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Development of Active Packaging Films Using Cacao (*Theobroma cacao* L.) Pods and Calamondin (*Citrus x microcarpa* Bunge) Peels

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This paper presents the properties of active films produced using cacao pods and calamondin peels. An active film is a type of packaging material that is made by incorporating the "active" ingredients for preservation on the packaging film rather than a direct application to foods. In this study, the conversion to active films was done by adding essential oils from lemongrass or calamondin peels, which are both known to exhibit antimicrobial properties. There were five film samples prepared for analysis: cacao-calamondin mixture; cacao-calamondin mixture with lemongrass essential oil; cacao-calamondin mixture with calamondin essential oil; cacao-calamondin mixture with encapsulated lemongrass essential oil; cacao-calamondin mixture with encapsulated calamondin essential oil; and two more samples for other analyses needing films from pure cacao and calamondin samples. The infrared vibration spectra revealed the presence of alcohol-, carboxylate, alkane-, and ether- containing compounds. The X-ray diffraction (XRD) patterns showed that the samples were amorphous and the Field Emission Scanning Electron Microscopy (FESEM) provided a visualization of the structures of the films produced. Other analyses conducted on the films included film thickness, moisture content, water activity, texture, oxidative stability, solubility, microbial, and antioxidant activity. The results are comparable to biodegradable films produced from other sources and the ones available in the market, which highlight the remarkable potential of films produced using cacao pods and calamondin peels activated by either lemongrass or calamondin essential oils to be further studied and developed for pilot and industrial productions.

Keywords: active packaging, biodegradable films, cacao pods, calamondin peels, characterization, essential oils

INTRODUCTION

The growing environmental concern about the use of packaging materials from non-biodegradable synthetic polymer sources channeled extensive studies on the development of new packaging materials that are based on naturally occurring biodegradable polymers. Packaging films made from fruits and vegetables are receiving much attention because of their availability, facile processing (Wu et al. 2019), and biodegradability. The commonly used parts of plants for the fabrication of packaging films are their waste materials such as fruit peels, which are known to contain rich amounts of biopolymers and bioactive compounds that are considered good substrates for packaging films (Andrade et al. 2016). However, films based on pure biopolymers have some limitations such as high solubility. Thus, one alternative to producing films with improved functional properties is mixing two plant polymer sources such as cacao and calamondin. Blending of natural polymers is an innovative approach to improve functional film characteristics (Ghaderi et al. 2019).The growing production of cacao and calamondin in the Zamboanga Peninsula will result in the undesirable accumulation of cacao pods and calamondin peels, which are considered waste materials. This situation, as well as the pressing environmental concerns on the use of plastic packaging, propelled the researchers to develop packaging films using cacao pods and calamondin peels. Cacao is grown in the tropics and the Philippines is among the countries in Asia that have an advantage for cacao production because of its strategic location and climatic condition. However, cacao is sold mainly for cocoa, the chocolate powder made from roasted and ground cacao seeds. This leaves up to 67 – 76% of unutilized cacao fruit parts consequently considered waste materials (Campos-Vega et al. 2018). This waste presents economic and environmental challenges that need to be addressed. Similarly, calamondin is another endemic plant in the Zamboanga Peninsula, which is known for its vitamin C content. It is sold mainly for its pulp and juice, but its peels and seeds are considered as waste. This study developed a packaging film from mixtures of the unutilized and waste materials of calamondin and cacao, which are its peels and pods, respectively. The film was converted into active packaging using lemongrass (*Cymbopogon citratus*) or calamondin (*Citrus x microcarpa* Bunge) essential oils.

Active packaging is a potential class of biodegradable plant-based packaging that is made by incorporating the active components for food preservation such as antioxidants and antimicrobial agents into the packaging material rather than a direct application of these components into foods (Almasi et al. 2020). Plant-based essential oils are generally recognized as safe (GRAS) food additives. They have received much attention recently because of their antioxidant and antimicrobial activities, which make them potential active agents in the production of active films. However, Atarés and Chiralt (2016) reported that the incorporation of essential oils poses challenges such as poor miscibility and phase separation during the film formation, the sensitivity of the bioactive compounds against environmental factors, and inconsistency in the transparency of the film. Moreover, a decreased shelf life of food due to the rapid migration of the active components in the film was reported in the study of Ribeiro-Santos et al. (2017). In the hopes of overcoming these challenges, essential oils were encapsulated using β-cyclodextrin to sustain the delivery system of the bioactive components. While there are several other coating materials including other polysaccharides, synthetic and biodegradable polymers, inorganic materials such as clays, silicates, and proteins such as gelatin, and casein (Capelezzo et al. 2018), the use of cyclodextrin was chosen because of its chemical and physical stability (Szejtli 1998) and because its central cavity has a hydrophobic character while its surrounding walls are hydrophilic. This allows cyclodextrins to form capsules, acting as a host for lipophilic compounds in their cavities and forming inclusion complexes (Rakmai et al. 2017). Among the cyclodextrins, β-cyclodextrin was used because it is 20 times cheaper than the other cyclodextrins and is perceived as economically viable in case of an upscaled production.

Several studies have been conducted on blending biopolymers to create a packaging film but to the best of the researchers' knowledge and reviews, no study on calamondin-cacao films activated by encapsulated lemongrass and calamondin essential oils has been conducted yet. This propelled the researchers to: 1) develop active packaging film using cacao pods and calamondin peels; and 2) determine the properties of the active packaging film developed. The development of a sustainable product will address not only the issues of the use of plastics but also the undesirable accrual of wastes from cacao and calamondin, which are evident in the province of Zamboanga del Sur. The study was also conducted to maximize the outputs that can be generated from the plant sources to provide potential additional income opportunities among local farmers and growers of cacao and calamondin.

MATERIALS AND METHODS

Preparation of Samples

The samples were provided by local cacao and calamondin growers. The cacao pods were acquired from BAC Jazz Farm, Malangas, Zamboanga Sibugay while the calamondin peels were acquired from Deli Foodline, Molave, Zamboanga del Sur. The samples were washed with tap water and dried until negligible moisture.

Extraction of Essential Oils

The calamondin (*Citrus x microcarpa* Bunge) and lemongrass (*Cymbopogon citratus*) oils were extracted via hydrodistillation using a Clevenger apparatus. This was done by passing steam into a round-bottomed flask containing 300 g of the sample for about 2 h. Condensate (water and oil) was allowed to separate directly and the oil was collected. The oil was then stored in preparation for the film formation.

Encapsulation of Essential Oils

Encapsulation of the essential oils was done by measuring approximately 0.90 g of the oil and mixing it in approximately 9 g of hydroxypropyl beta-cyclodextrin. The mixture was then transferred to a mortar added with 3 mL of absolute ethanol and kneaded for 1 h. The kneaded mixture was oven-dried for 24 h at 50°C and stored in a refrigerator for about 12 h.

Preparation of Active Films

The method for the preparation of active films was adopted from the methods described by Azmin et al. (2020) (pretreatment of the samples); Khorrami et al. (2021) (formation of films); and from Almasi et al. (2020) (conversion to active films).

About 100 g of the dried samples were soaked separately in 1 L of 2.5% Na_2CO_3 solution for 12 h. The solution was then cooked for 1 h, cooled to room temperature, and blended for homogenization. The active packaging film was prepared by dispersion of 1 g of alginate in 60 mL water. About 1 g of the cacao pods sample and 1 g of calamondin peels sample were then added to the mixture before the 2 g of xanthan gum and 10 mL of glycerin were added. The mixture was stirred until it was homogenous. About 0.1 g of the essential oil sample was then added to the mixture and allowed to settle to room

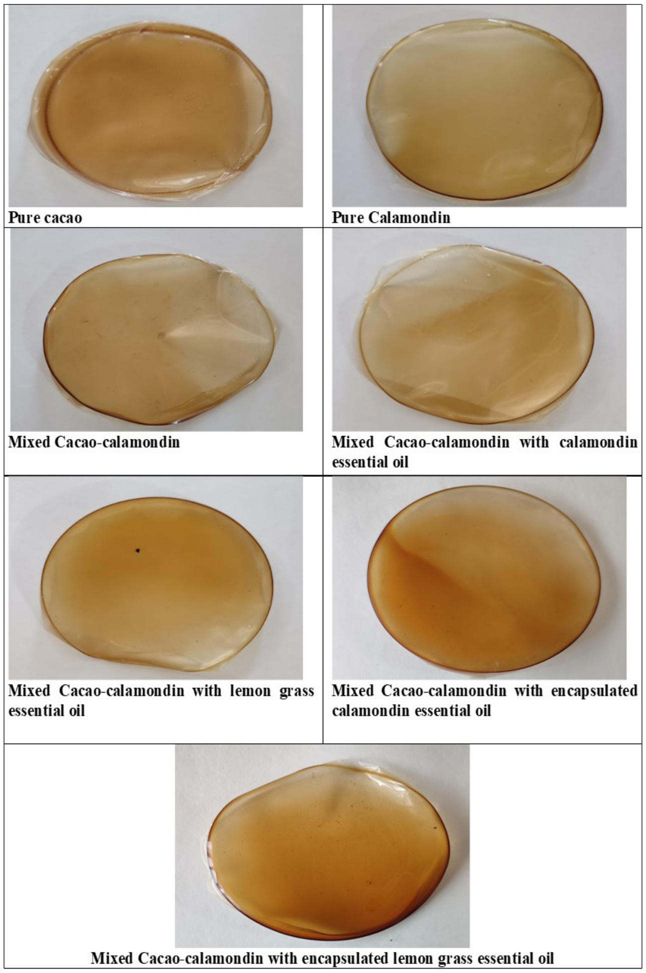

Fig. 1. Images of film samples.

temperature for 2 h before it was transferred to the oven and dried for 24 h at 70°C. The images of the different films produced are shown in Fig. 1.

Characterization of the Packaging Films

Fourier Transform Infrared (FT-IR) Spectroscopy

The structural interactions in the film samples were investigated using Perkin Elmer Fourier Transform Infrared (FT-IR) Spectrometer Frontier via Attenuated Total Reflectance (ATR) technique available at the Advanced Device and Materials Testing Laboratory (ADMATEL) of the Department of Science and Technology (DOST)-Industrial Technology Development Institute (ITDI). The spectra were collected over the wave number range of 4000 – 600 cm^{-1}. A total of 5 samples were sent for analysis: cacao + calamondin; cacao + calamondin + lemongrass essential oil; cacao+ calamondin + calamondin essential oil; cacao + calamondin + encapsulated lemongrass essential oil; and cacao + calamondin + encapsulated calamondin essential oil. There were 20 scans conducted for the 5 samples and a baseline correction was applied to the spectrum to improve its quality without distorting the band intensities in the final spectrum.

X-ray Diffraction (XRD) Analysis

The XRD patterns of the film samples were recorded using the X-ray diffractometer (Shimadzu LabX-6000) available at the Materials Science Division (MSD) of DOST I0TDI. The film samples were cut into circles with a diameter of 20 mm and about 3 – 4 pieces were piled up to fill the sample holder. The samples were then examined under a voltage of 40 kV and a current of 30 mA in the scanning range of 2.000 – 70.000 with a scanning speed of 1.0/min.

Morphology of Films

The surface morphology of the samples was evaluated using field emission scanning electron microscopy Dual Beam Helios Nanolab 600i available at ADMATEL DOST-ITD at an accelerated voltage of 15.00 kV with a beam current of 0.17 nA. The films were cut into small pieces and sputter-coated with Au prior to analysis to obtain high-resolution images.

Film Thickness

The thickness of the film samples was determined using a digital micrometer. Measurements were performed at 5 random points of each film sample to calculate the average value.

Moisture Content Analysis

About 5 g of the samples were analyzed using a Kern DBS 60 IR moisture analyzer set at 105°C available at the University of the Philippines Visayas-Regional Research Center (UPV-RRC).

Water Activity

Approximately 5 g of the samples were analyzed for water activity using Rotronic HP23-AW-A (quick mode) available at UPV-RRC.

Texture Analysis

The basic tensile strength of the 80 x 20 mm samples (50.00 mm target) was measured using Brookfield Ametel CT3-10K available at UPV RRC. The trigger load was set to 10 g with test and return speeds of 1.00 mm/s and 2.00 mm/s, respectively.

Oxidative Stability Test

The oxidative stability test was carried out using Metrohm 892 Professional Rancimat available at UPV-RRC, set at 100, 150°C under a constant gas flow of 20 L/h. The induction times were printed automatically by the apparatus software.

Solubility

The solubility of the film was measured according to Farahnaky et al. (2013). Initial dry weights of the film specimens (1 cm x 1 cm) were separately recorded. Each film was immersed in a beaker containing 50 mL of distilled water with periodical stirring for 2 h. Undissolved parts were collected and air-dried for another 24 h to obtain the final dry weight. The percentage of the total soluble matter was determined using the following equation:

$$\%\ Solubility = \frac{Initial\ dry\ mass - final\ dry\ mass}{Initial\ dry\ mass} x\ 100$$

Microbial Analysis

The microbial analysis of the films was conducted according to the method described in the Bacteriological Analytical Manual of the Food and Drugs Authority, where film-forming solutions were subjected to microbial analysis through BDJ Aquaculture and Environment Consultancy Services.

Antioxidant Property

The antioxidant activity of the films was assessed using the DPPH radical scavenging assay. A 100-mg film sample was dissolved in 2 mL of distilled water by continuous stirring for 2 min, and 1 mL of film extract solution was added to 0.2 mL of DPPH solutions (0.04 g/L) in ethanol. The mixture was vortexed vigorously and kept in the darkroom for 30 min at room temperature. The reduction in absorbance at 517 nm was determined via a UV–Vis spectrophotometer available at the Department of Chemistry, University of the Philippines Visayas. Finally, the antioxidant activity was measured as the percentage of DPPH free radical scavenging activity using the following equation (Zhu et al. 2018):

$$\%\ Free\ radical\ scavenging\ activity = \frac{|Absorbance\ of\ control - Absorbance\ of\ sample|}{Absorbance\ of\ control} x\ 100$$

Statistical Analysis

The data obtained were analyzed using JAMOVI—a free and open-source computer program for data analysis and statistical tests. Analysis of variance (ANOVA) was performed and significant differences between mean values were determined by Scheffe's test and Tukey's Honest Significant Difference (HSD) for the free radical scavenging activity. The results obtained were expressed as means ± standard deviation. Differences are considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Fourier Transform Infrared (FT-IR) Spectroscopy

The infrared vibration spectra of the films were determined to gain a better understanding of the relationship between the possible interactions or structural changes during the film formation. The infrared vibration spectra of the samples in Fig. 2 reveal the presence of alcohol-, carboxylate, alkane-, and ether-containing compound/s. The peak assignments of the samples are summarized in Table 1.

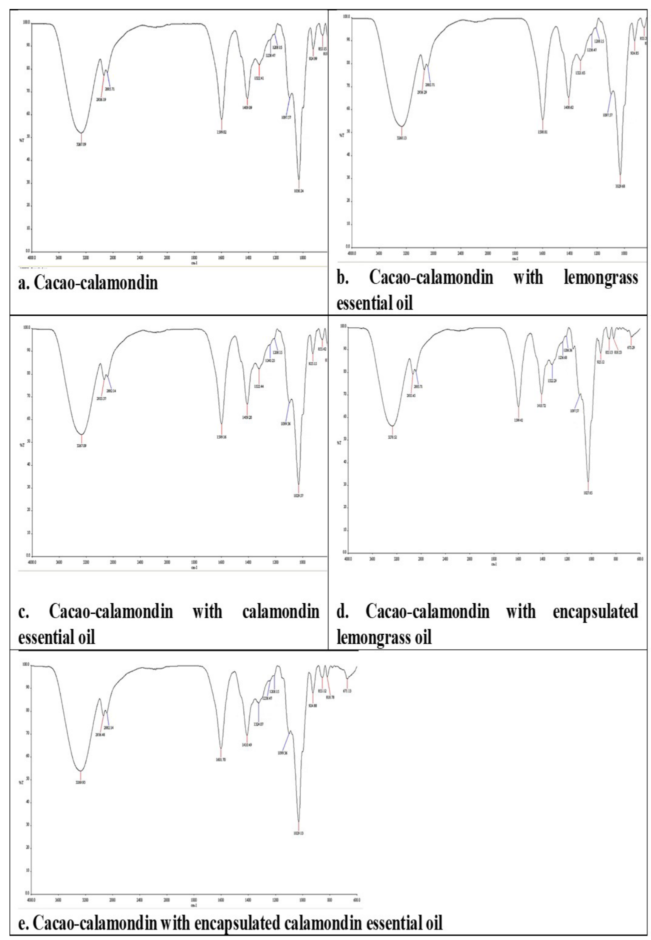

Fig. 2. FT-IR spectra of the various mixtures of cacao-calamondin films.

Table 1. Peak assignments in the infrared spectra of the various mixtures of cacao-calamondin films.

Standard Group Frequencies*	A	B	C	D	E	Structure/Compound Type*	Bonds*
3650–3200	3267.09	3268.13	3267.09	3270.52	3269.93	Alcohol	O-H stretch
3000–2840	2936.19	2936.29	2935.37	2935.45	2936.48	Alkane	C-H stretch
	2885.71	2885.71	2882.14	2885.71	2882.14		
1610–1550	1599.02	1598.81	1599.16	1599.42	1601.70	Carboxylate	(COO)- bend
1450–1400	1409.09	1408.62	1409.20	1410.72	1410.49		
1400–1250	1322.41	1321.65	1322.44	1322.29	1324.07	Alcohol	O-H bend
1250–1100	1238.47	1238.47	1240.25	1236.68	1238.47	Alkane	C-H skeletal vibration
	1208.18	1208.15	1208.15	1206.36	1208.15		
1100–1000	1097.57	1097.57	1099.36	1097.57	1099.36	Ether	C-O-C stretch
	1030.24	1029.68	1029.57	1027.65	1029.13		
940–900	924.99	924.85	925.15	925.12	924.88	Carboxylate	(COO)- bend
900–800	855.15	855.31	855.42	855.13	855.12	Alkane	C-H skeletal vibration
800–870	819.34	819.27	818.98	818.53	818.78	Ether	C-O-C stretch
< 700	674.90	671.42	670.74	675.29	671.13	Alcohol	O-H bend

A: Cacao-calamondin mixture; B: Cacao-calamondin mixture with lemongrass essential oil; C: Cacao-calamondin mixture with calamondin essential oil; D: Cacao-calamondin with encapsulated lemongrass essential oil; and E: Cacao-calamondin with encapsulated calamondin essential oil.

It can be seen in both Fig. 2 and Table 1 that the infrared spectra of the samples displayed similar absorption bands. This could mean that the concentrations of the essential oils utilized are too low to be detected via FT-IR. However, the absorption bands of the films produced are generally similar to the absorption bands of biodegradable films produced from poly(vinyl alcohol)-corn starch film where a broad band in the wavelength region between 3 600 and 3 000 cm^{-1} is attributed to O-H stretching, and two weak bands located between 2 950 and 2 850 are attributed to C-H stretching (Kumar et al. 2021).

X-ray Diffraction (XRD) Analysis

The X-ray diffraction analysis was conducted to determine whether the films have a crystalline or amorphous structure.

The samples were evaluated in the range of 2θ, from 2 – 70° . The resulting XRD patterns did not show sharp peaks (Fig. 3), indicating that the samples are relatively amorphous in nature and have a non-crystalline structure. These results are similar to the results of Shektaei et al. (2023) who performed the same test on biodegradable film from gellan and carboxymethyl cellulose (CMC) containing rosemary oil; Lee et al. (2019) for gellan-based films; and Jahit et al. (2016) for gelatin-CMC based film. Amorphous bioplastics have the advantage of flexibility and an accelerated rate for decomposition or composting. However, while all samples showed an amorphous structure, it can be observed that pure cacao and pure calamondin samples exhibited higher intensities compared to the mixed samples. This could be due to having more samples (2 g of pure cacao or calamondin compared to a 1:1 ratio for mixed samples) that diffracted the X-rays.

Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscopy provides a visualization of the samples and could reveal defects, cracks, voids, and micropores resulting in irregular surfaces. The mechanical strength of the films including tensile strength may be affected by the presence of cracks and micropores (Fig. 4). These micropores may interest microorganisms, which may accelerate the biodegradation process. Higher magnification images showed undissolved materials in the mixture (white). This is most likely due to the alginate component in the sample, which did not dissolve entirely before the film formation. This is mostly present in the cacao-calamondin sample (a), which is not evident in the cacao-calamondin with lemongrass oil (b). However, in (b), it can be noted that a crack (encircled red) is present, which may be due to an uneven distribution of the essential oils onto the surface of the film due to a lack of emulsifying agent in the mixture. This is not very evident in (c)—only the presence of an undissolved substance, which could most likely be alginate. Encapsulating lemongrass oil in (d) still yielded cracks and a few undissolved materials. Cracks were not evident in samples containing calamondin essential oils, indicating even distribution of the samples in the film.

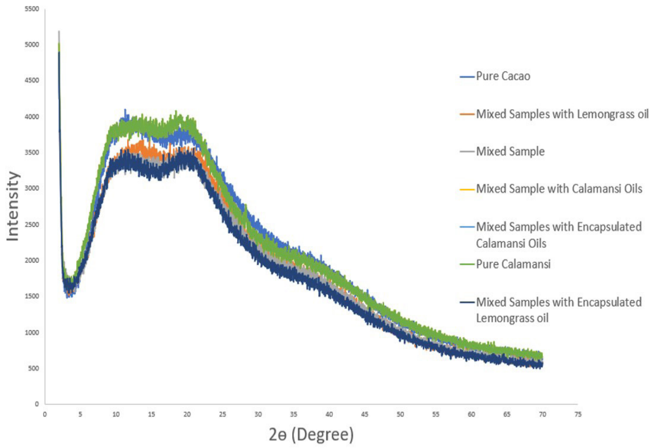

Fig. 3. XRD patterns of the various mixtures of cacao-calamondin films.

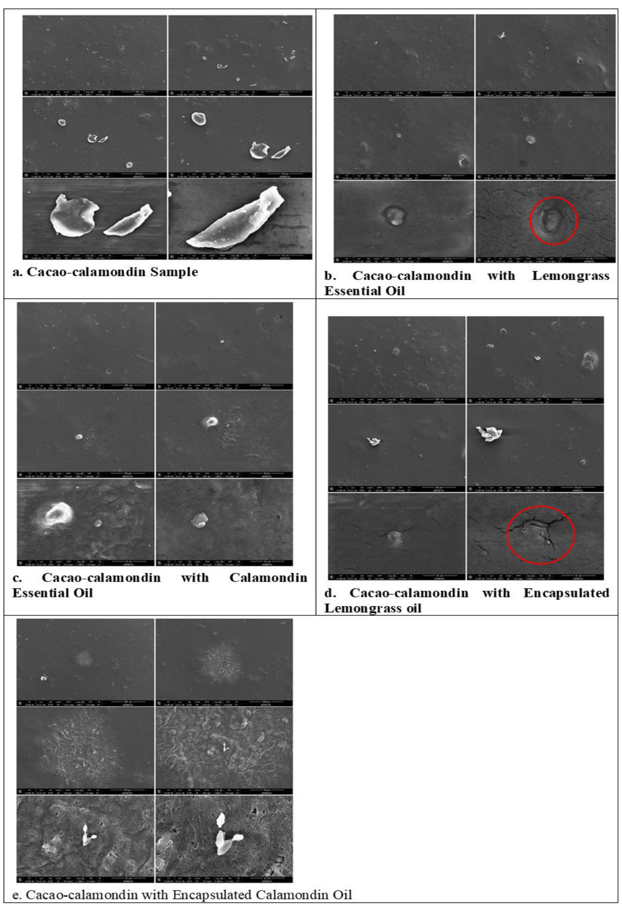

Fig. 4. FESEM images of the various mixtures of cacao-calamondin films showing undissolved materials (white) and cracks (red circle).

Film Thickness

The thickness of the film may control other physical film properties such as its mechanical strength. Generally, film thickness increases with increasing concentration. However, in this study, the concentration was held constant. The variation was done in the composition of the films such that essential oils were added to them. In Table 2, it can be seen that film thickness increased with the addition of other components in the film matrix. The addition of essential oils and encapsulated essential oils increased the thickness of the films. This increase is significant at alpha 0.05 as revealed from the statistical analysis, wherein the analysis of variance between groups showed a highly significant probability value of 0.00 less than 0.05 alpha level. Further, the post hoc analysis that was conducted to determine the multiple comparisons using Scheffe revealed that pure cacao film (sample A) and pure calamondin film (sample B) are significantly different from other samples but pure cacao film and pure calamondin film are not significantly different.

The determination of the thickness of the films is relevant as countries are imposing a ban on single-use packaging bags based on a certain level of thickness such as Africa and India, which ban the importation, production, use, and sale of plastic bags < 0.05 mm; Ethiopia, < 0.03 mm; Malawi, < 0.06 mm; and Sri Lanka, < 0.02 mm among others (Marichelvam et al. 2019). In the Philippines, the House of Representatives is still pushing to pass the single-use plastic bill. While this has not yet passed into law, and while the film that is being developed in this study is not categorized as "plastic," it is still important to note that all the values for the film thickness are above 0.1 mm, indicating their suitability for packaging.

Table 2. Film thickness of the various mixtures of cacao-calamondin films.

Sample	Description	Average Thickness (mm)		Grouping information using Scheffe's method (95% confidence)
		Mean	SD	
A	Pure cacao	0.113	0.02	A
B	Pure calamondin	0.101	0.05	A B
C	Cacao-calamondin	0.204	0.01	C D E F G
D	Cacao-calamondin with lemongrass oil	0.240	0.02	D C E F G
E	Cacao-calamondin with calamondin oil	0.241	0.01	E C D F G
F	Cacao-calamondin with encapsulated lemon-grass oil	0.252	0.02	F C D E G
G	Cacao-calamondin with encapsulated calamon-din oil	0.252	0.04	G C D E F

Note: Means that do not share a letter are significantly different.

Moisture Content Analysis

The moisture content analysis is conducted to determine the amount of moisture in the film samples. Moisture accelerates food spoilage. It is therefore necessary to ensure that the packaging material would not increase the moisture content of the substance being packaged. In the studies conducted by Dashipour et al. (2015), Hasheminya et al. (2019), and Xue et al. (2019), it was reported that the addition of essential oils would generally reduce the moisture content given that the addition of a hydrophobic material throughout the film matrix would reduce possible interactions of water molecules with the polymer chains. However, there were cases in which the moisture content was increased or significantly unchanged such as in the studies conducted by Evangelho et al. (2019), who reported moisture content values ranging from 18.81% to 21.93%; and Bajic et al. (2020), who reported moisture content values of their active packaging chitosan-based films ranging from 20.20% to 42.80%. This may be attributed to the formation of a porous structure, which may have facilitated the movement of water molecules in the polymer chains. Another reason for this increase in the moisture content of the films, despite the addition of a hydrophobic substance, is that essential oils are naturally volatile and tend to evaporate within the experimental conditions.

The moisture content of the films developed in this study mostly increased significantly upon the addition of essential oils. For instance, the addition of calamondin essential oil to the cacao-calamondin mixture (Sample E) significantly increased the moisture content relative to using only the cacao-calamondin sample (Sample C). This increase is not significant when the lemongrass essential oil was used, which can suggest a preference for the lemongrass essential oil. The statistical analysis also revealed that encapsulation of the essential oils (Samples F and G) significantly increased the moisture content relative to using pure cacao (Sample A) and pure calamondin (Sample B) samples, but this difference is not significantly relative to using unencapsulated essential oils. While the increase is mostly significant, the values are relatively lower than the ones that resulted from the aforementioned studies.

Water Activity

Water activity is a measure of the "unbound" water that is present in packaging films. It is a critical factor that could affect the shelf life of the films and the foods being packaged. Table 4 shows that the addition of essential oils generally decreased the water activity in the samples. Cacao-calamondin (Sample A) is significantly different from cacao-calamondin with lemongrass oil (Sample B), cacao-calamondin with calamondin essential oil (Sample C), and cacao-calamondin encapsulated with calamondin oil; however, cacao-calamondin is not significantly different from cacao-calamondin with encapsulated lemongrass oil (Sample D). For example, the addition of either lemongrass (Sample B) or calamondin essential oil (Sample C) to the cacao-calamondin mixture significantly decreased the water activity relative to using only the cacao-calamondin sample (Sample A). However, only the addition of encapsulated calamondin oil significantly decreased the water activity (Sample E) and not the encapsulated lemongrass oil (Sample D). This implies that encapsulating lemongrass oil will not cause any significant difference in the water activity of the cacao-calamondin sample. However, relative to the unencapsulated lemongrass (Sample B) and calamondin (Sample C) essential oils, encapsulating

Table 3. Moisture content of the various mixtures of cacao-calamondin films.

Sample	Description	Moisture Content (%) Mean	SD	Grouping information using Scheffe's method (95% confidence)
A	Pure cacao sample	8.22	0.03	A B C
B	Pure calamondin sample	9.12	0.02	B A C D
C	Cacao-calamondin	12.32	0.02	C A B D F G
D	Cacao-calamondin with lemongrass oil	15.19	0.05	D B C E F G
E	Cacao-calamondin with calamondin essential oil	17.77	0.03	E C D F G
F	Cacao-calamondin with encapsulated lemongrass oil	17.65	0.10	F C D E G
G	Cacao-calamondin with encapsulated calamondin oil	17.54	0.22	G C D E F

Note: Means that do not share a letter are significantly different.

Table 4. Water activity of the various mixtures of cacao-calamondin films.

Sample	Description	Water activity (%) Mean	SD	Grouping information using Scheffe's method (95% confidence)
A	Cacao-calamondin	0.511	0.003	A D
B	Cacao-calamondin with lemongrass oil	0.472	0.001	B C E
C	Cacao-calamondin with calamondin essential oil	0.473	0.002	C B E
D	Cacao-calamondin with encapsulated lemongrass oil	0.484	0.001	D B C
E	Cacao-calamondin with encapsulated calamondin oil	0.478	0.002	E B C D

Note: Means that do not share a letter are significantly different.

lemongrass oil significantly lowered the water activity. The lowest limit of water activity for microbial proliferation is 0.61. Below this value, no microbial proliferation can be assumed (Barbosa-Cánovas et al. 2020). The water activity values are within the expected range to ensure microbial safety.

Tensile Strength

Tensile strength is one of the most studied mechanical properties of biodegradable films. It is a measure of the film's resistance to tension forces. The desired values for tensile strength should be as high as possible because packaging should provide sufficient protection to the material during transportation or storage. The tensile strength values of the samples showed a highly significant probability value of 0.00 less than 0.05 alpha level (Table 5). Cacao-calamondin (Sample A) is significantly different from all other samples. The addition of both encapsulated and unencapsulated essential oils to the mixture significantly increased the tensile strength of the samples. Encapsulation of the essential oils decreased for encapsulated lemongrass (Sample D) relative to the encapsulated and unencapsulated calamondin essential oil samples (Samples C and E), which may be related to the presence of cracks on its microstructure. This decrease, however, is only significantly relative to the unencapsulated calamondin essential oil (Sample C) and not in the encapsulated calamondin essential oil (Sample E), which may suggest that encapsulation using either lemongrass or calamondin essential oil can still be done to enhance the tensile strength of the films.

Table 5. Tensile strength of the various mixtures of cacao-calamondin films.

Sample	Description	Peak Load (kg)	Work (mJ)	Deformation at peak load (mm)		Tensile Strength (Pa)	
				Grouping information using Scheffe's method (95% confidence)			
A	Cacao-calamondin	1.597	281.50	38.10 ± 0.03	A B	$1.1 \times 10^4 \pm 0.14 \times 10^2$	A
B	Cacao-calamondin with lemongrass oil	1.748	242.80	27.69 ± 0.20	B A C	$2.2 \times 10^4 \pm 3.24 \times 10^2$	B D
C	Cacao-calamondin with calamondin essential oil	2.741	251.20	22.18 ± 0.03	C B D	$5.5 \times 10^4 \pm 1.42 \times 10^2$	C E
D	Cacao-calamondin with encapsulated lemongrass oil	2.031	177.60	21.23 ± 0.32	D C	$4.4 \times 10^4 \pm 1.321 \times 10^3$	D B E
E	Cacao-calamondin with encapsulated calamondin oil	2.042	192.40	20.74 ± 0.02	E	$4.7 \times 10^4 \pm 0.93 \times 10^2$	E C D

Note: Means that do not share a letter are significantly different.

Oxidative Stability Test

Many compounds tend to undergo oxidation reactions in the presence of oxygen even at low temperatures, but there are also substances like films that, when subjected to isothermal conditions, exhibit a period of induction during which no reaction with oxygen occurs. This period is called the oxidation induction time or oxidative induction time. After this period, a reaction with oxygen occurs at an increasing rate.

The oxidative stability test was performed to measure the susceptibility of material to oxidation, which causes rancidity. In this study, the oxidation stability of the samples was tested at 100°C and 150°C and extrapolated using three temperature points: 4°C, 30°C, and 60°C. The induction time (time until secondary reaction products are detected) decreased with increasing temperature (Table 6). It is important to note that the extrapolations were based on the analysis conducted at 100°C and 150°C. This may suggest that if the analysis was conducted within a lower temperature range, higher induction time may be achieved. A higher induction time is preferred because oxidation can take place only after the induction period.

The statistical analysis revealed that only cacao-calamondin with encapsulated lemongrass oil (Sample D) is significantly different with all other samples. The encapsulated lemongrass essential oil (Sample D) increased the induction time significantly relative to the other samples while the encapsulated calamondin essential oil (Sample E) significantly decreased the induction time relative to the other samples. This may imply that lemongrass essential oil may be effective in acting as a barrier for oxidative degradation compared to calamondin essential oil.

Table 6. Oxidative stability test results of the various mixtures of cacao-calamondin films.

Sample No.	Description	Induction Time, h		Induction Time, h at Extrapolated T, °C			Grouping information using Scheffe's method (95% confidence)
		100°C	150°C	4	30	60	
A	Cacao-calamondin	0.44	0.39	0.57	0.53	0.49	A B C E
B	Cacao-calamondin with lemongrass oil	0.48	0.38	0.74	0.66	0.58	B A C
C	Cacao-calamondin with calamondin oil	0.49	0.4	0.75	0.67	0.59	C A B
D	Cacao-calamondin with encapsulated lemongrass oil	0.65	0.41	1.54	1.21	0.93	D
E	Cacao-calamondin with encapsulated calamondin oil	0.38	0.32	0.52	0.48	0.43	E A

Note: Means that do not share a letter are significantly different.

Water Solubility

The solubility of the films in water is an important parameter to measure especially for packaging materials that are meant to be in contact with high-moisture food products. The packaging film should maintain its integrity even in high moisture environments. Hence, in the development of packaging films, hydrophilicity and hydrophobicity are being considered as these can alter the solubility of the films in water. Cacao-calamondin with lemongrass oil (sample B) and pure calamondin film (sample G) are significantly different from other samples with sample B having the lowest solubility and sample G having the highest. This means that lemongrass essential oil is effective in lowering the solubility of the film in water. On the other hand, the highest solubility of the pure calamondin sample (Sample G) can be attributed to its high phenolic acids, which have high solubility in aqueous solutions (Cheong et al. 2012).

Cacao-calamondin with lemongrass oil and pure calamondin film are not significantly different. The water solubility of the samples generally decreased with the addition of essential oils. The mixed samples also have a relatively lower solubility in water compared to pure samples. Further, the results shown in Table 7 are similar to the active packaging films developed from sodium alginate/carboxymethyl cellulose containing shallot waster extracts in the study of Thivya et al. (2021), who reported that the solubility of their samples ranged from 42.85% to 71.49%.

Microbial Analysis

The microbial analysis was conducted to determine the types of microbes present in the film-forming samples and the effect of adding essential oils. The food pathogens selected here are the most common as specified by the Food and Drug Administration (FDA). Table 8 shows that the addition of essential oils decreased the number of colony-forming units per mL of the sample since essential oils have generally antimicrobial properties. Encapsulation of the oils further decreased the CFU/mL; however, it is interesting to note that the mixture of cacao and calamondin increased the number of salmonella and total coliforms compared to having pure cacao and calamondin samples. This could mean that the mixture provides nutrients and energy necessary for these microbes to grow. This, however, dramatically decreased with the addition of essential oils.

Table 7. Water solubility of the various mixtures of cacao-calamondin films.

Sample	Description	Solubility (%)		Grouping information using Scheffe's method (95% confidence)
		Mean	SD	
A	Cacao-calamondin	55.95	1.92	A C D E F
B	Cacao-calamondin with lemongrass oil	46.73	2.50	B G
C	Cacao-calamondin with calamondin essential oil	56.33	1.11	C A D E F
D	Cacao-calamondin with encapsulated lemongrass oil	57.39	1.57	D A C E F
E	Cacao-calamondin with encapsulated calamondin oil	58.51	2.60	E A C D F
F	Pure cacao sample	59.92	1.06	F A C D E
G	Pure calamondin sample	66.46	1.53	G B

Note: Means that do not share a letter are significantly different.

Table 8. Microbes in the various mixtures of cacao-calamondin films.

Sample	*E. coli*, CFU/mL	Salmonella, CFU/mL	Listeria	Cyclospora	Hepatitis A	Total Coliform, CFU/mL
Cacao-calamondin	ND	260	ND	ND	ND	160
Cacao-calamondin with lemongrass essential oil	ND	3	ND	ND	ND	15
Cacao-calamondin with calamondin essential oil	ND	3	ND	ND	ND	6
Cacao-calamondin with encapsulated lemongrass essential oil	3	13	ND	ND	ND	10
Cacao-calamondin with encapsulated calamondin essential oil	3	1	ND	ND	ND	4
Pure cacao	60	35	ND	ND	ND	90
Pure calamondin	ND	5	ND	ND	ND	60

Antioxidant Property

The free radical scavenging activity of the samples was evaluated using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay. Results showed that the pure cacao sample exhibited a significantly higher antioxidant property than the films developed using pure calamondin sample. Further, grouping information using Tukey's test revealed that cacao-calamondin with lemongrass oil has the significant highest free radical scavenging activity. Likewise, cacao-calamondin with encapsulated calamondin oil and cacao-calamondin with encapsulated lemongrass oil are significantly different with all other samples (Table 9). The sample containing the mixture of cacao and calamondin also exhibited a lower antioxidant property (2.01%) compared to the sample containing only calamondin (2.36%), but this difference is not statistically significant. The addition of essential oils generally increased the free radical scavenging activity of the films, and it was at its highest for the mixed sample containing lemongrass oil (Sample B). Encapsulation of oil significantly decreased its antioxidant activity relative to mixed samples containing unencapsulated oils.

Table 9. Antioxidant activity of the various mixtures of cacao-calamondin films.

Sample	Description	Free Radical Scavenging Activity (%)	Grouping information using Tukey's method (95% confidence)
A	Cacao-calamondin	2.01	A G
B	Cacao-calamondin with lemongrass oil	16.89	B
C	Cacao-calamondin with calamondin essential oil	12.51	A F
D	Cacao-calamondin with encapsulated lemongrass oil	7.26	D
E	Cacao-calamondin with encapsulated calamondin oil	9.36	E
F	Pure cacao sample	13.39	F C
G	Pure calamondin sample	2.36	G A

Note: Means that do not share a letter are significantly different.

CONCLUSION

Films from cacao pods and calamondin peels containing essential oils of lemongrass or calamondin peels were developed for active packaging and their antimicrobial properties were confirmed. The Fourier-transform infrared (FTIR) spectra revealed the presence of alcohol-, carboxylate, alkane-, and ether- containing compound/s. While the concentration of the oils may have been too low to be detected in the FTIR, increasing its value may enable its detection via FTIR. The X-ray diffraction (XRD) analysis revealed the amorphous structures of the films and field emission scanning electron microscopy (FESEM) provided a visualization of the structures of the films, which revealed the cracks and undissolved materials in the films. In terms of thickness, the results confirmed that it increased with the addition of other components in the film matrix. This was apparent in the mixtures containing both cacao and calamondin samples with encapsulated and unencapsulated oils. The tensile strength also increased with the addition of essential oils, but encapsulation of the essential oils decreased for the sample containing encapsulated lemongrass. For moisture content analysis, the addition of encapsulated and unencapsulated essential oils increased, which may be due to the formation of a porous structure that may have facilitated water movement across the polymer chains. On the other hand, the water activity analysis of the samples showed that the addition of essential oils decreased the water activity but encapsulating the oils slightly increased it relative to the mixture without oils and the mixtures containing unencapsulated essential oils. However, all the values are below the threshold value for microbial proliferation, therefore ensuring microbial safety. Similarly, the solubility of the films in water decreased with the addition of essential oils. The oxidative stability test revealed that the induction time is decreased with increasing temperature. This suggests that the sample may be better preserved at temperatures lower than 100°C. The microbial analysis also shows that the addition of essential oils decreased the number of colony-forming unit per mL of the sample, and that encapsulation of the oils further decreased the number of colonies formed in the films. This is consistent with the results of the antioxidant analysis, which also shows that the addition of essential oils generally increased the free radical scavenging activity of the films and it is at its highest for the mixed sample containing lemongrass oil. However, encapsulation of oils decreased its antioxidant activity relative to mixed samples containing unencapsulated oils. The addition of either calamondin or lemongrass essential oils would enhance the film's antioxidant and antimicrobial properties; but while the encapsulation of these oils generally decreased the colony-forming units in the samples, the antioxidant properties significantly decreased relative to the unencapsulated oils, suggesting that films containing either lemongrass or calamondin essential oils may be further studied for potential pilot production.

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