxing and the waxes produced were also weighed to calculate the yield using the equation below;

$$\text{Crude Yield} = \frac{Mass\ of\ the\ crude\ wax}{mass\ of\ sample} \times 100\%$$

### Physicochemical Parameters of Extracted Wax

The physicochemical analysis of the waxes extracted were carried out using the method described by (Anuj, Yuvraj, Veena & Nishi, 2013).

### Acid Value Determination

5.0g of wax was weighed into a dry conical flask of suitable size and 25ml of absolute ethanol was added and 2 drops of phenolphthalein indicator was added to the mixture. The mixture was then heated with shaking in water bath (65%) for 10 minutes and cooled. The cooled mixture was titrated against 0.1 N KOH solution until pink colour appeared (endpoint). The acid value and free fatty acid was calculated as;

$$Acid\ value = \frac{56.1\ x\ Nx\ V}{V}$$

Where, V = Volume of 0.1 N KOH solution used (mL)

N = Normality of KOH solution (determined by standardizing KOH solution with oxalic acid)

W = Weight of oil sample (g)

Free Fatty Acid= Acid value x 0.503

### Saponification Value Determination

2 g of the wax sample was weighed into a 250ml conical flask, and 25ml of alcoholic potassium iodide was added and dissolved in 250ml. A reflux condenser was attached and the flask content was heated on a boiling hot water for 1 hour with occasional shaking, while the solution was still hot, 3 drops of phenolphthalein indicator was added and then titrated with 0.5 N HCl. The same procedure was used for a blank (without the wax). The saponification value was calculated thus;

$$Saponification\ value = \frac{56.1\ x\ M\ x\ (B-S)}{W}$$

Where B = Volume of 0.5 M HC1 solution used for the blank (mL)

S = Volume of 0.5 M HC1 solution used for the oil sample (mL)

M = Molarity of HC1

W = Weight of oil sample (g)

**Ester Value**

Ester value was determined by the difference between the saponification value and the acid value and it showed the amount of potassium hydroxide consumed in the saponification of the esters.

*Ester Value* = Saponification value - Acid value

**Iodine Value Determination**

2g of the wax was weighed into a 250ml conical flask and 10ml of chloroform was added. 30ml Hannus solution was added, the flask was closed completely by parafilm and the solution was left for 30 minutes with shaking continuously. 10ml of 15% Potassium iodide was added and 10ml of distilled water was added. The iodine solution was titrated against 0.1N sodium thiosulphate solution using starch as an indicator. The same procedure was used for a blank (without the wax). The iodine value was calculated as;

$$Iodine\ value = \frac{12.69\ x\ N\ x\ (B-S)}{W}$$

Where B = Volume of sodium thiosulphate solution used for the blank (mL)

S = Volume of sodium thiosulphate solution used for the oil sample (mL)

N = Normality of sodium thiosulphate solution

W = Weight of oil sample (g)

**Melting Point of Extracted Wax**

The melting point of the waxes were carried out according to the method adopted by Bekele *et al.* (2016). The wax was melted by warming it in water bath at a temperature just sufficient to melt it and the melting points were determined by the capillary tube method.

**Characterisation of Waxes**

The characterization of the waxes extracted was carried out using Fourier transformed infrared spectroscopy (FT-IR) and Ultraviolet-Visible spectroscopy (UV-Vis).

## 3.0 RESULTS AND DISCUSSION

The physicochemical properties of the waxes are presented in Table 1 while the results of FT-IR and UV spectrum are presented in Figure 1-4.

The cassava bagasse was observed to have a higher concentrate of wax based on the percentage yield which was found to be 9.86% as compared to sugarcane bagasse whose yield was distinctively low (4.10%) although it is high when compared to the wax obtained by Gaoxiang *et al.,* 2016 (1.2%) and 0.53% obtained by Attard, McElroy, Rezende, Polikarpov, Clark & Hunt, 2015, the high yield of wax from cassava bagasse is dependent on the source sample.

Results of the physicochemical parameter of the waxes extracted from cassava bagasse wax (CBW) and sugarcane bagasse wax (SBW) are shown in Table 1 below. The Table presents the physical (colour, odour, state, nature and melting point) and chemical (acid value, saponification value, ester value and iodine value) characteristics of the waxes. They both have an agreeable odour and are viscous liquids at room temperature.

The melting point of a wax is the temperature at which the wax changes from a solid to a liquid state. The melting point of the waxes were 62.5ºC and 76ºC for cassava and sugarcane bagasse wax respectively, which was still within same range, this signifies that these waxes are clear of foulness and can be used for various purposes.

The acid value is the amount of KOH milligrams required to neutralize the free acidity present in one gram. It is a relative measure of rancidity as free acidity is normally formed during decomposition of glycerides (Bekele *et. al***,** 2016).

The acid values obtained are 29.15 mg/KOH/g and 6.7 mg/KOH/g for cassava and sugarcane bagasse wax respectively, the acid value obtained for cassava bagasse wax falls within the range of acid value for beeswax while the acid value of sugarcane bagasse wax falls within the range of Carnauba wax (Akoh & Min, 2002).

The saponification value (number) is the number of milligrams potassium hydroxide required to hydrolyze 1 g of the sample and it measures the amount of saponifiable matter present. Testing saponification value indicates the number of acids and ester group found in the waxes (Bernal, Jimenez, Delnozal, Toribio & Martin, 2005). The saponification values obtained was 275 mg/KOH/g and 202.6 mg/KOH/g for cassava and sugarcane bagasse waxes, respectively. Both waxes exceeded the international standard of saponification value for beeswax (Bekele *et al.,* 2017).

The ester value of wax determined by the difference between saponification value and acid value which indicates the amount of KOH consumed during saponification of esters (Bekele *et al***.,** 2017). The ester value obtained was 245.85 mg/KOH/g for cassava bagasse and 180.06 mg/KOH/g for sugarcane bagasse. The ester value of both samples exceeded the international limit for beeswax which could be attributed to the type of solvent used.

The iodine value is expressed as the grams of Iodine absorbed per 100g of lipid it indicates the degree of unsaturation. The greater the iodine value, the greater the number of C=C double bonds. An increase in iodine value indicates high susceptibility of liquid to oxidative rancidity due to high degree of unsaturation. The iodine value obtained in this study for both waxes was 22.54 g/100. This is similar to the iodine value for wool grease and lanolin.

Table 1. Physicochemical parameters of crude bagasse wax

| **Physicochemical parameter** | **CBW** | **SBW** |
|---|---|---|
| Colour | Pale brown | Yellowish Brown |
| Acid value (mg/KOH/g) | 29.15 | 6.7 |
| Saponification value (mg/KOH/g) | 275 | 202.6 |
| Ester value (mg/KOH/g) | 245.85 | 180.06 |
| Percentage yield (%) | 9.86 | 4.10 |
| Iodine value (g/100) | 22.54 | 22.54 |
| Free fatty acid | 14.66 | 3.45 |
| Melting point ($^oC$) | 62.5 | 76 |

**FT-IR Analysis Results**

Wax is a mixture of long chain aldehydes, long chain primary alcohols, fatty acids, hydrocarbons and esters. Following the extraction of the native waxes, spectroscopic study was carried out to examine its chemical composition. FTIR analysis of the wax samples revealed the presence of many organic functional group present in the waxes indicating their respective compounds. Comparison of the absorption frequency of various organic functional group revealed bands at 2911 $cm^{-1}$ and 2919 $cm^{-1}$ for cassava bagasse and sugarcane bagasse, respectively, this is characteristic of -CH stretch and bend, whereas bands at 1028$cm^{-1}$ and 1464$cm^{-1}$ for cassava bagasse wax and 1019$cm^{-1}$ and 1464$cm^{-1}$ for sugarcane bagasse wax signify $CH_2$ and $CH_3$ bands. Bands at 3382 $cm^{-1}$ and 3349 $cm^{-1}$ for cassava and sugarcane bagasse wax, respectively, indicated the presence of OH group in the wax samples. Similarly, bands at 1717$cm^{-1}$ for cassava bagasse wax and 1708$cm^{-1}$ for sugarcane bagasse wax were attributed to the presence of carbonyl group. Similar results were detected in the characterisation of sugarcane bagasse as reported by Mangesh & Lele (2012). According to Gaoxiang *et al.* (2016) the FTIR characterisation of sugarcane bagasse revealed the presence of many functional group such as alkanes, alcohol, fatty acids, aldehyde and small amount of lignin derivatives.

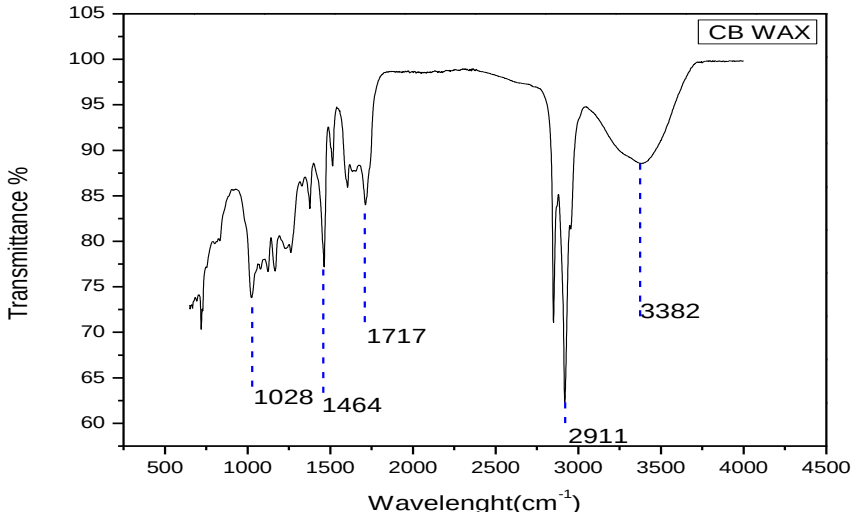


Figure 1: FT-IR Spectra of Extracted Cassava Bagasse Wax

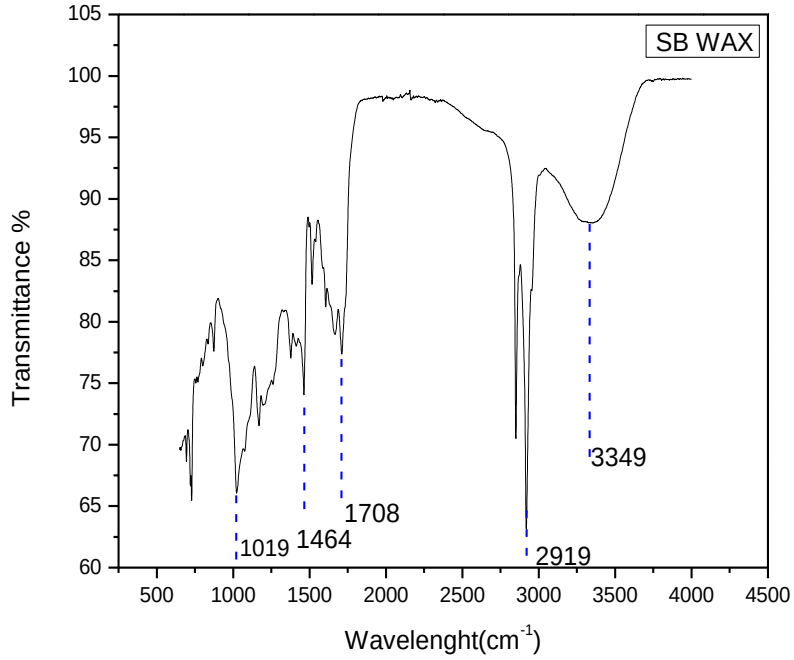


Figure 2: FT-IR Spectra of Extracted Sugarcane Bagasse Wax

**UV-Vis Analysis Results**

Fatty acids, conjugated dienes, and hydroperoxide developed as a result of lipid oxidation and absorb UV light at about 230nm. In the UV region (100- 400nm), cassava bagasse wax exhibited a maximum wavelength of absorption at 245nm while sugarcane bagasse wax indicated a maximum wavelength of absorption at 218nm, this connote the presence of conjugated dienes and trienes. Mangesh & Lele (2012) reported a close landermax of 230nm. The result was also in accordance with Athukorala, Mazza, & Oomah, 2009 where wax from (*Linumusitatissium*) strax has shown a similar pattern.

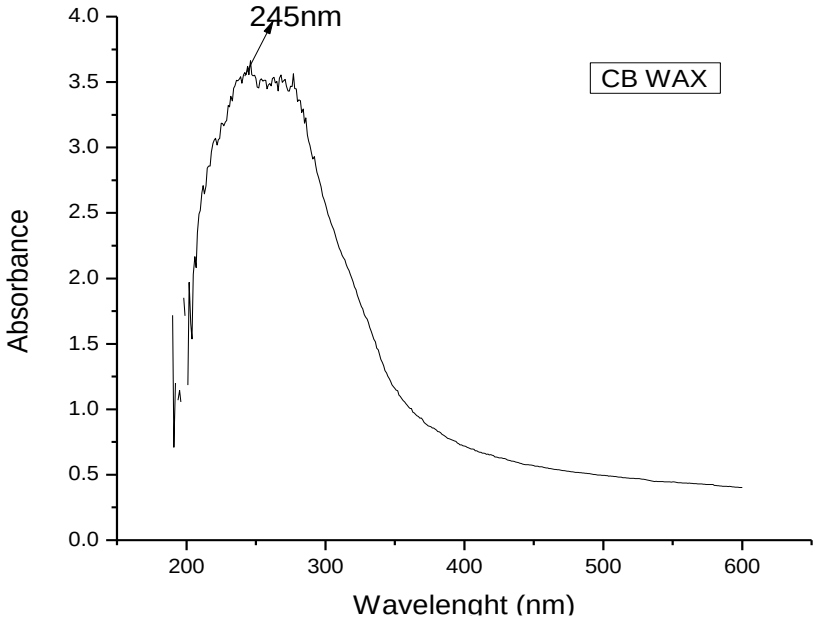


Figure 3: UV-Vis Spectra of Extracted Sugarcane Bagasse Wax

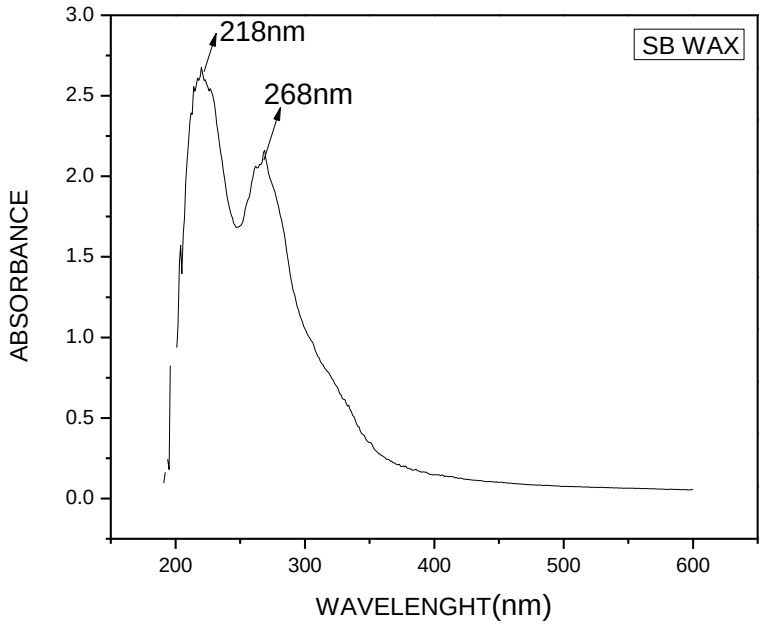


Figure 4: UV-Vis Spectra of Extracted Sugarcane Bagasse Wax

## 4.0 CONCLUSION

The crude waxes obtained from cassava bagasse and sugarcane bagasse were found to exhibit many classes of compound like alkanes, ester, alcohol and fatty acids in the crude wax. The major components of wax, is sec butyl isothiocyanates which has many beneficial effects that can be utilized for medicinal purposes. Fatty acids playing significant role in human were found to be present in wax. Apart from the medicinal and nutritional applications, wax can be used in food preservation as an edible coating for fruits and vegetables. Although, the wax obtained from the sugarcane bagasse was low (4.10%) compared to cassava bagasse wax (9.86%), both waxes have many compounds of biological and industrial importance therefore wax extracted from both waste products would be a potential alternative raw material for industries and help to minimize its deterioration to the environment.

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