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Socio-Economic And Physico-Mechanical Potential Of *Thaumatococcus Daniellii* And *Musa Paradisiaca* Leaves As Biodegradable Packaging For Food Risk Prevention

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Abstract
Non-biodegradable packaging is a source of environmental pollution and a public health problem. The production of environmentally friendly biodegradable packaging with defined physicochemical and mechanical characteristics remains a major challenge. The aim of this study was to assess the socio-economic and physico-mechanical impact of Thaumatococcus daniellii and Musa paradisiaca leaves, used as biodegradable food packaging. A survey conducted among 120 sellers of plant packaging provided information on socio-economic status and data on the plant species used. The physicochemical and mechanical properties including friability, opacity, absorption, pH and permeability were carried out using reference methods. The results indicated that Thaumatococcus daniellii leaves were the most marketed with 98%. Musa paradisiaca leaves (5 min to 50 min) wilted less quickly than Thaumatococcus daniellii leaves (2 min to 25 min) at temperatures between 60°C and 120°C. Fresh and flamed Thaumatococcus leaves (0.1 nm to 0.9 nm) remained more opaque than Musa paradisiaca leaves (0.1 nm to 0.5 nm). On average, Thaumatococcus leaves (0.09) absorbed more water than Musa leaves (0.07). All plant species were impermeable with pH values between 6.8 and 6.9. Thaumatococcus daniellii and Musa paradisiaca leaves have potential that can be exploited to make biodegradable packaging that complies with environmental standards.
Keywords:*Thaumatococcus daniellii, Musa paradisiaca, plant-based packaging, biodegradable packaging*

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I. Introduction

The use of non-biodegradable plastic packaging has become a major environmental problem worldwide, and particularly in Africa **(Al Mahmud *et al*., 2024; Kalle *et al*., 2023)**. These types of plastic packaging cause huge environmental problems, insalubrity and often clogging of gutters, particularly in cities and rural areas **(Mujinga *et al*., 2023; Onzo *et al*., 2014a)**. In addition, hot-packed foodstuffs in these plastic bags cause interactions that impair organoleptic quality and give rise to toxicological risks **(Al Mahmud *et al*., 2024)**. Despite the harmful consequences of using non-biodegradable plastic packaging, demand for and annual production of plastics have risen sharply in recent years **(Mujinga *et al*., 2023)**. In 2019 alone, an estimated 22 Mt of plastics were released into the environment. The majority (82%) is attributable to poor waste management through inadequate processes. The remaining share is due to abrasion and loss of microplastics (12%), litter (5%) and marine activities (1%) **(OECD, 2023)**.

Thus, it is necessary, to find alternative ways of packaging and protecting foodstuffs with materials that guarantee a healthy environment and improve health **(Mujinga*et al*., 2023 ; Gadhave *et al*., 2018).** Studies have shown that there is enormous potential for producing biodegradable packaging materials from plant sources in an economical and environmentally friendly way **(Mujinga*et al*., 2023; OECD, 2022)**.

Among these packaging materials, several leaves from plant species such as *Thaumatococcus daniellii*, *Musaparadisiaca*, Zea mays or *Tectona grandis* are used in Africa to cook, protect, transport or market food **(Odoom *et al.*, 2023 ; Ojekale *et al*., 2013**). These plant leaf species, enriched with antioxidants, also provide local populations with basic food, medicinal and technological products to satisfy their primary needs (**Al Mahmud *et al*., 2024**). In addition, these plant leaves impart characteristic flavors to the cooked foods that are packaged, and provide substantial income for many households (**Al Mahmud *et al*., 2024**).

The use of plant leaves as packaging materials offers an enormous opportunity to combat poverty and environmental pollution **(Ouétchéhou*et al.*, 2021**). Indeed, the importance of these packaging sheets lies in their biodegradability, their lower cost and their potential to improve the organoleptic qualities of foodstuffs **(Odoom *et al*., 2023 ; Onzo*et al.*,2014b**).

Consequently, the development of potentially biodegradable packaging materials as alternatives to plastic packaging is attracting increasing interest in the fight against environmental pollution **(Ouétchéhou *et al.*, 2021)**.

In addition, the replacement plant source of ecological, economic and health interest should have characteristics better than or equal to those of synthetic plastic **(Mujinga*et al*., 2023)**.Thus, the large-scale production and application of environmentally friendly packaging materials by applying one or more simple physicochemical treatments remains a major challenge (**Kalle *et al*., 2023**).

In Côte d'Ivoire, several works have been carried out on the diversity and ethnobotanical potential of plant leaf species used as food packaging **(Onzo *et al.*, 2014a; Onzo *et al.*, 2015).** Despite these few works carried out in Côte d'Ivoire no study relating to the characterization of inexpensive, sustainable and biodegradable packaging materials from locally available renewable edible leaf plant materials has yet been conducted.

The aim of this work was to assess the socio-economic and physico-mechanical impact of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves, used as biodegradable packaging for food risk prevention in Côte d'Ivoire.

II. Materials And Methods

Material

The plant material used in this study consisted of leaves of *Thaumatococcus daniellii* **(**attieké leaves**)** and *Musa paradisiaca* **(**banana leaves**) Figure 1**.

Figure 1: Plant species used as food packaging
***A:** Leaves of Thaumatococcus daniellii used as food packaging, **B:**Leaves of Musa paradisiacaused as food packaging.*

Method

Sampling

Size and survey

Samples of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves, each weighing one (1) kilogram, were taken from the fields, transported to the Biotechnologies and Molecular Biology laboratory at the Université Felix Houphouët Boigny and analyzed. In addition, a socio-economic survey was carried out among 120 plant leaf sellers to determine the use and economic value of these types of leaf packaging.

Sample selection criteria

Several species of plant leaves used as food packaging in Côte d'Ivoire, have been identified during previous work (**0nzo *et al*. 2015)**. Among these species, *Thaumatococcus daniellii* and *Musa paradisiaca* leaves are the plant wrappers most widely used in the agri-food industry in Côte d'Ivoire. In general, consumers prefer foods packaged with these two types of vegetable leaves, as they impart good presentation, texture and aroma to packaged foods. These two vegetable leaves are most often used to wrap foods such as attieké, akassa, Dokounou, abollo etc. (**Figure 1)**.

Physical and mechanical analysis

Before determining the physical and mechanical properties of the leaves, they were cleaned according to the traditional uses of the producers and users. Fresh leaves and those that had undergone flaming treatment were analyzed. Characteristics such as pH**,** moisture content, opacity, permeability, friability and water absorption were determined for vegetable leaves used as food packaging.

pH determination

The pH of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves was determined by immersing the pH meter's pre-calibrated glass electrode in 10 mL of supernatant. The supernatant was obtained after maceration of 10 g of *Thaumatococcus daniellii* or *Musa paradisiaca* sample in 75 mL of distilled water. The pH value displayed on the pH meter screen is taken as the pH of the sample.

Moisture content determination

Using the **AOAC (2000)** method, 5g of *Thaumatococcus daniellii* leaves and 5g of *Musa paradisiaca* leaves were weighed (**P0**) using a SARTORUIS BP 310S precision balance, Gottingen, West Germany. These 5g of *Thaumatococcus daniellii* leaves and 5g of *Musa paradisiaca* were dried in a branded oven (MEMERT, Schwabach West Germany), at 105°C for 24 h. On removal from the oven, the samples were cooled in a desiccator and the masses were weighed again (**P1**); the percentage moisture content was calculated according to the formula

$$\boxed{H = \frac{\mathbf{PO - P1}}{\mathbf{PO}} \times \mathbf{100}}$$

Determining packaging opacity

Opacity is a physical parameter that determines the relative permeability of vegetable film packaging to monochromatic light radiation. Opacity is used to assess the photo-protective effect of packaging on packaged foodstuffs. A high opacity for a package will better protect the foodstuff from photochemical deterioration.The opacity of *Thaumatococcus daniellii* and *Musa paradisiaca* leaves was determined by spectrophotometric measurement of absorbance at the following wavelengths : 330 nm, 500 nm, and 820 nm, corresponding, respectively, to the ultraviolet, infrared and visible light spectra. These wavelengths make it possible to assess the spectral range in which each package is most or least opaque.

In fact, to determine opacity, a volume of two (2) mL of pigment noise extract obtained after grinding each leaf was diluted to $1/10^{th}$ and $1/100^{th}$. The glass cuvettes, filled to 2/3 capacity, were inserted into the spectrophotometer's receiving tube, set to the chosen wavelength (330 nm, 500 nm and 820 nm), and the optical densities determined against a blank or controls. The monochromatic beam is then emitted onto the sample as soon as the control is started, and the opacity (OP) is determined from the optical density (OD) such that OD = log (OP) with $OP = 10^{DO}$. This opacity is determined by the following formula :

$$\boxed{\mathbf{OD = log\,(OP)}}$$

Determination of package permeability

The water permeability (**Pe**) of vegetable leaves was determined in order to assess the level of protection they provide foodstuffs against moisture. The test consisted in measuring the amount of water flowing through the tested leaves at 25°C after 4, 8, 16 and 24 hours of flow. Water permeability was determined by introducing 5 mL of water into a filter container containing 25^{cm2} of plant wrapping and fitted with a support for collecting the amount of water drained over time. Permeability was therefore determined according to the formula below, with **Pe :** flow rate of water ($g.cm^{-2}.h^{-1}$), **me :** mass of water flowing (g), **Sf :**

cross-sectional area of the leaf under consideration (^{cm2}), **te :** time taken for water to flow through the leaf (h). This permeability is determined by the following formula :

$$\boxed{Pe = \frac{me}{Sf.te}}$$

Determination of packaging friability

Friability characterizes the ease with which vegetable leaf packaging crumbles under the effect of heat. *Thaumatococcus daniellii* and *Musa paradisiaca* leaves were first cut and dried in a Pasteur oven at temperatures of 60°C, 80°C, 100°C and 120°C. For each of these temperatures (T°C), the different times required to obtain friability in the leaf packs were determined. The values recorded were used to plot the correlation between temperature and time required to obtain a given degree of friability (f(t) = T).

Determination of packaging water absorption capacity

The water absorption test on *Thaumatococcus daniellii* and *Musa paradisiaca* leaves enables us to assess the ability of these packages to keep food fresh. Absorption was measured over a temperature range from 35 to 105°C. The range was obtained by adding 15°C to the minimum temperature each time. This temperature range was chosen on the basis of the actual conditions under which vegetable leaves are used as food packaging.

Thus, 5x5 ^{cm2} square areas of each sectioned leaf were weighed (**mi**) and batches formed. Each batch was then immersed in a Marie bath at the indicated temperature for 20 minutes. After removal, all square surfaces were weighed again (**mf**). The variation in mass (**Δm**) of each sheet between before and after immersion was determined. This variation, which reflects absorption, is either a gain (**+Δm**) or a loss (**-Δm**) and is measured as follows: Δm**= (mf - mi) (g)**; with **mf:final mass** of immersed leaves, **mi: initial mass** of (non-immersed) leaves.

$$\boxed{\Delta m = (mf - mi)}$$

Statistical analysis

Survey data were processed manually and by computer. SPSS 20.0 was used to analyze questionnaire data. Excel was used to plot the curves. SPSS 20.0 was used for statistical analysis.

III. Results

Social and economic aspects of vegetable food packaging

The results of the survey showed that *Thaumatococcus daniellii* leaves were the most commercialized with 98%**(Figure 2**). The average daily income of between 0 and 5000 FCFA was mostly earned by women, with percentages ranging from 77% to 80%. Men, on the other hand, earned between 5,000 and 10,000 FCFA, with rates ranging from 92% to 96% **(Figure 3**).

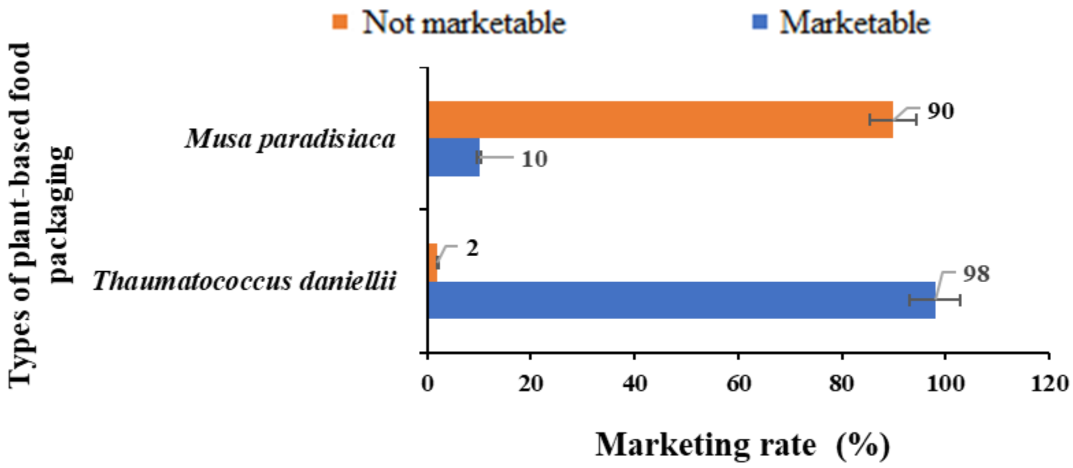

Figure2 : Level of marketing of plant-based packaging

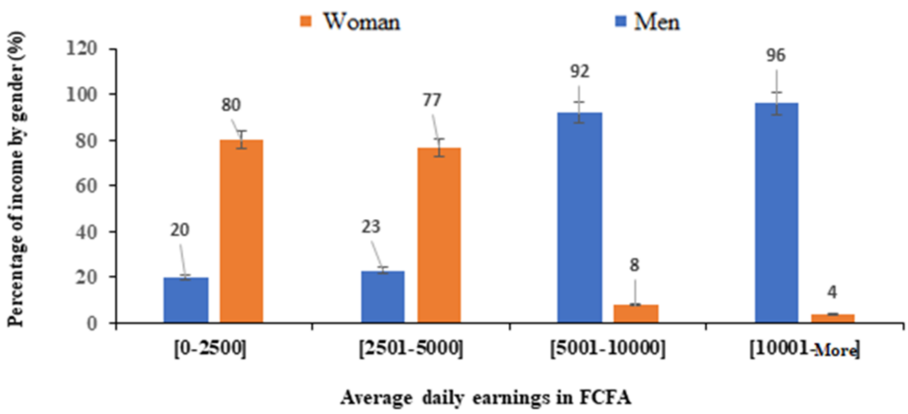

Figure3 : Daily income for plant packaging merchants

Physical and mechanical characteristics of vegetable packaging
Friability of vegetable food packaging

The results show that *Musa paradisiaca* leaves wilt less quickly than those of *Thaumatococcus daniellii*. The friability time of *Musa paradisiaca* leaves doubled (5 min to 50 min) at temperatures between 60°C and 120°C, compared with *Thaumatococcus daniellii* (2 min to 25 min) (**Figure 4)**.

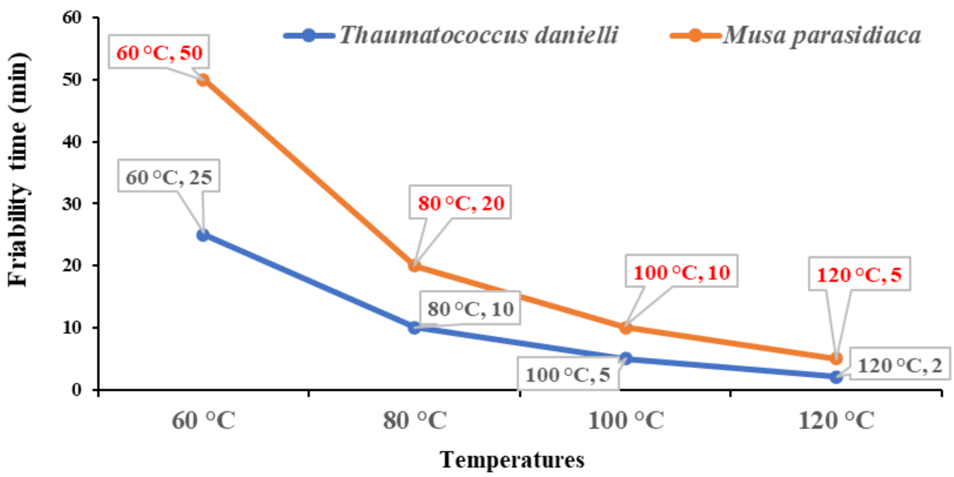

Figure4: Friability of vegetable food packaging

Opacity of vegetable food packaging

At each given wavelength, fresh *Thaumatococcus* and *Musa paradisiaca* leaves are more opaque than those that have undergone flambé treatment **(Table 1).** Fresh and flamed *Thaumatococcus* leaves (0.1 nm to 0.9 nm) remain more opaque than *Musaparadisiaca* leaves (0.1 nm to 0.5 nm).

Table1**: Opacity of plant-based food packaging**

Types of leaves analyzed diluted1/10th	Leaf Aspects	Wavelength range(nm)		
		330	500	820
T. daniellii	Fresh	0.9^a	0.3^a	0.2^a
	Outbreaks	0.7^b	0.2^b	0.1^b
M. paradisiaca	Fresh	0.5^c	0.3^c	0.1^c
	Outbreaks	0.4^d	0.2^d	0.1^d

Values with the same letters in the same column are not significantly different at the 5% threshold.

Absorption of food plant wrappings

Analysis of the results shows that for all temperature values (between 35 and 95°C), *Thaumatococcus* and *Musa paradisiaca* leaves absorb water (Δmmoy > 0) (**Figure 5).** However, on average, *Thaumatococcus* leaves (0.09) absorb more water than *Musa* leaves (0.07). Water uptake at 35°C and 80°C is identical and equal to 0.1 for both *Thaumatococcus daniellii* and *Musa paradisiaca*.

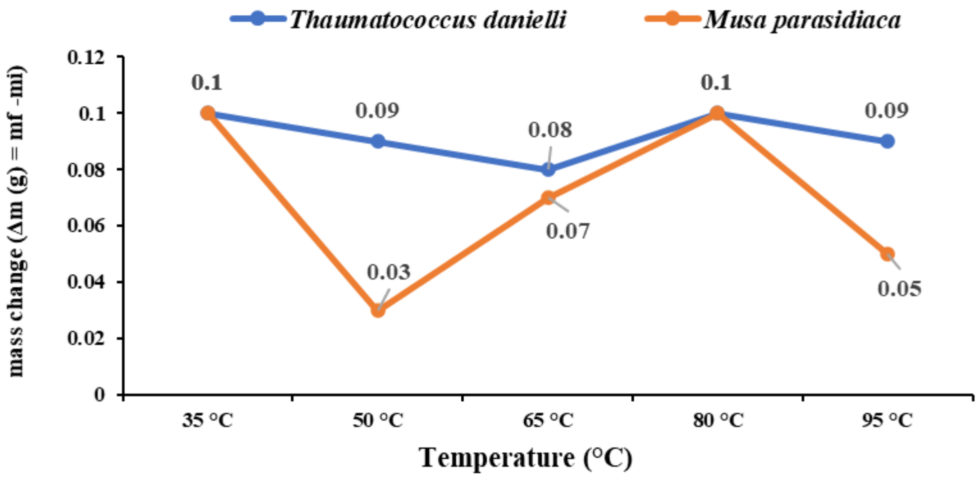

Figure5 : Absorption curve for plant-based food packaging

Moisture content, pH and permeability of vegetable food packages

We can see that for all plant species the pH is approximately neutral, with values between 6.8 and 6.9 (**Table II**). The moisture content of *Musa paradisiaca* (5.8) is higher than that of *Thaumatococcus daniellii* (4.5). The various tests carried out indicate that both types of plant leaf are impermeable.

TableII : Chemical and mechanical characteristics

Leaf types analyzed	Chemical and mechanical characteristics		
	pH	Humidity (%)	Permeability
T. daniellii	6.9	4.5	Waterproof
M. paradisiaca	6.8	5.8	Waterproof

IV. Discussion

Several foodstuffs are most often produced and/or sold from the leaves of different plant species **(Odoom *et al*., 2023 ; Ojekale *et al*., 2013**). Among these plant leaves, those of *Thaumatococcus daniellii* and *Musaparadisiaca* are the most widely used in Côte d'Ivoire as vegetable food packaging. Vegetable packaging is an important source of income for players in the sector and for the households that use it **(Al Mahmud *et al.*, 2024 ; Ouétchéhou*et al.*, 2021 ; Onzo*et al.*,2014a**).The results of the socio-economic survey indicated that *Thaumatococcus daniellii* leaves are the most widely traded, at 98%. This finding could be explained by the multiple uses of this type of leaf species (**OECD, 2022**).Indeed, *Thaumatococcus daniellii* leaves are used in traditional medicine for the treatment of various human and animal pathologies in Africa **(Ouétchéhou *et al.*, 2021**).

In addition, these types of plant leaves are used in the agri-food industry to produce, sell and protect foodstuffs. These same leaf species play an extremely important role in cultural practices (**Al Mahmud *et al*., 2024 ; Gadhave *et al*., 2018**).The survey data showed that the highest average income was earned by men. This is justified by the fact that the activity of selling vegetable food packaging is dominated by men and requires a great deal of physical effort or is often done on a large scale (**FAO, 2023**).

The results of this work also presented the physical and mechanical characteristics of these two types of foil. Thus, the ability of these plant leaves to resist heat indicated that, at equal temperatures, *Thaumatococcus daniellii* leaves reach their friability faster than those of *Musa paradisiaca*. This potentiality shows that it is prudent to pack warmer foodstuffs in *Musa paradisiaca* leaves, which could prevent food risk.Indeed, hot foods need to be packaged in heat-resistant food containers to avoid the transfer of certain toxic substances from the packaging to the food **(Al Mahmud *et al*., 2024 ; Hambleton, 2010)**.

Despite their good friability , *Musa paradisiaca* leaves are less opaque than those of *Thaumatococcus daniellii.* The higher the opacity of a package, the better it protects the food from photochemical degradation **(Ouétchéhou *et al.*, 2021**).This is because prolonged contact of solar ultraviolet rays with certain foodstuffs can lead to various reactions **(Andradya *et al*., 2023; Zhu *et al.*, 2020)**. Among these reactions, photo-oxidation is generally the cause of food spoilage and degradation **(Andradya *et al*., 2023; Jeantet, 2007)**. In all wavelength ranges, fresh leaves of both species analyzed were more opaque than heat-treated leaves.

This finding suggests that buckling may reduce the opacity of the packaging, and consequently its level of protection.In addition, this study has shown that water absorption is one of the main characteristics sought in a material used as food packaging. In fact, it provides information on the packaging's ability to keep food fresh. The results of this study even showed that for all temperature values (between 35 and 95°C), *Thaumatococcus* and *Musa paradisiaca* leaves absorb water (Δmmoy > 0).

Besides to this water absorption, these two species have a moisture content of 4.5 (*Thaumatococcus daniellii*) and 5.8 (*Musa paradisiaca*) respectively. This water absorption and moisture content of the two plant species used as packaging could favour microbial contamination of these packages. With approximately neutral pH values of between 6.8 and 6.9, both types of plant leaf are also impermeable to water. In the event of microbial contamination, the neutral pH of these plant leaves could contribute to the selection of neutrophilic microorganisms.

This study shows the need to valorize these types of biodegradable plant packaging **(Al Mahmud *et al*., 2024 ; Gadhave *et al*., 2018)**. However, a microbiological challenge and compliance with good hygiene practice measures remain necessary to guarantee better food safety and risk prevention.

V. Conclusion

This study has shown that the trade and use of vegetable leaf packaging contributes to improving the living conditions of those involved. It showed that *Musa paradisiaca* leaves have potential that can be used to design food packaging that is resistant to physical stress and chemically inert. On the other hand, *Thaumatococcus daniellii* leaves offer better protection against ultraviolet rays, and can therefore be used in the design of photo-protective packaging. In addition, the short friability time of *Thaumatococcus daniellii* leaves may make them practical, easily biodegradable packaging. This study could constitute a database for the valorization of these biodegradable leaf species with a view to protecting the environment.

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References

[1] Kalle Mm, Barry I, Bah Al, Keita M, Berete A, Duonamou L. Recycling Plastic Waste Into Eco-Friendly Paving Stones Using Local Materials Such As Sand, Clay And Laterite In Faranah (Republic Of Guinea). Am. J. Innov. Res. Appl. Sci. 2023. 17 : 117-128.

[2] Mujinga Ka, Mabika Kw, Nkashama Mjc, Mwamba Tbv, Kidimbu Dsi. « Plastic Packaging Issues In Kinshasa: Factors For The Adoption Of Ecological Behavior By Households », French Review Of Economics And Management. 2023. 4 : 292–316.

[3] Oecd (2022), Global Plastics Outlook: Economic Drivers, Environmental Impacts And Policy Options, Oecd Publishing, Paris, Https://Doi.Org/10.1787/De747aef-En. 201 P.

[4] Ouétchéhou R, Dabadé Ds, Vieira-Dalodé G, Sanoussi Af, Fagla-Amoussou Ab, And Azokpota Paulin. Bio-Based Packaging Used In Food Processing : A Critical Review. African Journal Of Food Science. 2021. 15 : 131-144.

[5] Al Mahmud Mz, Mobarak Mh, Hossain N. Emerging Trends In Biomaterials For Sustainable Food Packaging: A Comprehensive Review. Heliyon, 2024. 10 : E24122
[6] Ocde (2023), Global Plastics Outlook: Scenarios For Action To 2060, Editions Ocde, Paris, Https://Doi.Org/10.1787/C5abcbb1-Fr.
[7] Gadhave Rv, Das A, Mahanwar Pa, Gadekar P.T. Starch Based Bio-Plastics: The Future Of Sustainable Packaging. Open Journal Of Polymer Chemistry. 2018. 8 : 21-33.
[8] Aoac. Official Methods Of Analysis (13th Edn). Association Of Official Analytical Chemist: Washington, D C. 2000. 771p
[9] Fao. The Status Of Women In Agrifood Systems. Rome. 2023. Https://Doi.Org/10.4060/Cc5343en
[10] Hambleton A. Study Of The Transfer Of Small Molecules Through Edible Films Encapsulating Active Substances (Flavor). Thesis To Obtain The Degree Of Doctor Of Food Science From The University Of Burgundy, 2010. 121 P.
[11] Jeantet R, Croguennec T, Schuck P, Brule G. Science Des Aliments, 2 : Biochimie, Microbiologie, Procédés, Produits. Editions Tec Et Doc; 2007. 2-449.
[12] Andradya A.L, Barnesb P.W, Bornmanc J.F, Gouind T, Madroniche S, Whitef C.C, Zeppg R.G, Jansen M.A.K. Oxidation And Fragmentation Of Plastics In A Changing Environment; From Uv-Radiation To Biological Degradation. Epa Public Access, Sci Total Environ. 2023. 10 : 1-21.
[13] Zhu L, Zhao S, Bittar Tb, Stubbins A, Li D. Photochemical Dissolution Of Buoyant Microplastics To Dissolved Organic Carbon: Rates And Microbial Impacts. J. Hazard. Mater. 2020. 383, 121065. [Pubmed: 31518809]
[14] Odoom W, Osei-Asibey Rk, Tei-Mensah E, Ayebeng G, Asante Ta. Determination Of Phytochemicals In Thaumatococcus Daniellii (Sweet Prayer Leaves) And Musa Paradisiaca (Plantain Leaves) As A Food Packaging Material. International Journal Of Technology And Management Research. 2023. 8 : 1-14.
[15] Ojekale Ab, Oladipupo Al, Adeola As, Folorunso Os, Azeez Ii, Andy Ro. Volatile Constituents, Antioxidant And Insecticidal Activities Of Essential Oil From The Leaves Of Thaumatococcus Danielli (Benn.) Benth From Nigeria. Iosr Journal Of Pharmacy, 2013. 3 : 01-05.
[16] Onzo Fc, Aka S, Azokpota P, Benie Ckd, Dje Km, Bonfoh B. Diversity Of Traditional Foodstuffs Packaged In Plant Leaf Packaging In Ivory Coast, Agronomie Africaine. 2015. 27 : 155 -172 (2015).
[17] Onzo Fc, Aka S, Azokpota P, Dje Km, Bonfoh B. Mapping Of Plant Leaf Species Used As Food Packaging In Côte D'ivoire. Journal Of Scientific Research Of The University Of Lomé (Togo), Series A. 2014a. 16 : 19 - 36.
[18] Onzo F, Azokpota P, Agbani Gf, Hounhouigan Dj, Kossou D. Physicochemical, Phytochemical And Toxicity Characteristics Of Plant Species Used As Food Packaging In West Africa. International Journal Of Biological And Chemical Sciences. 2014b. 8 : 1504 -1516