PHYSICOCHEMICAL ANALYSIS OF BIOFILMS FROM *Cocos nucifera* Linn. (COCONUT) FLOUR AND *Manihot esculenta* Crantz(CASSAVA) STARCH WITH POLYVINYL ALCOHOL (PVA)

ABSTRACT

The province of Northern Samar, its municipalities including Pambujan, is a place abundant of *Manihot esculenta* Crantz (cassava), and *Cocos nucifera* Linn. (coconut). However, its industry is mainly limited to food production. This study developed biofilms from cassava starch (CasS), and coconut flour (CoF) with polyvinyl alcohol (PVA). The physicochemical properties were analyzed to determine their pH, solubility, moisture content, and tensile strength. Functional groups were investigated using FTIR, and the antibacterial properties were evaluated. The results showed that both biofilms with PVA were weakly acidic and insoluble in all solvents. Meanwhile, CoF biofilm contained higher moisture content than the CasS biofilm. CoF biofilms also carried more weight and higher force than CasS biofilm. Hydroxyl, alkyl, and alkene functional groups were identified for both biofilms.Meanwhile, both biofilms showed no inhibitory effect against *E. coli*, and *S. aureus*. Therefore, the developed biofilms with PVA showed good physicochemical properties and can be used for packaging applications. The slight acidity can prolong their shelf-life. Moreover, both will not easily dissolve. CoF biofilm comprised more water than CasS but both are still suitable for packaging due to their tensile strength attributed to the presence of the functional groups. However, CasS, CoF, and PVA do not have antibacterial properties. More physicochemical tests, further characterization, and incorporation of antibacterial agents were recommended.

Keywords: biofilm, flour, physicochemical analysis, polyvinyl alcohol, starch

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I. INTRODUCTION

The Philippines is an agricultural country. In fact, most Filipinos live in rural areas and support themselves through agricultural activities. Almost 10 percent of the country's gross domestic product or GDP in 2021 was attributed from the 1.76 trillion gross value addedor

GVAof the agricultural sector (Statista, 2022). Both cassava and coconut are products of this sector, and are mainly used for food production.

Cassava, also called manioc, mandioca, or yuca in other parts of the world, is a perennial plant that produces edible roots (OECD, 2016). These roots contain high starch and carbohydrate contents (Abotbina *et al*., 2022). Accordingly, cassava starch is already used to create biodegradable films (Souza *et al*., 2012). Meanwhile, coconut tree or "The Tree of Life," as Filipinos call it, produces a fruit enclosed in a hard shell (Ahuja *et al*., 2014). Inside this fruit are thirst-quenching juice, and edible meat. This meat is also processed to make flour which contains more fat, protein, and fiber compared to wheat flour (WebMD, 2023).

This work developed biofilms from cassava starch, and coconut flour with polyvinyl alcohol (PVA). Biodegradable films or biofilms are not new and play an important role in food packaging. But due to its components such as starch, biofilms possess poor physical and mechanical properties (Gupta *et al*., 2022). Therefore, plasticizers such as glycerol and polyvinyl alcohol (PVA) are added. Glycerol helps in changing the interface between adjacent molecules in the polymetric starch chain (Almeida *et al*, 2022).Another plasticizer that eliminates brittleness as well as high water sensitivity of biofilms is PVA, therefore, improvingits stability, elasticity, and edibility. Polyvinyl alcohol is exceptionally compatible with starch because of the hydroxyl groups it contains. These functional groups create the intermolecular and intramolecular hydrogen bonds with thermoplastic starch and increases the quality of the formulation(Syamani *et al.*, 2020).

Zehra (2022) found that the addition of PVA increased both the moisture content and solubility of Water Chestnut Starch film. In terms of tensile strength, Ezeoha and Ezenwanne (2013) and Larotonda (2004) observed that glycerol made the films more malleable. Additionally, PVA also strengthens the biofilms because of the intermolecular interactions of the components through hydrogen bond formation (Syamani *et al.*, 2020). On the other hand, further reinforcements of acids and essential oils promoted antibacterial activity to biofilms.

Thus, this study generated products as well as processes for practical, and economic advantages. People from the business, and farming sectors could potentially benefit from these products. Particularly, farmers could expect more demand for their crops; businessmen could manufacture and market these biofilms; and ordinary people could gain extra income for selling their previously discarded cassava starch and coconut pulp.

II. METHODOLOGY

The *Manihot esculenta* Crantz (cassava), and *Cocos nucifera* Linn. (coconut) used in this study were collected at Barangay Cababto-an, Pambujan, Northern Samar. Pre-treatment methods, extraction of samples, development of biofilms, and the physicochemical tests, and FTIR characterization were conducted at the Bio-Physical Laboratory Complex, College of Science

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while the antibacterial susceptibility tests were done at the Integrated Research Laboratory both situated at the University of Eastern Philippines, University Town, Northern Samar.

Isolation of Cassava Starch

Afoakwa's (2011) sedimentation method was employed, with modifications. The roots were manually grated, then milled with 800 mL of water using blender. The slurry sample was filtered through a clean cheese cloth. The solids that retained by the cloth were washed with distilled water and further filtered through cheese cloth. The starch sedimented overnight and the liquid was discarded. The starch was dried under room temperature until completely dry.

Preparation of Biofilm from Cassava Starch with PVA

The preparation was done using Cui-Lim and Bangco's (2017) method, with modifications. PVA solution was prepared. The solution was used to prepare the starch dispersion at 10% (w/v). Drops of sorbitol, and glycerol were added as plasticizer. Starch, and PVA biocomposites were heated to 40°C-60°C and held to allow gelatinization. Upon completion, the solution was casted on a petri dish. The prepared solution was dried in an oven at 100 °C for 15 minutes. Then, it was further dried for 3-5 days. Dried films were peeled and stored in a desiccator at room temperature until experimentation.

Making of Coconut Flour

Coconut meat was scraped and transferred to a blender with distilled water. Blended on high speed until the pulp was smooth. Strained the contents of the blender through a cheese cloth. Saved the pulp retained inside of the cloth for the flour. Preheated the oven to 150 degrees Fahrenheit (77^{o}C). Transferred the coconut pulp to the baking sheet and spread over the parchment paper. Baked the pulp for one and a half hour until it felt completely dry to the touch. Transferred the dehydrated pulp to a grinder. Blended until it appeared finely ground. Stored the flour in an airtight container until use.

Preparation of Coconut Flour Solution

To make the coconut flour solution, coconut flour and distilled water were mixed and boiled for 5-7 minutes. Filtered the solution, discarded the residue, and kept the filtrate until use.

Preparation of Biofilm from Coconut flour with PVA

The preparation of biofilm from coconut flour with PVA was also done using Cui-Lim and Bangco's (2017) method, with modifications.

Determination of the Physicochemical Properties of Biofilms from Cassava Starch, and Coconut Flour with PVA

pH

The pH of the samples was determined using a pH meter. It was done in three trials.

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Solubility

Three (3) solvents were used, namely; hexane, water, and ethanol. Each biofilm was be placed into a clear test tube. Then, each test tube was added with each solvent. The test tubes were observed to determine the solubility of the samples. The results were recorded either as soluble or insoluble. Three trials were done for solubility determination.

Moisture Content

Films were conditioned at 58% relative humidity (RH) and 25 ℃ for seven (7) days. The weight difference was determined before and after drying of the equilibrated films in an oven at 105 ℃ for 24h (Nafchi *et. al.*, 2012; Dagalea, Lim, 2021).

The formula for moisture content is:

$$Moisture\ content\ (\%) = \frac{initial\ weight\ of\ film - final\ dried\ weight\ of\ film}{initial\ weight\ of\ film}\ x\ 100$$

Tensile Strength

Caballa and Getalado's (2019) method was used, with modifications. In this test, the ends of the biofilm were hooked in a spring scale gauge above and in a carrier below. Then, different weights were put into the carrier until the biofilm broke. The quantity of the weight as well as the force was recorded. This test was done in three trials.

Determination of Functional Groups using Fourier-Transform Infrared Spectroscopy

The characterization was carried out using the FTIR. Strips of cassava starch biofilm, and coconut flour film were placed into the FTIR machine and analyzed for their functional groups.

Determination of Antibacterial Property

In determining the antibacterial properties of the films, Dagalea's (2018) procedure was used. First, all equipment were sterilized using autoclave. Then to determine the antibacterial activity of the biofilms against *Escherichia coli*, and *Staphylococcus aureus*, the Kirby Bauer antimicrobial test was employed. In this test, each of the subculture pure isolates of the bacteria was aseptically harvested into the surface of the cultured plates by using inoculation loops. Next, filter papers, as positive controls, were soaked with the solution of Clindamycin for gram-positive bacteria, and with Cefalexin for gram-negative bacteria, and as negative controls in distilled water. Filter papers were also soaked in the biofilm solutions. The soaked discs were aseptically and carefully impregnated into the surface of nutrient agar using sterile tweezers. Each disc was be

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placed with a space from each other. The inoculated plates were incubated at 37ºC for 18-24 hours. After this, the plates were inspected for the existence of any clear Zone of Inhibition around the discs. Lastly, any Zone of Inhibition was measured using a Vernier caliper.

III. RESULTS AND DISCUSSION

As shown in Table 1, the pH of the cassava starch biofilm was 6.25 while the pH of the coconut flour biofilm was 6.09 which were both interpreted as weakly acidic. Just like foods, a slight acidity of the developed biofilms can prolong their shelf-life which means that microorganisms would take a little more time to grow in them.

Additionally, all biofilms were insoluble to both polar, water and ethanol, and non-polar, hexane, solvents. If the developed biofilms will be used as a commercial food packaging, it will not easily dissolve. However, the additionof PVA significantly increased the solubility of the composite films (Zehra *et al.,* 2022). While when ferulic or cinnamic acid is added,the film's water solubility (WS) was reduced (Ordonez *et al.,* 2021) and citric acid can enhance the water solubility of the film (Wu *et al.,* 2017).

In terms of moisture content (MC), coconut flour biofilm has more water content than cassava starch biofilm as MC is directly associated to the water content of a sample or product (Mermelstein, 2009). PVA increased free hydroxyl groups which permitted to produce hydrogen bonds with water molecules and hence, moisture content is increased (Zehra *et al.,* 2022).

Results on tensile strength revealed good values. Thus, the developed biofilms can be used for packaging because when stretched, they render better tensile strength and is not easily torn (Aminingsih *et al.,* 2021). PVA contributed to the increase in the strength and flexibility of the composite (Zehra *et al.,* 2022).

Table 1. Summary of the Physicochemical Properties of Biofilms from Cassava Starch, and Coconut Flour with PVA

Parameters	Cassava Starch Biofilm	Coconut Flour Biofilm
pH	6.25	6.09
Solubility	Insoluble	Insoluble
Moisture content	16.23%	43.15%
Tensile strength	89.5g, 0.6 N	137.8 g, 1.07 N

Figure 1 shows that the cassava starch biofilm registered an IR spectra of -OH at 3294.69 cm-1, -CH at 2934.26 cm-1, and C=H at 1646.26 cm-1 while coconut flour biofilm registered an IR spectra of -OH at 3306.72 cm-1, -CH at 2942.09 cm-1, and C=H at 1712.86 cm-1. PVA, with an exceptional compatibility with starch (Judawisastra *et al.,* 2017) enhanced the properties of the developed biofilms given its higher strength, tear, and flexibility (Karaogul *et al.,* 2018).

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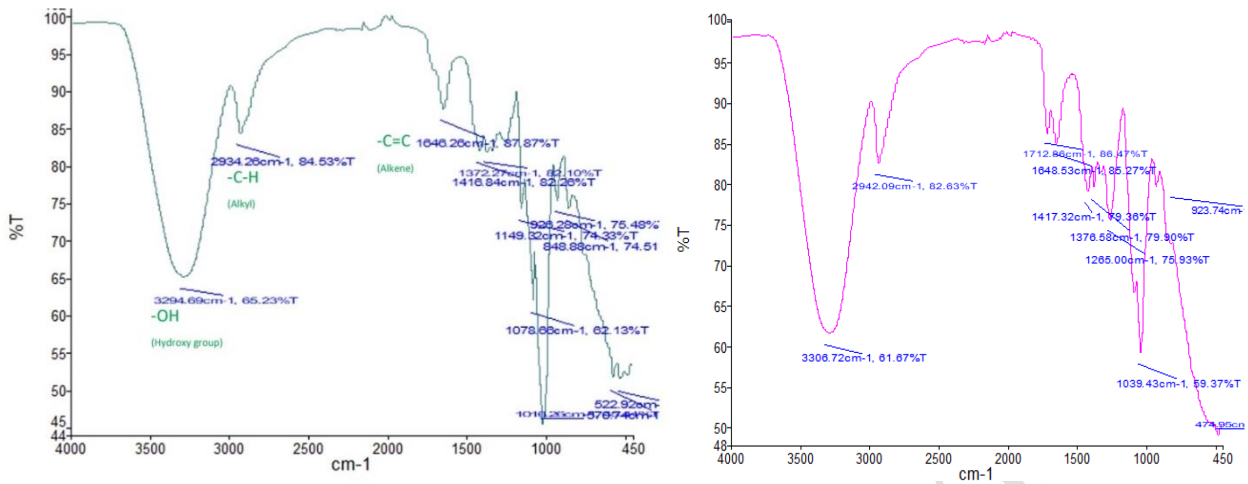

Figure 1. FTIR Spectra of Cassava Starch Biofilm, and Coconut Flour Biofilm with PVA

Table 2 shows that the developed biofilms from cassava starch, and coconut flour with PVA have no inhibitory effects against *E. coli*, and *S. aureus*. Meanwhile, the positive controls, Cefalexin and Clindamycin, have 30.7 mm and 31.0 mm Zone of Inhibitions against *E. coli*, respectively. Moreover, Cefalexin and Clindamycin, have 26.0 mm and 32.0 mm Zone of Inhibitions against *S. aureus*, respectively. Results of all the susceptibility tests suggest that cassava starch, coconut flour, as well as PVA do not have antibacterial properties. However, further reinforcements of acids and essential oils promoted antibacterial activity to biofilms. Both cinnamic and ferulic acids granted antibacterial activity to the starch films against *E. coli* and *L. innocua* (Ordonez *et al*., 2021). While films with essential oils (EO) such as cinnamon EO also generated a zone of inhibition because CEO contains a high concentration of trans-cinnamaldehyde, a well-known antimicrobial compound with linalool, eugenol, and other phenolic compounds, which work against the possible microbial growth (More *et al*., 2020).

Table 2. Comparative Chart of the Zone of Inhibition of Cassava Starch Biofilm, and Coconut Flour Biofilm with PVA against *E. coli* and *S. aureus*

	Zone of Inhibition against *Escherichia coli* (mm)
Cassava Starch Biofilm	0
Cefalexin (+ control)	30.7
Distilled Water (- control)	0
Coconut Flour Biofilm	0
Cefalexin (+ control)	31.0

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Distilled Water (- control)	0
	Zone of Inhibition against *Staphylococcus aureus* (mm)
Cassava Starch Biofilm	0
Clindamycin (+ control)	26.0
Distilled Water (- control)	0
Coconut Flour Biofilm	0
Clindamycin (+ control)	32.0
Distilled Water (- control)	0

IV. CONCLUSION

The findings of this study revealed that cassava starch, and coconut flour can be used to develop biofilms with additives and plasticizers such as glycerol, sorbitol, and PVA. Furthermore, the developed biofilms showed good physicochemical properties with the presence of alkyl, alkene, and hydroxyl functional groups. Hence, they can be used for packaging applications for foods, and even drugs or medicines. However, cassava starch, coconut flour, as well as PVA do not have antibacterial properties.

These findings necessitate for more physicochemical tests such as color evaluation, texture analysis, film thickness, and shelf-life as well as characterization like TGA, SEM, and UV-Vis on biofilms with different concentrations of cassava starch, coconut flour, and PVA. Also, the incorporation of proven antibacterial agents, acids and essential oils for instance, can enhance the formulation.

The results of this work serve as a call for the business and farming sectors, and government agencies to develop the industry. As a starting point, relevant funding of programs and projects can be initiated. This will primarily give economic benefits to the people.

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