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Original

Layer-by-Layer Dip Coating of Pili Kernel with Shellac-Rosemary Extract Blend for Reduced Rancidity

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Abstract: Pili kernels are prone to oxidative deterioration and rancidity, impacting their shelf life and consumer appeal. Coatings play a pivotal role in preserving quality and extending shelf life, which improves the characteristics of food products. In this study, we coated pili kernels and adapted the layer-by-layer dip coating technique. The coating consists of shellac as the formable matrix embedded with rosemary extract as the anti-rancidity agent. Pili kernels coated with a larger number of coating layers are shown to have lower peroxide and free fatty acid values. These values, which are indicative of levels of rancidity, suggest favourable results in the use of shellac-rosemary extract blend as a coating for pili kernels.

Keywords: **Layer-by-layer dip coating, pili kernel, shellac-rosemary extract blend.**

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1. Introduction

Coating of food products with functional materials has been widely practiced for the preservation and improvement of food quality (Mehyar et al., 2012; Hira et al., 2022; Hernández-López et al., 2020; Lara et al., 2020; Suhaimi et al., 2021). These coatings can be applied in liquid form (Yousuf et al., 2018) and are mostly natural biocompatible materials such as polysaccharides, proteins, and lipids that can be incorporated with active ingredients. In recent years, food coatings have been utilized to increase the shelf life, provide safety, improve food appearance, and enhance flavor.

Coatings typically consist of formable matrices that take the shape of the product. When applied to solid foods, it should be viscous enough to create a continuous layer to maximize its protective effect over the surface. Common coating methods involve spraying (Yan et al., 2020) and dipping (Ma et al., 2021; Jo et al., 2014; Chitravathi et al., 2014; Khorram et al., 2017). Both methods are simple and low-cost. However, spraying does not ensure evenness of the coating, especially due to the chaotic trajectory of the particles. For mass production, not all sides of the product might be coated considering that the spray source is mounted at rest.

The dip coating technique offers a simple process in which the product is immersed in a solution and air-dried afterward. Moreover, the layer-by-layer dip coating technique can be adapted in which the coating of one layer is air-dried first before the application of another layer.

One of the substances used in the food coating industry is shellac. Shellac (or lac resin) (Wang et al., 1999) is a natural polymer secreted by *Kerria lacca* insects found in the trees of India, Burma, Thailand, and Southern China. It is labeled by the US Food and Drugs Administration (US FDA) as “generally recognized as safe”. Shellac and shellac-based materials have been used in a variety of products such as varnish (Šimůnková et al., 2018), dielectric (Thue, 2011), food glaze (Pacheco-Torgal et al., 2016), and food packaging (Du et al., 2019) among others. Shellac is insoluble in water (Thombare et al., 2022) and is thus desirable for preventing moisture in food systems. Studies have shown that shellac embedded with active ingredients such as tannic acid (Ma et al., 2021) and carvacrol (Yan et al., 2020) have been effective in improving the quality of fruits.

Rosemary (Rosmarinus *officinalis*) has been widely utilized in the food industry due to its antioxidant, antibacterial, and antifungal properties (Brandt et al., 2023; Rashidaie Abandansarie et al., 2019; Babuskin et al., 2015; Martínez et al., 2019; Ibrahim & Al-Ebady, 2014). It is recognized by the US FDA as GRAS which makes it a desirable component for food products. Rosemary extracts can be prepared using food-grade solvents such as water, ethanol, hexane, and acetone (Xie et al., 2017). Its three major phenolic constituents, namely, carnosic acid, carnosol, and rosmarinic acid are responsible for rosemary’s antioxidative effect (Loussouarn et al., 2017; Zhang et al., 2012).

Various researches reported on the embedding of rosemary into different matrices. Chitosan coating with rosemary extract has been effective in decreasing the rate of browning, softening, and degrading sensory properties of fresh-cut pears (Xiao et al., 2010). Rosemary-containing carboxymethyl cellulose (CMC) coating helped in increasing the oxidative and microbial stability of smoked eel (Choulitoudi et al., 2017). Cassava starch films incorporated with rosemary extracts were also used in developing food packaging (Piñeros-Hernandez et al., 2017).

Rosemary extracts embedded in matrices can be applied as coatings on nuts. The pili kernel (*Canarium ovatum*) produced by the pili tree has a high economic value in the Philippine market as it can be sold as a flavored snack or as a raw material for pastries and confectioneries. Due to this, the pili kernel has a high potential for export. However, the entire pili nut weighs nearly four times more than the kernel alone (Gallegos et al., 2013), which increases the cost of the whole nut, particularly when transportation costs are calculated by mass. If the pili kernel is the only ingredient of interest, it will be practical to discard the shell since its mass may significantly add to the cost of transportation.

Major export markets for pili nuts include countries such as the United States of America, the United Kingdom, the United Arab Emirates, and Canada. Just recently, the European Union has opened its doors for pili nut export (Da-Afid, 2023). Considering the distance of these countries to the Philippines, exportation of pili may involve long periods of travel, and this makes the pili kernel prone to rancidity. This can result in the loss of potential market. Limited research is available with the aim of maintaining raw pili kernel quality (De Jesus et al., 2021). In practice, pili kernels are cooked before transport to prolong shelf life. This reduces the potential uses of pili kernels, especially in products where pili kernels need to avoid rancidity.

Studies on coating resulting in reduced rancidity and extended shelf life have been shown in the past years (Bhan et al., 2022; Felicia et al., 2022; Oyom et al., 2022; Rohasmizah & Azizah, 2022). Rancidity refers to the off flavors in food resulting from the breakdown of oils and fats. The two main causes of rancidity include oxidation and hydrolysis. Oxidation occurs when oxygen attacks triglycerides while hydrolysis occurs when triglycerides, react with water, resulting in the release of free fatty acids. Rancidity is rampant among nuts due to the high content of unsaturated fatty acids.

In this study, we determined the peroxide value and free fatty acid value of the oil of the coated pili kernels, which are both indicators of rancidity. We developed a coating that consists of a shellac matrix with rosemary extract as the anti-rancidity agent. We also described the different characteristics of the coating such as thickness, hardness, and color.

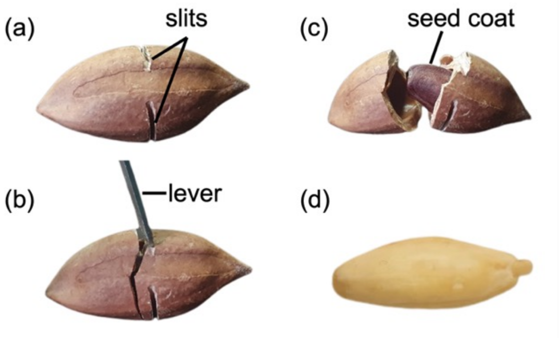

Figure 1. Cracking of pili shells to obtain the bare pili kernel. (a) Shelled pili with slits. (b)-(c) Cracking of pili shells using a lever. (d) Pili kernel after the testa (seed coat) was manually removed.

2. Materials and Methods

2.1 Materials

The shelled pili nuts were harvested at a plantation in Barangay Sabang Tabok, Lavezares, Northern Samar, Philippines. The dewaxed bleached shellac powder was procured from Ohvem Sales Corporation (Chhattisgarh, India). Ethyl alcohol (95%) was purchased from ANM Chemical Resources (Laguna, Philippines) and served as a solvent for the dewaxed bleached shellac. Ground rosemary leaves from McCormick were purchased at a local grocery store in Los Baños, Laguna, Philippines. All reagents used are of food/USP grade. A bare pili kernel was obtained from the shelled pili nut as shown in Figure 1.

2.2 Pre-coating Preparation

A transverse slit was made across the three corners of the pili shell using a hand saw (Figure 1a). A stainless lever was used to crack the shell and obtain the pili kernel (Figure 1b-1d). To remove the testa (seed coat), the pili kernels were blanched in boiling water for three minutes. The loosened testa was removed by manual peeling. The peeled pili kernels were then exposed to room temperature and were air dried.

2.3. Preparation of Shellac-rosemary Extract Blend

To create the coating, the shellac-rosemary extract blend was prepared using ethyl alcohol as its solvent. The shellac served as the matrix, and it was embedded with rosemary extract as the functional material.

Shellac powder was mixed with rosemary extract in ethyl alcohol. In a beaker, 25 g of shellac powder was mixed with 100 mL of rosemary extract to obtain a shellac-rosemary blend. The solution was subjected to magnetic stirring for 45 minutes at room temperature. The beaker was afterward kept in a desiccator for 24 hours to allow the setting of undissolved shellac powder. The homogeneous shellac-rosemary blend coating was collected via decantation.

Rosemary extract was prepared by mixing ground rosemary with ethyl alcohol. In a beaker, 25 g of ground rosemary leaves was mixed with 250 mL of 95% ethyl alcohol. The mixture was subjected to a commercial blender at room temperature for 2 minutes until a green heterogeneous mixture was obtained. The mixture was transferred to a beaker which was kept at a desiccator for 24 hours to allow settling of fine rosemary sediments. The rosemary extract was separated from the undissolved sediments via decantation.

2.4 Coating of Peeled Kernels using Layer-by-layer Dip Coating Technique

The peeled pili kernels were coated with shellac-rosemary extract blend using the layer-by-layer dip coating technique. In this technique, the pili kernels were dipped in the blend for three seconds. After dipping, the pili kernels were air-dried to allow ethyl alcohol to evaporate. The air-dried sample produced a hardened coating which corresponds to a single coating layer. For multiple layers of coating, it is necessary that the previous coating is dry before repeating the dipping process. The dipping process was done depending on the desired number of layers (e.g., 1, 3, and 5). The result is a hard transparent coating taking the form of the pili kernel.

2.5 Characterization

Reflectance spectra of the uncoated pili kernel and the pili kernels with 5 layers of shellac-rosemary blend coating were obtained using a UV-2600 SHIMADZU UV-VIS Spectrophotometer. The tests were done with 3 trials each. The presence of compounds in the shellac-rosemary blend coating was confirmed using a Shimadzu IR Prestige-21 Fourier Transform Infrared Spectrophotometer.

All coated pili kernels were placed in a room with a constant temperature of 27 degrees Celsius for 14 days. For the peroxide value (PV) and free fatty acid value (FFAV) analyses, 30 g of pili kernels were prepared for oil extraction using the solvent extraction method at a 1:4 sample-to-solvent ratio. PV was determined using a modified method of AOAC 965.33 (iodometric titration) while the FFAV was determined using a modified method of AOAC 940.28 (alkaline titration).

The thickness of the coating was determined by analysing the cross-sectional area of the coated pili kernel. Using ImageJ software, thickness measurements around the pili kernel were obtained using 30 data points. The hardness of the coating with 1, 3, and 5 layers was measured in 5 trials using a Sauter analog durometer Shore D.

3. Results and Discussion

We successfully coated pili kernels with shellac-rosemary extract blend ("coating blend") using layer-by-layer dip coating technique. Figure 2 shows uncoated and coated pili kernels with 1, 3, and 5 layers of coating blend. During the process of layer-by-layer dip coating, the coating blend was applied to two different substrates—bare pili kernel during the coating of the first layer, and the coated pili kernel during the succeeding layers. The addition of succeeding layers resulted in different pili coating thicknesses per layer.

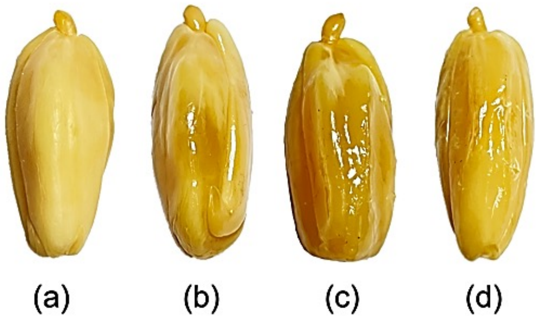

Figure 2. (a) Uncoated pili kernel and (b)-(d) coated pili kernel with 1, 3, and 5 layers of coating blend.

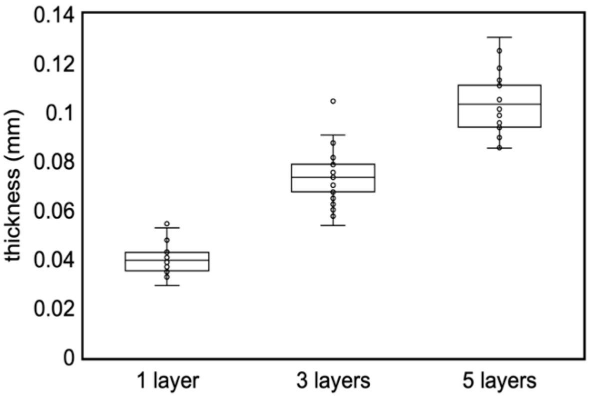

Figure 3. Thickness of coated pili kernels with varying number of layers. Using the least square method, the relationship between the number of layers and the thickness can be described as $y = 0.0158x + 0.0249$ where x is the number of layers and y is the thickness, with a coefficient of fitness of $R^2 = 0.9989$.

Figure 3 shows the relationship of the pili coating thickness with the varying number of layers. Using the least square method, the relationship between the number of layers and the thickness can be modelled as a linear function resulting in $y = ax + b$, where x is the number of layers and y is the thickness, a = 0.0158 mm/mm and b = 0.0249 mm, with a coefficient of fitness of $R^2 = 0.9989$. Based on the linear model, we can take a as the best estimate for the thickness of a single coating layer on a coated pili kernel, while ax + b where x = 1 is the best estimate for the first layer of coating, i.e., the coating on a bare pili kernel. Table 1 shows the estimated coating thickness based on the model using a bare pili kernel and a coated pili kernel as substrates. For the bare pili kernel, the estimated coating thickness is 0.0407 mm (which is close to the average thickness for 1 layer of coating, which is 0.0401 mm) while for the coated pili kernel, it is 0.0158 mm. This might suggest that the ethyl alcohol-dissolved shellac has a stronger adherence to the pili kernel surface than to the hardened form of itself.

Table 1. Comparison of the coating thickness per layer during application of coating to a bare pili kernel and a coated pili kernel.

Substrate	Estimated coating thickness per layer based on the model (mm)
Bare pili kernel	0.0407
Coated pili kernel	0.0158

Figure 4 shows the normalized reflectance plots of uncoated pili, pili with shellac only, and pili with shellac-rosemary blend coating in the visible region. The reflectance of the uncoated pili and shellac-coated pili is shown to increase from 400 nm to 700 nm, indicating a characteristic color in the orange to red region. The shellac coating itself is orange in color which accounts for the high reflectance from 550 nm to 700 nm. In (c), the weakened reflectance in the red region with broad dips from 595 nm to 630 nm and 630 nm to 700 nm indicates the presence of green rosemary in the coating. In (c), the weakened reflectance in the red region with broad dips from 595 nm to 630 nm and 630 nm to 700 nm indicates the presence of green rosemary in the coating.

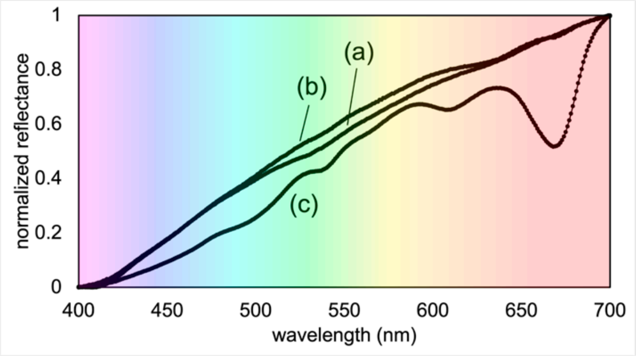

Figure 4. Normalized reflectance versus wavelength plots of (a) uncoated pili, (b) pili with shellac coating, and (c) pili with shellac-rosemary blend coating.

Figure 5 shows the relative hardness of the coating of the pili kernels with respect to the single layer of coating. The hardness is shown to increase with the number of layers. A study by Jo et al. has shown that shellac-based coatings have been used to maintain the hardness of apples during storage (Jo et al., 2014). ‘Ruby Red’ grapefruit coated with shellac wax (Yan et al., 2020) and mangoes coated with shellac and tannic acid (Ma et al., 2021) exhibited greater firmness during storage as compared to those which are not coated.

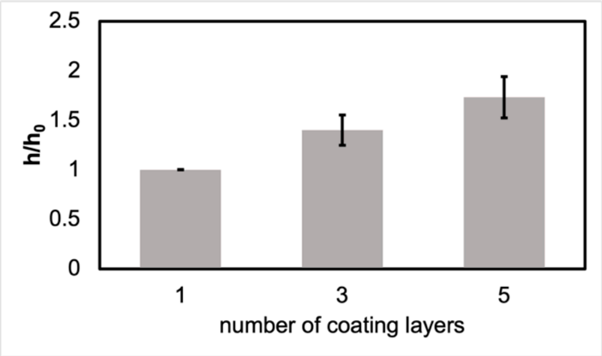

Figure 5. Relative hardness (h/h0) of coated pili kernels with respect to the single layer of coating. Note that the hardness of the uncoated pili kernel is too soft, thus not within the detectable range of the equipment.

Figure 6 shows the FTIR spectroscopy plots of uncoated pili, pili with shellac only, and pili shellac-rosemary blend coating. Similar FTIR spectra can be observed between pili with shellac coating and shellac-rosemary coating. The broad peak from 3340 cm^{-1} to 3500 cm^{-1} is due to the OH stretching vibration. Strong peaks found at 2927 cm^{-1} and 2856 cm^{-1} can be attributed to CH2 asymmetric and symmetric vibrations, respectively. The strong signal found at 1708 cm^{-1} is due to the C=O stretching vibration of esters, while its shoulder at 1635 cm^{-1} can be due to the C=C stretching vibration of vinyl. Weak signals at 1462 cm-1 and 1413 cm^{-1} can be attributed to the CH2 bonds present in shellac’s ester chain. A series of peaks at 1290 cm^{-1}, 1247 cm^{-1}, 2117 cm^{-1}, 1151 cm^{-1}, and 1109 cm^{-1} can be attributed to the presence of C-O bonds in both shellac and ethanol. The absorption peak at 1045 cm^{-1} may be due to the C-C stretching vibration. Stretching vibration at 900 cm^{-1} and bending vibration at 761 cm^{-1} may be due to C=C and C-H present in linoleic acid, oleic acid, or triglycerides in the pili nut. The peaks at 943 cm^{-1} (CH2 from alkenes), 883 cm^{-1} (C=C), and 719 cm^{-1} (CH2) can be attributed to shellac. A summary of the peaks of the FTIR spectra is shown in Table 2.

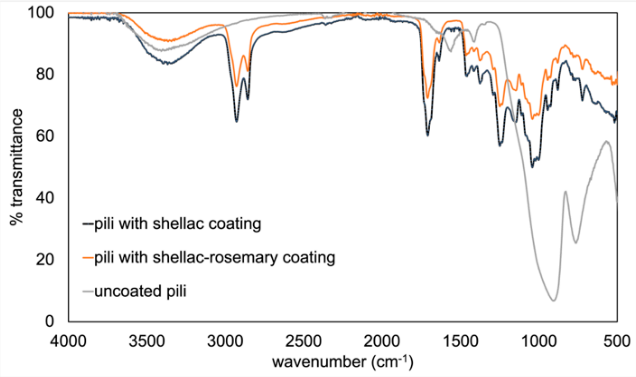

Figure 6. Percent transmittance versus wavenumber of uncoated pili, pili with shellac coating, and pili with shellac-rosemary blend coating.

Table 2. Peaks of infrared spectra of the detected compounds on the uncoated and coated pili kernel samples.

wave number (cm^{-1})	vibrational mode	component	material
3340 to 3500	OH stretching vibration	ethanol	coating
2927	CH2 asymmetric vibration	shellac	coating
2856	CH2 symmetric vibration	shellac	coating
1708	C=O	shellac	coating
1635	C=C stretching	shellac	coating
1560	N-H	protein (amino acid)	pili nut
1462	CH2 bend	shellac/ ethanol	coating
1413	CH2	shellac	coating
1375	CH3 bend	ethanol	coating
1290	C-O stretching	shellac	coating
1247	C-O	shellac	coating
1170	C-O	shellac	coating
1151	C-O	ethanol	coating
1045	C-C	shellac	coating
1028	C-O stretching	ethanol	coating
943	O-H carboxylic acids	shellac	coating
900	C=C stretching	linoleic acid /oleic acid	pili nut
883	C-H	shellac	coating
761	C-H	tri glycerides	pili nut
719	CH2	shellac	coating

Figure 7 shows the peroxide value (PV) and free fatty acid value (FFAV) of the coated pili kernels relative to the uncoated pili kernel. The values of the relative PV and FFAV are lower at a higher number of coatings. This shows that the production of peroxide and fatty acid, which is associated with rancidity is minimized due to the presence of coating.

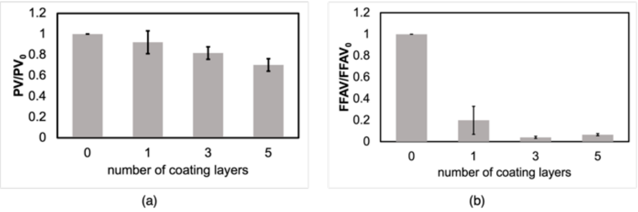

Figure 7. (a) Relative amount of peroxide value (PV/PV_0) and (b) relative amount of free fatty acid value ($FFAV/FFAV_0$) with respect to uncoated pili.

4. Conclusions

The researchers were able to coat pili kernels with shellac-rosemary extract blend coating via the layer-by-layer dip coating technique. The hardness of the coating was shown to increase as the number of layers increased. The thickness of one coating layer was greater when it was applied onto a bare pili kernel as compared to an already coated pili kernel. The normalized reflectance of pili with shellac-rosemary extract coating may indicate the presence of rosemary extract due to the dip in the red region. Peroxide value and free fatty acid value tests are lower with a larger number of shellac-rosemary blend coatings which shows a potential increase in the shelf life of the coated pili kernels.

As an extension of this study, peroxide and free fatty acid values can be monitored over time to demonstrate the coating's ability to extend the shelf life of pili nuts. Additionally, further characterization of the antimicrobial activity of rosemary extract embedded in the shellac matrix can be explored.

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Conflict of Interest

The authors do not have any type of conflict of interest to declare.

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