

Chemical, Mechanical, Antioxidant, and Antimicrobial Properties of Paper Prepared from Cocoa Bean Shell Using Polyethylene Glycol

RH. Fitri Faradilla, Tamrin*, Muhammad Nuh Ibrahim, Sri Rejeki, Arwan Siala, Firmansyah

Food Science and Technology, Faculty of Agriculture, Universitas Halu Oleo, Kendari, Indonesia

*Corresponding author: tamrin@uho.ac.id

Received July 05, 2019; Revised August 09, 2019; Accepted August 17, 2019

Abstract Cocoa bean shell is an underutilised residue which can be processed into paper-like active packaging because of its fiber and phytochemical compounds that have antioxidant and antimicrobial properties. In this study, a minimal process was applied in making paper from cocoa bean shell. Paper making involved milling for size reduction, mixing with polyethylene glycol (PEG 1000 g/mole) as a plasticiser, and drying at low temperature (60°C). Chemical and heat treatment were avoided to preserve the antioxidant and antimicrobial properties of the shell. Since there was no cellulose isolation process, the resulted papers contained various components other than cellulose, such as hemicellulose, lignin, and inorganic materials. The addition of PEG in concentration of 5% improved the tensile strength and elongation of the paper. The presence of plasticiser did not affect the paper thermal stability, antioxidant capacity, and antimicrobial activity. The paper samples reduced DPPH radicals by approximately 75% and showed antimicrobial activity against *E. coli*, *S. aureus*, and *B. cereus*.

Keywords: cocoa bean shell paper, antioxidant, antimicrobial, packaging

Cite This Article: RH. Fitri Faradilla, Tamrin, Muhammad Nuh Ibrahim, Sri Rejeki, Arwan Siala, and Firmansyah, "Chemical, Mechanical, Antioxidant, and Antimicrobial Properties of Paper Prepared from Cocoa Bean Shell Using Polyethylene Glycol." *Journal of Food and Nutrition Research*, vol. 7, no. 8 (2019): 579-583. doi: 10.12691/jfnr-7-8-5.

1. Introduction

During the processing of a high value cocoa bean, various low value by-products, such as cocoa pods, mucilaginous pulp, and cocoa bean shell are also produced in relatively high quantity. Among these by-products, the utilisation of cocoa bean shell is the least studied. One of the reasons could be the proportion of cocoa bean shell that is the lowest compared to cocoa pods, mucilaginous pulp, and beans. The fraction of cocoa bean shell is about 10% of the total cocoa bean mass [1]. Indonesia is one of the largest cocoa producers in the world with the annual cocoa production of more than 800,000 tonnes [2] and availability of cocoa bean shell in Indonesia is abundant. This shell residue is mostly used as organic compost because their use as animal feed is limited due to high theobromine content [1,3].

Despite limited utilisation, the cocoa bean shell has high potential to be developed into higher value added products. Cocoa bean shell is known to have high antioxidant capacity and antimicrobial activity due to the presence of polyphenols [4]. Moreover, our previous study shows that this material is mainly composed of lignocelluloses with the cellulose content approximately 22% (dw), which is relatively higher in comparison to

algal biomass, orange peels, and lemon peels [5]. This makes the shell a potentially high source of raw material for paper making.

Although unlike plastic, paper is biodegradable, but paper production is not always eco-friendly. Most of the paper products are made from woods that grow for 10-20 years before they can be harvested. However, the wood availability is decreasing due to increase in deforestation [6]. The rate of paper production is higher than the rate of wood regeneration. Therefore, alternative sources for paper making are needed [6]. China is an example where paper from non-wood fibers is produced in large quantity [7]. The fibers used can be from straw, reed, cotton, bagasses, and bamboo [7]. However, there are no studies reported for paper made from cocoa bean shell fiber.

In traditional paper making, the biomass is cooked in alkaline and bleaching solution to remove impurities and collect cellulose [8]. This process may be applied to cocoa bean shell, but the phenolic compounds that have functional properties may most likely get damaged. In order to preserve the bioactive compounds of cocoa bean shell, the paper should be prepared with minimum contact with chemicals and heat treatment. Thus, in this research we aimed to characterise the properties of cocoa bean shell papers that were minimally processed. We also evaluated the effect of polyethylene glycol addition as a plasticiser. Results of this research are important to further

develop functional paper-based packaging from cocoa bean shell.

2. Materials and Methods

2.1. Materials

Cocoa bean shell was obtained from Kalla Kakao Industri, Kendari, Indonesia. Polyethylene glycol with molecular weight 1000 g/mole was purchased from Merck.

2.2. Paper Production

Dried cocoa bean shell was ground into fine flour. The flour was added with distilled water and milled in a ball mill for three hours. The milled shell was then added with polyethylene glycol (PEG) in the concentration of 0 (control), 5, 15, and 30% of shell flour. The resultant suspension was then poured into plastic petri dishes and dried in the oven (60°C, 12 hours).

2.3. Analysis

Prepared paper samples were characterised for their chemical, thermal, morphological, mechanical, antioxidant, and antimicrobial properties. **Chemical properties** of the paper samples were analysed using Fourier-transform infrared spectroscopy (FTIR) (TENSOR 27) and scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM-EDX) (JSM-6510) based on method ASTM E 1252-13 and ASTM E1508-12a, respectively. Samples were made into KBr pellets prior to analysis with FTIR.

Thermogravimetric analysis (TGA) was used to determine the **thermal properties** of the papers. Thermographic analysis (TGA) (TGA Mettler Toledo) was conducted using ASTM E 1131-14. The initial heat treatment was done from 50°C to 600°C with temperature increments of 10°C/min under nitrogen gas (50 mL/min). Samples were held for 5 mins at 600°C under nitrogen gas (50 mL/min) and continued with heat treatment up to 900°C (10°C/min) under oxygen gas (50 mL/min).

Morphology of the paper samples was characterised using scanning electron microscopy (SEM) (JSM-6510) after coating them with platinum for 45 s.

Mechanical properties (tensile strength and elongation at break) of the papers were analysed using tensile test (Shimadzu AGS-10kNG) using ASTM D882-11 with stretching speed 0.05 mm/min and grip distance 16 mm. The samples were conditioned at 23°C and 50% RH for 40 hours. The test was conducted at 22°C and 53% RH.

Antioxidant capacity was evaluated by the DPPH ((2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay. The DPPH solution was prepared by diluting 4 mg DPPH with absolute ethanol in a 100 mL volumetric flask. The solution was incubated for 2 hours in the dark before using. Paper sample (1 g) was macerated in absolute ethanol (16 mL) for 6 hours. After 6 hours, the filtrate was collected and the residue was re-macerated for another 6 hours. The filtrate from the second maceration was combined with first one. The extract was then mixed with DPPH solution in the ratio of 1:1 and incubated in the dark for 30 minutes. Absorbance was read using a

spectrophotometer at 517 nm. The antioxidant capacity data was compared between samples using one-way ANOVA and Tukey's test ($\alpha = 95\%$) (Minitab Pro 16.2.0.0).

Antimicrobial activity of the paper sample was analysed against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* in nutrient agar (NA). Samples were cut with paper hole punch for uniform size. The samples were placed on the NA that was swabbed with the bacterial culture. The media was then incubated for 24 hours at 37°C.

3. Results and Discussion

3.1. Chemical Properties

Chemical properties of the paper samples were characterised using FTIR and SEM-EDX. FTIR was used to detect the main chemical compounds in the papers, while SEM-EDX was used to determine elements present in the paper samples semi quantitatively. **Figure 1** shows the FTIR spectra of the samples. Cocoa bean shell is known to be rich in fibers, that include but not limited to, cellulose, hemicellulose, and lignin [4,9]. The FTIR spectra of the control paper show the wide peak at 3400-3200 cm^{-1} that indicate the presence of -OH groups. The -OH peak together with C-O(H) peak at 1050 cm^{-1} are fingerprints for the presence of cellulose [10]. Hemicellulose was indicated by the peak at 1730 cm^{-1} for its acetyl and uronic ester groups. Peak at 1730 cm^{-1} may be due to the ester linkage of carboxyl groups of ferulic and p-coumaric acids of lignin. Lignin was also indicated by the peak at 1540 cm^{-1} (the vibration of aromatic ring) and 1254 cm^{-1} (guaiacyl ring breathing with C=O stretching) [11].

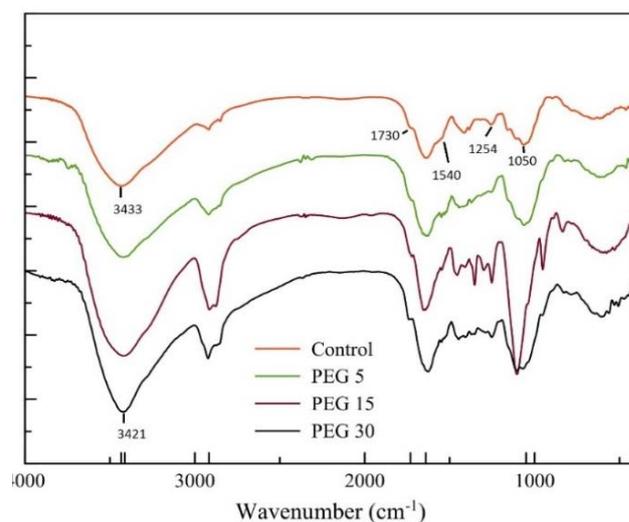


Figure 1. FTIR of paper samples.

The addition of 5, 15, and 30% of PEG modified the FTIR spectra of the samples relative to the control (**Figure 1**). PEG and cocoa bean shell components, especially the cellulose, may interact through hydrogen bonding. According to [12], hydrogen bonding interaction can be evaluated by IR spectra. The indication of hydrogen bonding is marked by peak shifting, increase in peak intensity, peak boardening, and the distortion of the

peak asymmetry [12]. As can be seen in Figure 1, the peak for -OH shifted from 3433 cm^{-1} in the control film to 3421 cm^{-1} for the PEG-plasticised samples. The intensity of the peak increased and the symmetry level changed. Thus, it could be concluded that the PEG formed hydrogen bonding with -OH groups of the cocoa bean shell. An obvious change could also be observed at the peak around 1050 cm^{-1} , which corresponds to C-O(H) group (Figure 1).

Besides lignocelluloses and PEG, the paper also contained several elements, such as Mg, Al, Si, S, K, Ca, and Zr, as indicated by SEM-EDX results in Table 1. These elements are from the cocoa bean shell since there was no added additive during the control paper making. One should note that the sample contained relatively high zirconium element (approximately 17.3%). The presence of minute amount of zirconium in food products has been well known since zirconium is 'the twentieth most common element on the Earth's surface' [13]. Zirconium itself also does not play any biological value and there is no adverse effect to human health reported [13]. However, according to [14], zirconium is relatively immobile in soil, hence the plant absorption of this metal is limited. Therefore, our finding, where the cocoa bean shell paper contained about 17% zirconium, should not be ignored. Further investigation is needed to discover the source of zirconium, the mechanism on how this metal was absorbed in large quantity in cocoa plant, and the implication of this metal to products derived from this cocoa plant.

Table 1. Elements in the Control Sample

No	Elements	Content* (% mass)
1	C	41.09 ± 0.28
2	O	31.55 ± 0.59
3	Mg	0.83 ± 0.02
4	Al	1.74 ± 0.02
5	Si	1.11 ± 0.04
6	S	1.05 ± 0.02
7	K	3.40 ± 0.29
8	Ca	1.97 ± 0.11
9	Zr	17.26 ± 0.39

*semi quantitative.

3.3. Thermal Properties

Thermal properties of the paper samples were analysed using TGA. The TGA curve is shown in Figure 2. The initial degradation temperature (T_i) of the control sample was around 200°C . This was similar to the degradation temperature of other non-wood biomass, such as bagasse, rice straw, and rice hulls [15]. However, the control paper sample degraded at lower temperature than the paper made of pineapple leaves fiber [16] and pulps from *Prunus amygdalus* and *Tamarisk sp.* [17] that have initial degradation temperature in the range of $230\text{--}250^\circ\text{C}$. The control paper had lower T_i than the later examples because the control sample was made of the whole cocoa bean shell, while paper made from pineapple leaf and the pulp undergo process to remove materials other than cellulose. The whole cocoa bean shell still contained protein and carbohydrate other than cellulose that would degrade at lower temperature than cellulose [4,18].

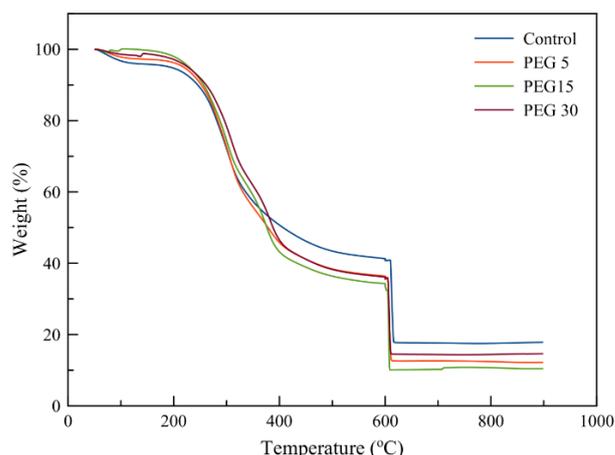


Figure 2. TGA curve of paper samples.

The addition of PEG did not change the initial degradation temperature of the paper (Figure 2). Hence, the thermal stability of the paper was not affected by the present of plasticiser. Our previous study [19] also showed similar results, where the use of PEG 1000 g/mole or higher have no effect on the initial degradation temperature of nanocellulose film from banana pseudo-stem. This was the reason PEG was chosen as the plasticiser in this study.

Although PEG did not affect the initial degradation temperature of the paper, it changed the TGA curve of the samples (Figure 2). The presence of PEG was indicated in TGA curve by a sudden drop at temperature closer to 355°C , which indicated the PEG decomposition [19]. This decomposition was more obvious in PEG 30 since this sample contained the highest amount of PEG.

The addition of PEG also affected the interaction between paper and water. Since PEG is polar, this plasticiser could hold water and its water holding ability can be inferred from the TGA curve in Figure 2 and Table 2, where the initial mass reduction at $50\text{--}150^\circ\text{C}$, which was the evaporation of moisture, was lower in PEG plasticised paper than in the control paper.

Table 2. Composition of the paper samples based on TGA results

	Unit	Highly Volatile Materials ^{a)}	Medium Volatile Materials ^{b)}	Combustible Matter ^{c)}	Inorganic Residues
Control	%	3.65	54.46	23.25	18.65
PEG5	%	2.61	41.84 & 18.97	23.49	13.09
PEG15	%	-	38.00 & 27.38	21.60	13.03
PEG30	%	-	35.95 & 26.31	21.38	16.37

a) Decomposition of organic material at $50\text{--}150^\circ\text{C}$ in N_2 gas

b) Decomposition of organic material at $150\text{--}600^\circ\text{C}$ in N_2 gas

c) Non-decomposed organic material up to 600°C in N_2 gas.

Another important information that can be deciphered from the TGA results is the amount of inorganic residue in the paper samples. There was about 13-18% of inorganic residue left after the TGA heating process (800°C) (Table 2). These residues were most likely composed of inorganic elements that were listed in Table 1. The presence of inorganic residues could affect the quality of the paper. The residue may act as filler that would improve the mechanical properties of the paper or disrupt the fiber network that adversely affects the paper strength [20]. Further investigation is needed to determine how exactly these residues affect the paper quality.

3.4. Morphology and Mechanical Properties

As mentioned earlier, paper samples in this research were prepared from the whole cocoa bean shell in order to preserve the bioactive compound in the raw material. As a result, the papers contained numerous amounts of compounds that are naturally present in the cocoa bean shell and caused uneven surface as can be seen in Figure 3. Heterogeneous components in the papers was also suspected to cause relatively large pores. The presence of PEG in the paper matrix was not obvious from the SEM images. This was desirable since it indicated that there was no phase separation between PEG and the paper matrix.

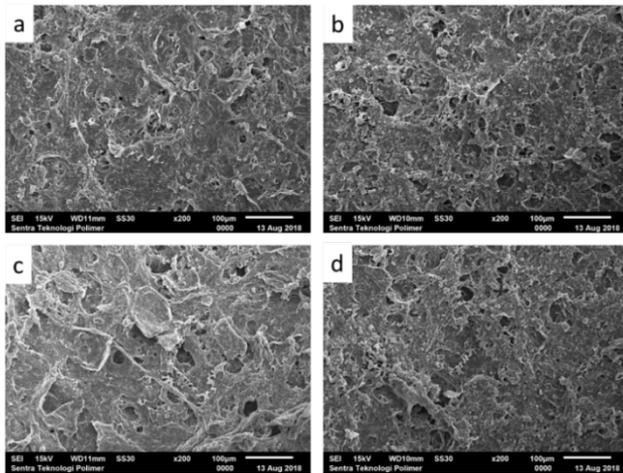


Figure 3. Morphology of the papers: a) control, b) PEG 5, c) PEG 15, d) PEG 30

The effect of PEG on the mechanical properties of the paper is shown in Table 3. The control paper had tensile strength of 4.54 MPa and strain at break 1.15%. The strength of the paper can be comparable with the paper made from the mixture of cassava bagasse and kraft paper that was reported by [21]. However, the control sample of the paper could not be folded as it was brittle and breaks when folded.

The addition of PEG could slightly improve the flexibility of the paper at low concentration (strain at break of PEG 5 was 1.35%). At higher concentration, PEG with molecular weight 1000 g/mole did not improve the strain at break of papers. However, all the plasticised papers were not brittle and foldable. The foldability of a paper is important, especially when the paper is intended for use in food packaging.

Table 3. Tensile strength and strain at break of paper samples

Samples	Tensile strength (MPa)	Strain at break (%)
Control	4,54 ± 0,91	1,15 ± 0,35
PEG 5	6,76 ± 1,27	1,35 ± 0,45
PEG 15	5,65 ± 1,34	1,07 ± 0,50
PEG 30	4,87 ± 0,61	1,10 ± 0,05

Tensile strength of the paper improved due to the addition of PEG. Although, the tensile strength of the plasticised papers decreased with increase in the concentration of PEG. At higher concentration, PEG may have trapped more moisture from the environment, which caused the paper to be softer. Thus, it can be concluded that the addition of PEG with molecular weight

1000 g/mole at concentration of 5% was the optimum amount for improving the mechanical properties of cocoa bean shell paper.

3.4. Antioxidant Capacity and Antimicrobial Activity

The primary aim of minimal processing of cocoa bean shell for paper production was to preserve the bioactive compounds that according to [4] have antioxidant and antimicrobial properties. Figure 4 shows the antioxidant capacity of the papers. As shown in Figure 4, the control sample had relatively high antioxidant capacity, which was approximately 83% and the presence of PEG did not significantly ($p > 0.05$) affect the antioxidant capacity of the papers. Plasticised samples had antioxidant capacity in the range of 69-77%. Thus, the process of paper making in this study had successfully preserved the antioxidant property of the cocoa bean shell. This also shows that the papers made from cocoa bean shell have great potential for use in packaging of fatty food and provide protection to the product from oxidation.

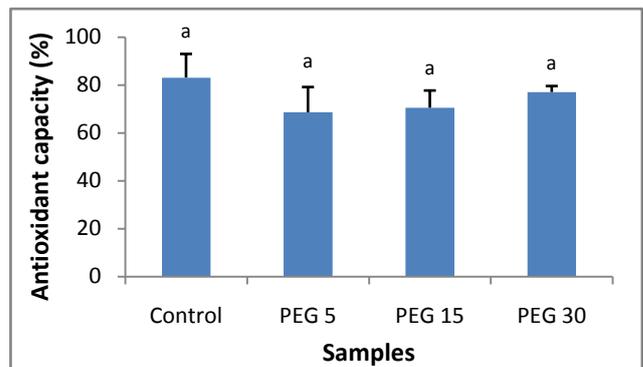


Figure 4. Antioxidant capacity of paper samples. Means that share the same letter are statistically the same ($p > 0.05$)

Antimicrobial property of the papers was evaluated against *E. coli*, *S. aureus*, and *B. cereus*. After one day incubation, the bacteria colonies covered almost entire surface of the agar media, except where the paper sample was placed. Figure 5 shows an example of how the *E. coli* colonies did not grow on the area with paper attached. This was also observed for other bacteria in all paper samples, which indicated that the papers had antibacterial properties. The addition of PEG neither improved nor reduced the antimicrobial properties of the cocoa bean shell papers.



Figure 5. Paper samples against *E. coli*. A control, B PEG 5, C PEG 15, and D PEG 30

4. Conclusion

Foldable papers that had antioxidant capacity and antimicrobial activity had been successfully made from cocoa bean shell with minimal processing and without chemical extraction and heat treatment. The paper samples contained compounds that are naturally present in cocoa bean shell, such as cellulose, hemicellulose, lignin, and inorganic materials. The paper samples prepared in this study also contained zirconium at relatively high quantity, which needs further investigation. The addition of plasticiser in paper making was crucial since the unplasticised paper was brittle and unfoldable. PEG with molecular weight 1000 g/mole as plasticiser improved the mechanical properties of the paper, especially in the concentration of 5%. This plasticiser did not affect thermal stability, antioxidant capacity, and antimicrobial activity of the paper. The paper extract could reduce the DPPH radical by approximately 75% and inhibit the growth of *E. coli*, *S. aureus*, and *B. cereus*.

Acknowledgements

The authors thank Program Insentif Riset Sistem Inovasi Nasional (INSINAS) for the research funding.

References

- [1] Adeyina, A., et al., Performance and physiological response of weaner rabbits fed hot water treated cocoa bean shell-based diet. *Res J Anim Vet Sci*, 2010. 5: p. 53-7.
- [2] Pusat Data dan Sistem Informasi Pertanian, Outlook Kakao Komoditas Pertanian Subsektor Perkebunan. 2016, Pusat Data dan Sistem Informasi Pertanian Kementerian Pertanian.
- [3] Olubamiwa, O., et al., Effect of boiling time on the utilization of cocoa bean shell in laying hen feeds. *International Journal of Poultry Science*, 2006. 5(12): p. 1137-1139.
- [4] Nsor-Atindana, J., et al., Quantification of total polyphenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) Bean Shells. *Pakistan Journal of Nutrition*, 2012. 11(7): p. 574.
- [5] Ververis, C., et al., Cellulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. *Bioresource Technology*, 2007. 98(2): p. 296-301.
- [6] Ashori, A., Nonwood fibers—A potential source of raw material in papermaking. *Polymer-Plastics Technology and Engineering*, 2006. 45(10): p. 1133-1136.
- [7] Hammett, A., et al., Non-wood fiber as an alternative to wood fiber in Chinas pulp and paper industry. *Holzforschung*, 2001. 55(2): p. 219-224.
- [8] Robertson, G.L., *Food packaging: principles and practice*. 2005: CRC press.
- [9] Redgwell, R., et al., Dietary fibre in cocoa shell: characterisation of component polysaccharides. *Food Chemistry*, 2003. 81(1): p. 103-112.
- [10] Yang, H., et al., Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel*, 2007. 86(12-13): p. 1781-1788.
- [11] Pelissari, F.M., P.J. do Amaral Sobral, and F.C. Menegalli, Isolation and characterization of cellulose nanofibers from banana peels. *Cellulose*, 2014. 21(1): p. 417-432.
- [12] Turhan, K., F. Sahbaz, and A. Güner, A spectrophotometric study of hydrogen bonding in methylcellulose-based edible films plasticized by polyethylene glycol. *Journal of Food Science*, 2001. 66(1): p. 59-62.
- [13] Reilly, C., *Metal contamination of food: its significance for food quality and human health*. 2008: John Wiley & Sons.
- [14] Shahid, M., et al., Behavior and impact of zirconium in the soil-plant system: plant uptake and phytotoxicity, in *Reviews of Environmental Contamination and Toxicology Volume 221*. 2013, Springer. p. 107-127.
- [15] Nassar, M.M., Kinetic studies on thermal degradation of nonwood plants. *Wood and fiber science*, 2007. 17(2): p. 266-273.
- [16] Laftah, W.A. and W.A.W.A. Rahaman, Chemical pulping of waste pineapple leaves fiber for kraft paper production. *Journal of Materials Research and Technology*, 2015. 4(3): p. 254-261.
- [17] Mechi, N., et al., Preparation of paper sheet from cellulosic fibres obtained from *Prunus amygdalus* and *Tamarisk* sp. *Cellulose chemistry and technology*, 2016. 7(8): p. 863-872.
- [18] Martínez, R., et al., Chemical, technological and in vitro antioxidant properties of cocoa (*Theobroma cacao* L.) co-products. *Food Research International*, 2012. 49(1): p. 39-45.
- [19] Faradilla, R.H.F., et al., Effect of polyethylene glycol (PEG) molecular weight and nanofillers on the properties of banana pseudostem nanocellulose films. *Carbohydrate Polymers*, 2018.
- [20] Xiao, Z., Papermaking Process Online Measurement and Control of Paper Ash Content. *Sensors & Transducers*, 2014. 174(7): p. 229.
- [21] Matsui, K.N., et al., Cassava bagasse-Kraft paper composites: analysis of influence of impregnation with starch acetate on tensile strength and water absorption properties. *Carbohydrate Polymers*, 2004. 55(3): p. 237-243.

