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Incorporation of papaya (*Carica papaya* L.) leaf extract into cornhusk for glutinous rice snack packaging application

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Abstract

The present work was performed to evaluate the potency of papaya leaf extract (PLE) to improve the characteristics of cornhusk as a packaging material for glutinous rice snack (GRS). Total phenolic, tannin, and saponin contents of PLE were 7.41 ± 0.65 mgTAE/mL, 1.80 ± 0.70 mgTAE/mL, and 11.78 ± 0.36 mgDE/mL, respectively. The presence of bioactive compounds on the surface of GRS was confirmed by Fourier transform infrared spectroscopy analysis. Characteristic bands for saponin were caused by the stretching vibration of C=O (at 1744 cm^{-1}) as well as C–O and C–C vibrations (at 1051 cm^{-1}). Tannin was identified as C–O asymmetric stretch vibration at 1051 cm^{-1} and C–H out of plane vibration at 926 and 866-867 cm^{-1}. The antioxidant activity of PLE was found to be 49.53 ± 2.67%. The reductions of total plate counts (TPC), yeast and mould counts (YMC), and *Aspergillus flavus-A. parasiticus* counts on PLE-incorporated cornhusks after 24 h were in the range of 0.2 - 1.2 log CFU/cm^2, and retained the loads below 2 log CFU/cm^2 during 14-d storage. PLE decreased the water vapour transmission rate of the cornhusk due to the particles of the extract adhering to the cornhusk surface, as supported by the result of the scanning electron microscopy of PLE-incorporated cornhusk. The incorporation of PLE also increased elongation without reducing the tensile strength of the cornhusk significantly. There were reductions of TPC, YMC, and *Aspergillus flavus-A. parasiticus* counts of GRS ranging from 0.4 - 2.3 log CFU/g using PLE-incorporated cornhusks during storage. GRS rancidity was minimised by PLE-incorporated cornhusks. Owing to its bioactive compound, PLE could be incorporated into the cornhusk to improve packaging characteristics and controls microbial contamination of GRS while retarding the rancidity.

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Introduction

In recent years, the use of agricultural waste-based packaging materials has risen in importance as an alternative to conventional plastics, and has challenged researchers with their degradability and functionality concerns. Packaging materials from agricultural waste sources such as cornhusk, bamboo fibre, citrus rind, hay, coconut shell, and mycelium of mushrooms are naturally biodegradable. A so-called biodegradable packaging employs polymer that can be deteriorated under microbial, aerobic, and anaerobic exposures. Cornhusk is one of the commercially available (sold through an online shopping cart and/or traditional marketplace) biodegradable packaging that is widely used for a variety of traditional food products in many countries. Various intermediate moisture food (IMF) products from Indonesia such as *dodol* and *wajit* are wrapped in cornhusk (Wahyuningsih *et al.*, 2016). Also, traditional IMF products such as *tamale*, *chuchitos*, and *bollos* from American and Caribbean regions are also wrapped in cornhusk (Tompkins and Sternberg, 2004; Argueta, 2010; Long, 2015). This packaging material is also usually applied by many other countries over the world to wrap brown sugar block (Pilla, 2011), as well as meat and fish, before, during, and/or after cooking (Raghavan, 2006). Not only as food wraps, but they can also be found as dining plate or dining plate cover to give an aesthetic decoration for various traditional and modern dishes.

However, cornhusk has high microbial loads due to the lack of handling at the post-harvest stage; consequently, the food, which comes into contact

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with it can be highly contaminated and spoiled. Moulds predominantly detected on cornhusk are *Aspergillus flavus* (Siriacha *et al*., 1994) and *Fusarium verticillioides* (Venturini *et al*., 2011). Therefore, this natural packaging material should be improved in terms of its functionality and stability during usage.

The addition of antimicrobial agents into packaging material is expected to be more effective than that directly formulated into food systems. The mode of action of the antimicrobial agent can be insensitive due to the interaction of antimicrobial active compounds and food components. Their direct use may also change the food taste and other sensory attributes; therefore, incorporating natural antimicrobial agents in food packaging materials can be a promising alternative to effectively limit food contamination while controlling their release into food. Previous work by Matan *et al*. (2011) developed a breakthrough to incorporate some essential oils into areca palm leaf sheath as a natural food packaging by the dipping treatment.

Papaya (*Carica papaya* L.) is one of the traditional medicinal plants with beneficial secondary metabolites derived from various parts including leaf, fruit, branch, seed, and flower. As further reviewed by Sukoco *et al*. (2019), biodegradable packaging, edible coatings, and edible films can be used as a carrier of PLE to provide antimicrobial activity while improving functional packaging properties. To this end, the present work developed a simplified extraction process as it will be useful for small-to-medium-food and/or packaging industries that are looking for affordable, safe, and impactful antimicrobial alternatives. Those industries that mainly use cornhusk during processing and/or post-processing will gain benefit from this approach to make either the cornhusk or food wrapped sold more storage-stable/shelf-stable. Given that the rocketing food prices are now influencing the ability of people in lower-to-middle-income countries to buy food, pushing food products to stay longer on display and the use of enhanced packaging have become indispensable.

Therefore, the present work was carried out to characterise the microbiological, physical, mechanical, and structural properties of cornhusk incorporated with PLE. The microbiological properties and free fatty acid (FFA) content of GRS, as well as the traceability of bioactive compounds through their functional groups in GRS, were also evaluated. GRS, a toffee-like sweet traditional Indonesian snack, was chosen as a model because it is commonly wrapped in cornhusk, but has not been incorporated with PLE. GRS is highly susceptible to microbial contamination and rancidity.

Materials and methods

Materials

Fresh papaya (var. Calina IPB California) leaves at the age of six months were used. Papaya leaves at the lowest stalk position were collected from the teaching farm of Agronomy and Horticulture, IPB University, Indonesia. Dried cornhusks (var. Pioneer) were collected from farmers in Gresik, East Java, Indonesia. Microbiological media used were plate count agar (PCA), potato dextrose agar (PDA), dichloran-glycerol agar (DG18), and *Aspergillus flavus* and *Aspergillus parasiticus* agar (AFPA). All microbiological media were purchased from Oxoid (England). All other chemicals were of analytical grade, and obtained from commercial sources.

Extraction of papaya leaves

Papaya leaves were extracted as carried out by Vuong *et al*. (2013) with some modifications. The water-to-leaf ratio used was 2:1 (v/w). Briefly, the crushed papaya leaves (1.5 kg) were juiced with 3 L of distilled water and then stirred for 20 min at 70 ± 2°C. The juice was filtered through two layers of cheesecloth. Without centrifugation process, the supernatant was stirred for 4 h at 70 ± 2°C. The aqueous extract obtained was then filtered again through two layers of cheesecloth, and kept at 4 ± 2°C for no longer than 2 d.

Determination of bioactive compounds of PLE

Total phenol and tannin contents were determined as described by Makkar *et al*. (1993) at 725 nm using a UV-Vis spectrophotometer. The results were expressed as mg of tannic acid equivalents per mL of extract (mgTAE/mL). Saponin content was determined according to Hiai *et al*. (1976) at 554 nm using UV-Vis spectrophotometer. The result was expressed as mg of diosgenin equivalents per mL of extract (mgDE/mL).

Determination of antioxidant activity of PLE

The antioxidant assay was performed according to Brand-Williams *et al*. (1995). Briefly, 2 mL of extract was added to 2 mL of 2,2-diphenyl-1-

picrylhydrazyl (DPPH). DPPH stock solution (0.1 mM) was prepared using 95% ethanol. The mixture was shaken vigorously using a vortex, and stored for 30 min in a dark place. The absorbance of 1 mL of mixture was read at 515 nm using a UV-Vis spectrophotometer (HITACHI U-2900, Japan). The standard curve was linear between 62.5 and 1,000 µM ascorbic acid. The antioxidant activity was quantified as the percent inhibition of DPPH radical using Eq. 1:

DPPH radical scavenging activity (%) =
[(Absorbance of control – Absorbance of sample) / Absorbance of control] × 100 (Eq. 1)

Preparation of PLE-containing cornhusks

In most small- and medium-scale GRS industries, the dried cornhusks were only soaked in hot water and air-dried at room temperature for 24 h (commercial packaging). In the present work, after soaking the dried cornhusks in hot water for 1 min at 90 ± 2°C, cornhusks were soaked in PLE at various concentrations (25, 50, 75, and 100%) for 15 min at room temperature (25 ± 2°C). The cornhusks were then well-drained in a porous basket for 24 h at room temperature (25 ± 2°C) before using them to wrap the GRS. This is commonly applied by GRS industries to reduce the moisture content of the cornhusks in order to prevent the rancidity of GRS. The microbial loads and other characteristics of treated- and untreated-cornhusks were evaluated on days 1, 3, 5, 7, and 14 of storage.

Preparation of GRS

GRS was made by mixing 2 kg of glutinous rice flour, 1 kg of raw mung bean, 1.5 kg of coconut milk, 1 kg of sugar, 0.5 kg of brown sugar, and 3 L of filtered water. The mixture was heated for 5 h at 85 ± 5°C and stirred continuously until the mixture changed into a sticky and elastic form. GRS was stuffed in several types of packaging after conditioning for 30 min at room temperature of 25 ± 2°C. The wrapped GRS was dried under direct sunlight for 2 - 4 h to reach a moisture content of under 20%.

Microbiological analysis

Microbiological tests were conducted with several media by counting the microbial count of the treated- and untreated-cornhusks, as well as GRS wrapped in commercial packaging and cornhusks treated with PLE. A sample suspension was prepared by adding a piece (1 × 1 cm) of investigated cornhusk into 10 mL of diluent, whereas 25 g of GRS was also prepared and diluted in 225 mL of diluent. A 0.1% peptone water was used as the diluent. The sample suspension made with investigated cornhusk was then serially diluted from 10^{-1} to 10^{-3}, while serial dilution from 10^{-1} to 10^{-4} was applied for sample suspension made with GRS.

For the determination of TPC, PCA was used and prepared according to the method of ISO 4833-1 (ISO, 2013) with slight modification in incubation temperature. The incubation was carried out for 3 d at 28°C. PDA was used for culturing yeasts and moulds from the sample according to the Pharmacopeial Harmonized Methodology (Valls and Nacente, 2011). PDA was also supplemented with a 10% sterile solution of tartaric acid. Inoculated plates were then incubated for 5 d at 28°C. A pour-plate method was used for both PCA and PDA.

The enumeration of xerophilic moulds in DG18 medium was carried out as described by ISO 21527-2 (ISO, 2008) with slight modification in incubation temperature. The incubation was conducted for 5 d at 28°C. For the detection of *Aspergillus flavus-A. parasiticus* counts, the diluted sample was cultured in AFPA according to Pitt *et al*. (1983) with slight modification in incubation temperature. The plates were incubated for 2 d at 28°C. Spread-plate method was used. These media were also supplemented with chloramphenicol. Plates with 10 to 150 colonies on AFPA, DG18, and PDA were counted, and plates containing between 15 and 300 colonies on PCA were also counted. The results were expressed as log CFU/cm^2 for microbial count present in cornhusk, and log CFU/g for microbial count present in GRS.

Determination of water vapour transmission rate

The method used for measuring WVTR was adapted from ASTM E96-00 (ASTM, 2000). A test cup was used in this measurement to mount the sample to the top of the cup so that there was an air gap at about 4.0 cm between desiccant (calcium chloride) and the sample surface. All samples (a round shape with a diameter of 3 cm) were previously conditioned in a desiccator containing saturated magnesium nitrate (RH 53%, at 25°C) for 2 d. After measuring the initial weight of the test cup containing desiccant and sample, the cup was placed into the desiccator containing saturated potassium chloride (RH 84%, at 25°C). The cup was periodically

weighed every 2 h during 24 h sample incubation. A plot of weight gained against time was used to determine the WVTR. WVTR was reported as a unit of gram per meter squared per day ($g/m^2/day$).

Determination of mechanical properties

These analyses were carried out using a universal testing machine (Instron 3369, United States) following the ASTM method C1557-03 (ASTM, 2013) with slight modification in load cell. A 100 N of load cell was used in this measurement. The tensile speed used was 3 mm/min. The sample was prepared in the form of rectangular strips (75 × 20 mm). Tensile strength was expressed as megapascal (MPa) while the elongation as percentage (%).

Structural observation

The morphological structure was done using a scanning electron microscopy (JEOL JSM 5310 LV, Japan). In brief, the sample was coated with gold metal by using sputter coater (IB2, Japan). The sample was then fixed to aluminium stubs with double-sided adhesive carbon tape. The image was taken at an electron-accelerating voltage of 5 kV, and at 150× magnification.

The crystallinity measurement was performed using an X-ray diffractometer (Shimadzu XRD-7000 MAXima). The sample was tested in the angular range from 5 to 40° (2θ) using CuKα radiation (λ = 0.154 nm) at a voltage of 40 kV, a current of 30 mA, and a scan speed of 2°/min. Crystallinity was reported as percentage (%) and determined as the ratio between the intensity of integrated crystalline and total intensity.

Free fatty acids (FFA) analysis

The FFA content of GRS was measured according to Paquot (1979). Samples were reported as % FFA as lauric acid.

Fourier transform infrared (FTIR) spectroscopy

Detection of bioactive compounds of PLE on the surface of GRS was performed using FTIR (Bruker TENSOR 37) as described by Sani *et al*. (2018). FTIR was used to identify the band position and functional groups of bioactive compounds.

Spectra were obtained by averaging 32 scans at 4 cm^{-1} resolution over the spectral range of 4000 to 400 cm^{-1}.

Statistical analysis

A one-factor completely randomised design was used. Data were statistically analysed by One-way analysis of variance (ANOVA) and continued by Duncan's multiple range test (DMRT) (α = 5%). Software SPSS version 16.0 for windows was used for the analysis.

Results and discussion

Bioactive compounds of PLE

The total phenolic content (7.41 ± 0.65 mgTAE/mL) observed in PLE was lower than that obtained by Kothari-Chhajer *et al*. (2016) of 10.457 mgTAE/g. Tannin was also obtained in PLE at approximately 1.80 ± 0.70 mgTAE/mL, and this result ascertained that tannin was not only found in methanolic and ethanolic extracts of papaya leaves (Poh and Jien, 2017). Previous study has reported that methanol could extract the highest total phenolic content from plant materials rather than using solvents with a higher or lower polarity index than methanol (Ferhat *et al*., 2017). The solvent system is the key driving force in the phenolic extraction of plant materials due to its ability to interact with diverse phenolic characteristics ranging from non-polar to polar by nature that might consequently turn their solubility.

PLE yielded 11.78 ± 0.36 mgDE/mL of saponin during prolonged extraction time. As reported by Vuong *et al*. (2015), the highest saponin was obtained from the extraction process of 25 min as compared to that of 5, 10, 15, and 20 min. Given the simplified extraction procedure, the extraction time for 4 h in the present work was therefore used to obtain the maximum amount of saponin from fresh papaya leaves. However, a long extraction time can influence the thermolabile compounds such as phenolic and tannin; therefore, selecting suitable solvents, as well as adjustment of temperature and time will be a further challenge to efficiently extract bioactive compounds from papaya leaves.

Antimicrobial activity of cornhusk incorporated with PLE

Results from four microbial growth parameters (Figure 1) revealed that the treatments of hot water alone or in combination with PLE suppressed the microbial viability during the storage of the cornhusk. Interestingly, the cornhusks treated with the

combination of hot water and PLE at all concentrations (25, 50, 75, and 100%) showed less TPC than that with hot water alone during 14-d storage ($p < 0.05$), as shown in Figure 1A.

Figure 1A shows that the combination of hot water and PLE at various concentrations (25, 50, 75, and 100%) significantly lowered the TPC after 24-h storage, *i.e*., 1.2, 0.7, 0.65, and 0.3 log CFU/cm^2, respectively. In particular, the TPC of cornhusks treated with PLE at concentrations of 50, 75, and 100% remained stable on days 3, 5, and 7 of storage. The TPC of PLE 50%-treated cornhusk was 1.05 to 1.15 log CFU/cm^2, and the TPC levels of cornhusks treated with PLE 75 and 100% were identified to suppress the microbial count below 1 log CFU/cm^2 until 7-d storage. These PLE concentrations (50, 75, and 100%) also maintained the TPC of the cornhusks below 2 log CFU/cm^2 during 14-d storage. The TPC levels of PLE-treated cornhusks were found to be statistically significant when compared with the untreated cornhusk and hot water-treated cornhusk ($p < 0.05$). Both treatments showed TPC of more than 2 log CFU/cm^2 after 7-d storage ($p < 0.05$).

Figure 1B shows that the combination of hot water and PLE at concentrations of 25 and 50% had lower YMC after 24-h storage, *i.e*., 1 and 0.7 log CFU/cm^2, respectively. Moreover, the combination with PLE at concentrations of 75 and 100% showed an extreme reduction up to 0 log CFU/cm^2 after 24-h storage, and it was statistically significant when compared with the other treatments ($p < 0.05$). PLE concentrations of 75 and 100% showed lower YMC during storage of cornhusks in the range from 0.6 to 0.8 and 0.6 to 0.75 log CFU/cm^2, respectively. On the other hand, YMC levels of untreated cornhusk and hot water-treated cornhusk were more than 1 log CFU/cm^2 after 24-h storage, and more than 2 log CFU/cm^2 after 14-d storage.

In the case of YMC on DG18, Figure 1C shows the combination of hot water and PLE at concentrations of 75 and 100% gave YMC below 1 log CFU/cm^2 after 24-h storage, *i.e*., 0.6 and 0.5 log CFU/cm^2, respectively. Both concentrations maintained YMC of cornhusks below 2 log CFU/cm^2 during 14-d cornhusk storage in the range of 1.20 to 1.75 log CFU/cm^2. More than 2 log CFU/cm^2 of YMC was observed after 14-d storage of untreated cornhusk, hot water-treated cornhusk, and PLE 25%-treated cornhusk. The untreated cornhusk was the most susceptible to contamination, with the YMC value of more than 2 log CFU/cm^2 during storage.

Figure 1D demonstrates that no *A. flavus* and *A. parasiticus* were able to survive in cornhusks until 7-d storage when treated with the combination of hot water and PLE at various concentrations (25, 50, 75, and 100%), whereas the treatment of hot water alone showed no contamination up to 3-d storage. About 1 to 1.45 log CFU/cm^2 of *Aspergillus flavus-A. parasiticus* counts was observed in untreated cornhusk during the storage period. From the result on day 1 of storage, either hot water alone or in combination with PLE at all concentrations showed inhibitory effects against *A. flavus* and *A. parasiticus* that are indigenously present in cornhusks. The *Aspergillus flavus-A. parasiticus* counts of PLE-treated and untreated cornhusk were comparable on day 14 of storage. The contamination of *A. flavus* and *A. parasiticus* in PLE-treated cornhusks is likely to have originated from the environment of the material warehouse where the cornhusks were stored.

The present work found that the treatments with PLE at concentrations of 75 and 100% resulted in higher antimicrobial activity. This result implied that the bioactive compounds of PLE could be substantial contributor to impairing the growth of undesirable microorganisms. The efficacies of saponin as an antifungal have been reviewed by Arif *et al*. (2009), and its ability can be related to the amphiphilic nature of saponin to penetrate through the fungal cell wall. Tannin has also been reviewed to cause the death of the bacterial cell through the disruption of the bacterial cell wall, and the severity of the damage depends on the cell wall components (Kaczmarek, 2020). In the case of yeast inhibition, saponin destroys the membrane through the binding of the sterols, and leakage of the cytoplasm components out of the cell (Zhang *et al*., 2006). This indicated that PLE could be used as an antimicrobial active substance for other various packaging models to inhibit the growth of a wide range of unwanted microorganisms.

WVTR of cornhusk incorporated with PLE

The thickness of the cornhusks was not significantly different across all treatments ($p > 0.05$). The average thickness was 0.22 mm with a standard deviation of 0.01 mm. Based on the result of thickness, the data of WVTR obtained (Table 1) were supposed to be not affected by the thickness of the cornhusk, but it was only influenced by the treatments given.

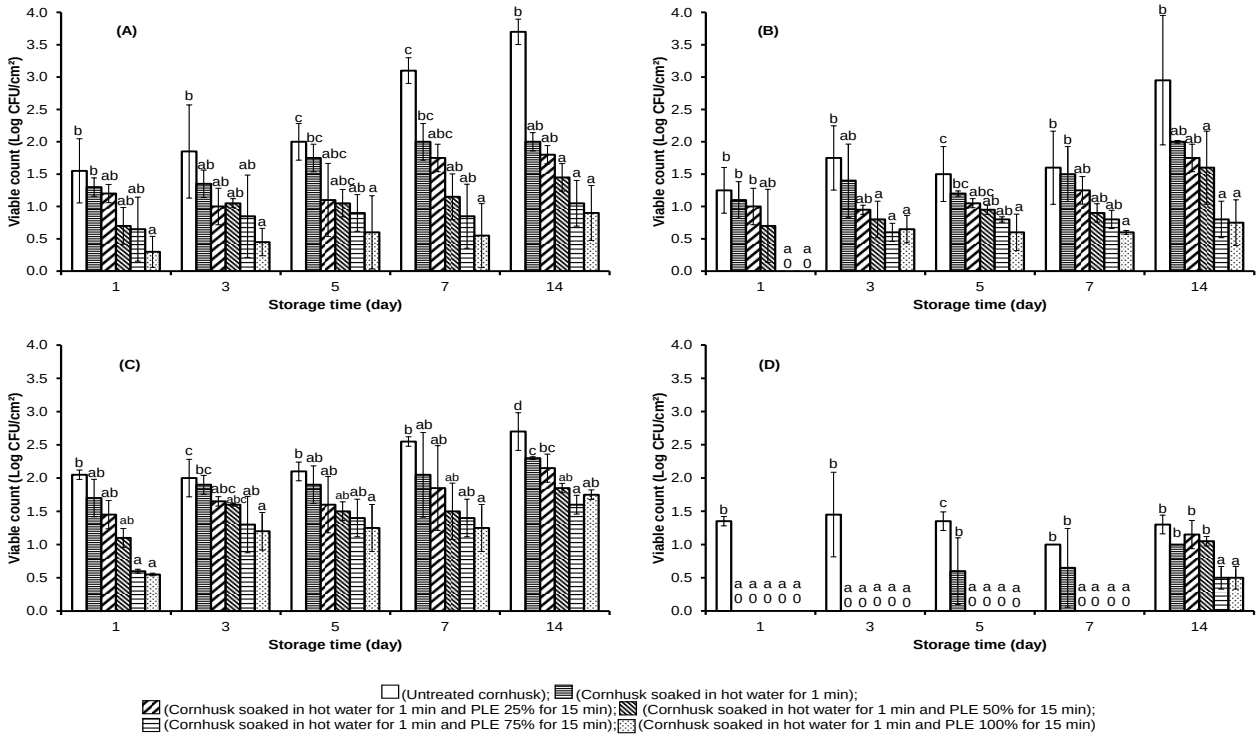

Figure 1. TPC **(A)**, YMC on PDA **(B)**, YMC on DG18 **(C)**, and *Aspergillus flavus-A. parasiticus* counts **(D)** of untreated- and treated-cornhusks. 0 (zero) means that the contamination was 0 Log CFU/cm² in the sample. Error bars represent the standard deviation of the mean (n = 4).

Table 1. WVTR, tensile strength, and elongation of untreated- and treated- cornhusks.

	Sample	Storage time (day)				
		1	3	5	7	14
WVTR (g/m²/day)	T1	29.41 ± 0.15^{b}	28.64 ± 0.77^{b}	28.73 ± 1.20^{c}	28.06 ± 0.24^{b}	27.89 ± 1.12^{b}
	T2	29.17 ± 0.55^{b}	28.77 ± 1.05^{b}	27.42 ± 0.41^{bc}	25.95 ± 0.73^{ab}	25.13 ± 1.56^{b}
	T3	28.20 ± 1.68^{ab}	25.95 ± 2.05^{a}	25.11 ± 0.12^{ab}	26.09 ± 1.66^{ab}	24.69 ± 0.90^{b}
	T4	28.61 ± 0.27^{ab}	26.58 ± 0.73^{ab}	25.90 ± 0.32^{ab}	25.47 ± 1.76^{ab}	24.52 ± 1.98^{b}
	T5	26.99 ± 0.51^{a}	25.01 ± 0.32^{a}	24.06 ± 1.37^{a}	23.26 ± 1.07^{a}	20.17 ± 0.07^{a}
	T6	27.43 ± 0.26^{ab}	24.26 ± 0.29^{a}	24.33 ± 1.85^{a}	22.95 ± 1.07^{a}	19.78 ± 1.37^{a}
Tensile strength (MPa)	T1	17.55 ± 1.60^{b}	16.96 ± 1.97^{b}	16.64 ± 1.23^{d}	16.99 ± 0.57^{c}	15.58 ± 1.03^{a}
	T2	11.27 ± 3.70^{a}	8.36 ± 2.39^{a}	7.97 ± 1.06^{a}	8.53 ± 2.52^{a}	11.18 ± 1.53^{a}
	T3	10.13 ± 1.82^{a}	10.15 ± 2.67^{a}	8.66 ± 2.60^{ab}	12.16 ± 0.07^{b}	11.67 ± 2.20^{a}
	T4	10.20 ± 2.43^{a}	9.79 ± 0.93^{a}	12.02 ± 0.84^{bc}	14.92 ± 2.12^{bc}	14.70 ± 2.12^{a}
	T5	10.36 ± 0.35^{a}	12.12 ± 1.36^{a}	10.95 ± 1.35^{abc}	13.17 ± 0.16^{b}	12.96 ± 0.49^{a}
	T6	13.51 ± 3.21^{ab}	12.23 ± 1.25^{a}	12.80 ± 1.14^{c}	13.75 ± 1.36^{bc}	14.61 ± 2.66^{a}
Elongation (%)	T1	4.89 ± 3.06^{a}	3.89 ± 0.58^{a}	2.80 ± 0.80^{a}	2.84 ± 0.47^{a}	3.23 ± 0.07^{a}
	T2	7.41 ± 0.94^{ab}	4.98 ± 0.25^{a}	3.87 ± 2.11^{a}	3.48 ± 0.86^{a}	3.55 ± 0.37^{a}
	T3	14.66 ± 3.25^{c}	3.87 ± 1.16^{a}	3.27 ± 0.95^{a}	4.19 ± 1.24^{a}	3.39 ± 0.21^{a}
	T4	12.64 ± 0.43^{bc}	3.39 ± 0.98^{a}	3.85 ± 0.80^{a}	3.24 ± 1.28^{a}	3.59 ± 0.80^{a}
	T5	13.21 ± 3.34^{bc}	4.65 ± 2.27^{a}	4.50 ± 2.37^{a}	3.84 ± 0.40^{a}	3.58 ± 0.21^{a}
	T6	16.35 ± 1.43^{c}	4.68 ± 0.54^{a}	5.23 ± 0.58^{a}	3.42 ± 0.20^{a}	3.75 ± 0.01^{a}

T1: untreated cornhusk; T2: cornhusk soaked in hot water for 1 min; T3: cornhusk soaked in hot water for 1 min and PLE 25% for 15 min; T4: cornhusk soaked in hot water for 1 min and PLE 50% for 15 min; T5: cornhusk soaked in hot water for 1 min and PLE 75% for 15 min; and T6: cornhusk soaked in hot water for 1 min and PLE 100% for 15 min). Values are mean ± standard deviation (n = 4). Means with different lowercase superscripts in the same column for each sample are significantly different ($p < 0.05$).

Table 1 shows that the various treatments given had a significant impact on WVTR value ($p < 0.05$). The combination of hot water and PLE at various concentrations (25, 50, 75, and 100%) posed lower WVTR during 14-d storage as compared to the treatment of hot water, alone and untreated. On day 14 of storage, the combination of hot water and PLE at concentrations of 75 and 100% resulted in the lowest WVTR values, 20.17 and 19.78 g/m^2/day, respectively. This result indicated that the barrier property of the cornhusk could be greatly improved by the addition of PLE. The particles of the extract may impede the movement of the water vapour through the cornhusk. The water-cellulose system of the cornhusk allows the particles of the extract to be incorporated during the soaking process (Sukoco *et al*., 2019). Lipophilic compounds that are still likely bound to saponin provide a better barrier against water vapour. This was in agreement with the result obtained by Schmidt *et al*. (2013) who used stearic acid as a lipophilic agent to reduce the water vapour permeability of cassava starch-based film. Since saponin may improve water vapour barrier performance, focus on the optimum saponin extraction can be taken in the future to attest to its ability in controlling water vapour transport.

Mechanical properties of cornhusk incorporated with PLE

Table 1 also presents significant differences among treatments given on the tensile strength of cornhusk stored on days 1, 3, 5, and 7 ($p < 0.05$), while no significant difference was found on day 14 ($p > 0.05$). This can be a consequence of the moisture content of cornhusk on day 14 of storage which was not significantly different ($p > 0.05$) in the range of 9.56 to 10.14%. The intra- and inter-molecular associations such as hydrogen bonds in the presence of water led to a decrease in the value of tensile strength (Bertuzzi *et al*., 2012). The presence of PLE also possibly weakens the tensile strength of the cornhusk because the OH-cellulose system of the cornhusk can interact with particles of extract. As shown in the present work, the tensile strength values of the cornhusks treated with hot water alone or in combination with PLE at various concentrations (25, 50, 75, and 100%) were lower than that with untreated cornhusks during 14-d storage. In this regard, there is only a strong OH-cellulose bond and a closer distance of the bond between cellulose. Such a system strengthens the cohesiveness of the cornhusk, thereby increasing the tensile strength value of the untreated cornhusk. This result agreed with Unar *et al*. (2010) that the absence of plasticisers such as water increased tensile strength due to the system having no dipole interactions between polymer and plasticiser.

Elongation of cornhusk stored on day 1 showed a higher value significantly ($p < 0.05$) when treated with hot water alone or in combination with PLE at various concentrations (25, 50, 75, and 100%) as compared to untreated cornhusk (Table 1). This result agreed with Rezaei and Motamedzadegan (2015) that the lower the tensile strength, the higher the elongation, and vice versa. On days 3, 5, 7, and 14 of storage, the treatments of hot water alone or in combination with PLE at different concentrations also had higher elongation than untreated cornhusk, but not significantly different ($p > 0.05$). Water did not exhibit its plasticising effect since there was no polymer added in the present work which was responsible for retaining the water in the cornhusk during storage. Other plasticisers such as glycerol, sorbitol, and poly(ethylene glycol) 400 in combinations with natural polymers (pectin, starch, and whey protein isolate) are possible to be used for further study.

Structural properties of cornhusk incorporated with PLE

The structures of untreated cornhusk, as well as cornhusk treated with hot water alone or in combination with PLE 100%, are compared (Figure 2). The pores (white circle) of the untreated cornhusk were clearly observed as shown in Figure 2A, thus indicating no water molecules on the surface of the untreated cornhusk. Figure 2B indicates that hot water treatment affected the surface of the cornhusk by entering the OH-cellulose system which is depicted as water separating two cellulose parts (red circles), whereas Figure 2C illustrates that the particles of PLE are shown like impurities that adhered to and covered the cornhusk surface. This model was also investigated in a work by Liu *et al*. (2016) who observed that the essential oil dispersed on the surface of the blend films made from oregano essential oil in poly(lactic acid)/poly(trimethylene carbonate).

The crystallinity measurement convinced the structural observation. In the present work, the crystallinity values of untreated cornhusk, cornhusk

soaked in hot water alone, and cornhusk soaked in hot water and PLE 100% were 19.44 ± 0.41, 17.25 ± 1.68, and 16.62 ± 1.39%, respectively ($p > 0.05$). Cornhusk soaked in hot water and PLE 100% possessed the lowest crystallinity. Water is one of the most effective plasticisers that can decrease the degree of crystallinity (Vieira *et al*., 2011). It is likely that the PLE also decreased the cornhusk crystallinity due to phenolic, tannin, saponin, and flavonoid compounds, which are potential plasticisers. This result paralleled that of elongation where PLE-treated cornhusks exhibited higher value as compared to that without PLE on day 1 measurement (Table 1).

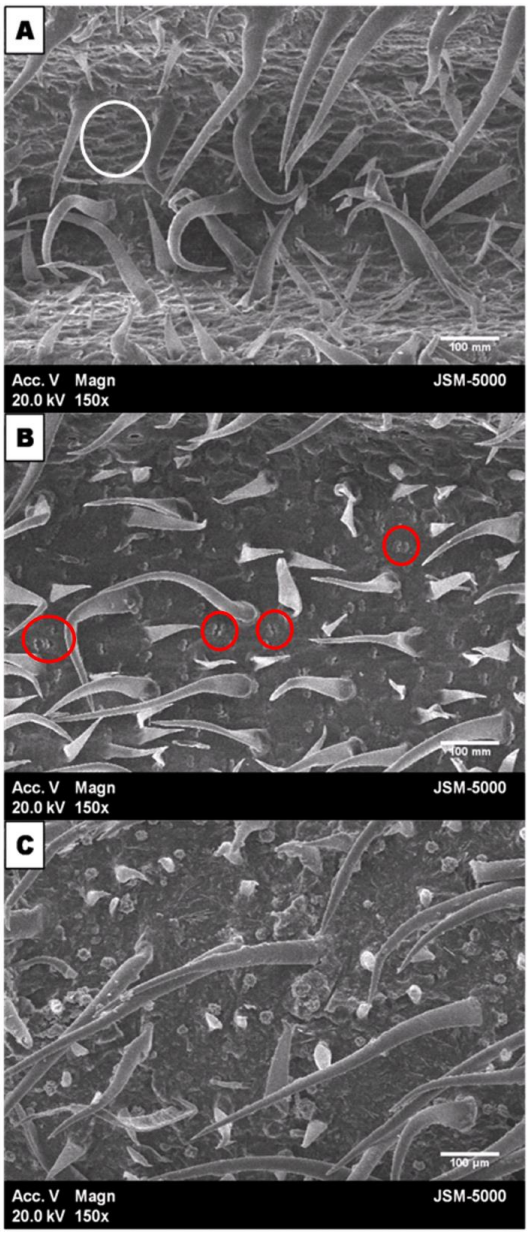

Figure 2. Morphology of untreated cornhusk **(A)**, cornhusk soaked in hot water for 1 min **(B)**, and cornhusk soaked in hot water for 1 min and PLE 100% for 15 min **(C)**.

Microbial decay of GRS

Cornhusks treated with the combination of hot water and PLE at concentrations of 75 and 100% were selected for further investigation due to outstanding characteristics in terms of microbiological, mechanical, and water vapour barrier improvements. Cornhusk soaked in hot water was also used as a comparison, which is mostly applied as commercial packaging by the small- and medium-scale industries.

Figure 3A shows that the TPC values of GRS on day 1 of storage were 2.55 log CFU/g (wrapped in PLE 100%-treated cornhusk), 3.35 log CFU/g (wrapped in PLE 75%-treated cornhusk), and 4.6 log CFU/g (wrapped in a hot water-treated cornhusk or named a commercial packaging). This result indicated that microbial decay happened immediately after the cooking process. Other than raw mung bean, packaging types is obviously also considered to cause rapid microbial deterioration of GRS. Interestingly, TPC values of GRS wrapped in cornhusk treated with the combination of hot water and PLE at concentrations of 75 and 100% were significantly lower during GRS storage than using the commercial packaging ($p < 0.05$). This paralleled the TPC values of the packaging used (Figure 1A). Since PLE-treated cornhusks had lower TPC (0.3 to 1 log CFU/cm^2) than commercial packaging (1.3 to 2 log CFU/cm^2), the TPC of GRS wrapped in PLE-treated cornhusks also became minimal.

TPC of GRS wrapped in PLE 75%-treated cornhusk on day 7 of storage reached 4.1 log CFU/g, and this value was more than the acceptable range for TPC value determined by Indonesian National Standard (abbreviated as SNI) 7388 (SNI, 2009), *i.e.*, 4 log CFU/g. Whereas PLE 100%-treated cornhusk retained the TPC of GRS in the accepted range until 14 days of storage (3.85 log CFU/g). In contrast, the use of commercial packaging did not seem to maintain the TPC of GRS during storage.

PLE at concentrations of 75 and 100% significantly suppressed the growth of yeasts and moulds in GRS ($p < 0.05$), as shown by the YMC either on PDA or DG18 (Figures 3B and 3C, respectively). Cornhusks treated with PLE at concentrations of 75 and 100% consecutively provided lower YMC (on PDA) of GRS up to 3.15 and 2.2 log CFU/g after 24 h storage. Also, lower YMC (on DG18) by approximately 3.6 and 3.7 log CFU/g were found in GRS after 24-h storage. YMC of GRS wrapped in cornhusks treated with PLE and wrapped in commercial packaging gave a similar

increasing trend between PDA and DG18. Despite the YMC values over 2.3 log CFU/g (SNI 7388) (SNI, 2009) observed in GRS wrapped in PLE-treated cornhusks on day 1 of storage, the present work emphasised that the increase in the surviving yeasts and moulds was greatly different as affected by the packaging used.

Figure 3D also indicates that the PLE at 75 and 100% concentrations were less effective against aflatoxigenic fungi in GRS. There was no significant difference in *Aspergillus flavus-A. parasiticus* counts on day 1 of GRS storage wrapped in all packaging types ($p > 0.05$). Significant results were obtained after storing GRS for over 24 h ($p < 0.05$). Cornhusk treated with PLE 100% exhibited a slight increase in *Aspergillus flavus-A. parasiticus* counts of GRS from 2.10 log CFU/g (day 1) to 2.85 log CFU/g (day 14). Whereas *Aspergillus flavus-A. parasiticus* counts of GRS wrapped in cornhusk treated with PLE 75% rocketed from 1 log CFU/g (day 1) to 3.20 log CFU/g (day 14). The highest *Aspergillus flavus-A. parasiticus* count was observed in GRS wrapped in commercial packaging with a value of more than 3 log CFU/g during the storage period.

These results confirmed that PLE had moderate or even low anti-yeast and antifungal activities, especially antifungal activity against *Aspergillus* species. Fungi of the genus *Aspergillus* are tolerant to an extreme environment (Mandal *et al*., 2022) as they can produce diverse classes of active metabolites to modulate varying mechanisms of their resistance. Bautista-Baños *et al*. (2000) also found that PLE showed medium antifungal activity against pathogenic fungi *Pestalotiopsis* spp., *Alternaria* spp., and *Fusarium* spp. However, the activity of PLE against undesirable microorganisms can still be attributed to the mechanism of action of bioactive compounds such as saponin and tannin (Zhang *et al*., 2006; Arif *et al*., 2009; Kaczmarek, 2020). The release of such bioactive compounds from packaging to food surfaces allows PLE to slow down or inhibit the undesirable growth of microorganisms. Since the shortage in the extraction efficiency to obtain the optimum level of bioactive compounds can affect fungal growth, further studies are necessary to optimise the extraction condition for the practical application of PLE in a cornhusk. Further studies on the synergistic combination of PLE with other antimicrobial compounds of herbs, spices, or other plant sources may also be possible to tackle this drawback.

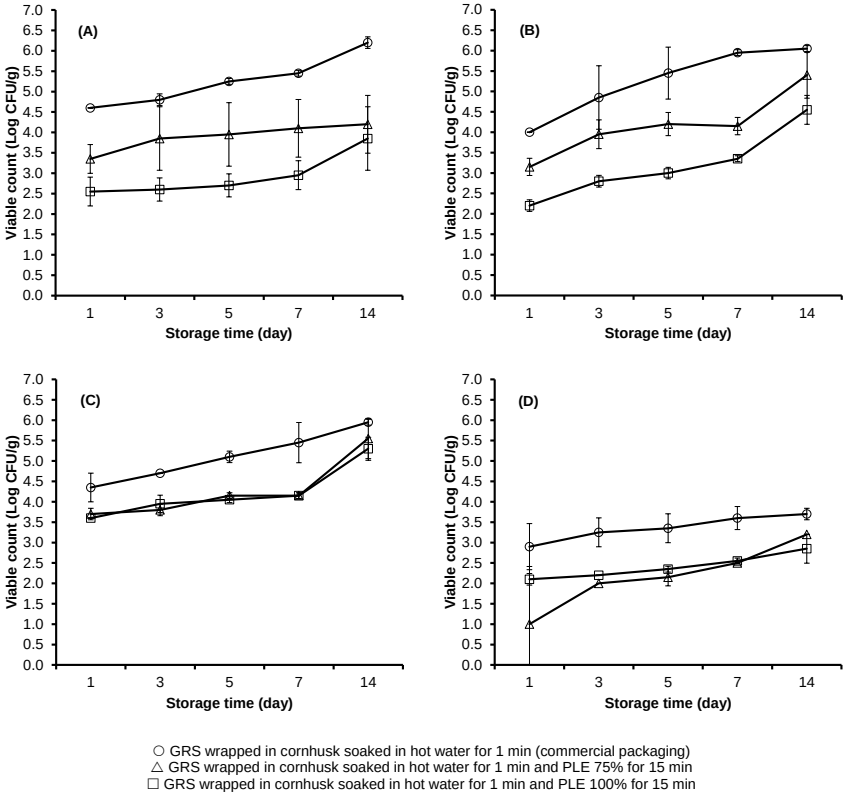

Figure 3. TPC **(A)**, YMC on PDA **(B)**, YMC on DG18 **(C)**, and *Aspergillus flavus-A. parasiticus* counts **(D)** of GRS wrapped in various packaging types. Error bars represent the standard deviation of the mean ($n = 4$).

Free fatty acids (FFA) content of GRS

A different trend was observed on the changes of FFA in GRS during 14-d storage. The FFA values of GRS using commercial packaging, PLE 75%-treated cornhusk, and PLE 100%-treated cornhusk were 0.83 ± 0.01, 0.54 ± 0.02, and 0.72 ± 0.02, respectively (day 1); 0.82 ± 0.02, 0.60 ± 0.03, and 0.77 ± 0.03, respectively (day 3); 0.77 ± 0.01, 0.55 ± 0.01, and 0.72 ± 0.03, respectively (day 5); 0.69 ± 0.01, 0.60 ± 0.01, and 0.62 ± 0.01, respectively (day 7); and 0.69 ± 0.01, 0.60 ± 0.02, and 0.62 ± 0.01, respectively (day 14).

PLE-treated cornhusks showed significantly lower FFA in GRS on days 1, 7, and 14 of storage than GRS wrapped in commercial packaging ($p < 0.05$). However, the GRS wrapped in cornhusk treated with PLE 75% demonstrated the lowest FFA content ($p < 0.05$). This could mainly be due to the moisture content of GRS during storage. As previously stated, the sun-drying process should be able to reach a moisture content of wrapped GRS lower than 20%. Cornhusk treated with PLE 100% resulted in a higher (different up to 1.5%) moisture content of GRS during storage than that of using cornhusk treated with PLE 75% ($p > 0.05$). Both treatments produced higher moisture content of about 19% during the early stage of storage, while untreated cornhusk had a significantly lower value (16%) on moisture content ($p < 0.05$). The particle of the PLE accumulated on the cornhusk surface (Figure 2C) kept the moisture content of GRS higher, particularly during initial storage, due to the constraint of the water vapour moving out of the packaging during the drying process (Table 1).

On one hand, there was an increasing trend on FFA in GRS wrapped in cornhusk treated with PLE 75% after 5-d storage. On the other hand, GRS wrapped in cornhusk treated with PLE 100% indicated decreasing trend after 3-d storage. Higher PLE concentration is believed to provide stronger antioxidant activity for the cornhusk. It was found that the PLE exhibited the average inhibition of 49.53 ± 2.67% (IC_{50}: 267.03 ± 6.32 µg ascorbic acid/mL) in scavenging DPPH free radical through the electron-donating ability. Therefore, the present work suggested cornhusk treated with PLE 100% because of the FFA trend recorded which may affect the FFA in GRS after prolonged storage (more than 14 d). Similarly, Sharma *et al*. (2021) reported that the higher concentration of *Commiphora wightii* extract incorporated into the edible films, the lower the FFA of chicken nuggets until two months. The accumulation of PLE on the surface of GRS also certainly contributed to the decrease in FFA content during storage.

Detection of bioactive compounds on GRS surface by FTIR

The FTIR spectra revealed that the addition of PLE into packaging material changed the spectrum range that could present the best prediction. Figure 4A shows the peak of GRS wrapped in commercial packaging at 3395 cm^{-1} corresponding to the OH stretch band, and slightly shifted to 3368 cm^{-1} in GRS wrapped in cornhusks treated with PLE 75 and 100% (Figures 4B and 4C, respectively). This indicated that chemical interactions occurred when two or more compounds were mixed. Long-chain linear aliphatic compounds were identified at 2978, 2923, and 2926 cm^{-1} by the peak of GRS wrapped in commercial packaging, cornhusk treated with PLE 75%, and cornhusk treated with PLE 100%, respectively. Those peaks refer to the fat content of the sample. The presence of carbon dioxide, as shown at peaks 2361 and 2337 cm^{-1} (Figures 4B and 4C), was not the problem, and this did not interfere with bands of investigated compounds.

The present work used gallic acid as a reference compound which is commonly used as a standard for the measurement of total phenol and tannic acid contents. In the fingerprint region, the sharp peaks of 867 cm^{-1} (Figure 4B) and 866 cm^{-1} (Figure 4C) were similar to the sharp peak of gallic acid at 867 cm^{-1} (Figure 4D), corresponding to C-H out-of-plane band (aromatic). By comparing the FTIR spectrum of the GRS wrapped in commercial packaging with that of spectra of gallic acid and GRS wrapped in PLE-treated cornhusks, the presence of bioactive compounds in GRS wrapped in PLE-treated cornhusks was observed. For instance, tannin chemically contains aromatic rings.

Ricci *et al*. (2015) reported peaks of tannin at 3370, 2925 - 2927, 1630, 1411, 1260, 1148, 1050, 921, and 864 cm^{-1}. The present work used those peaks as approach peaks to indicate the presence of tannin in GRS. Figure 4B shows the experimental peaks at 3368, 2923, 1639, 1416, 1263, 1135, 1051, 926, and 867 cm^{-1}. Figure 4C shows the experimental peaks at

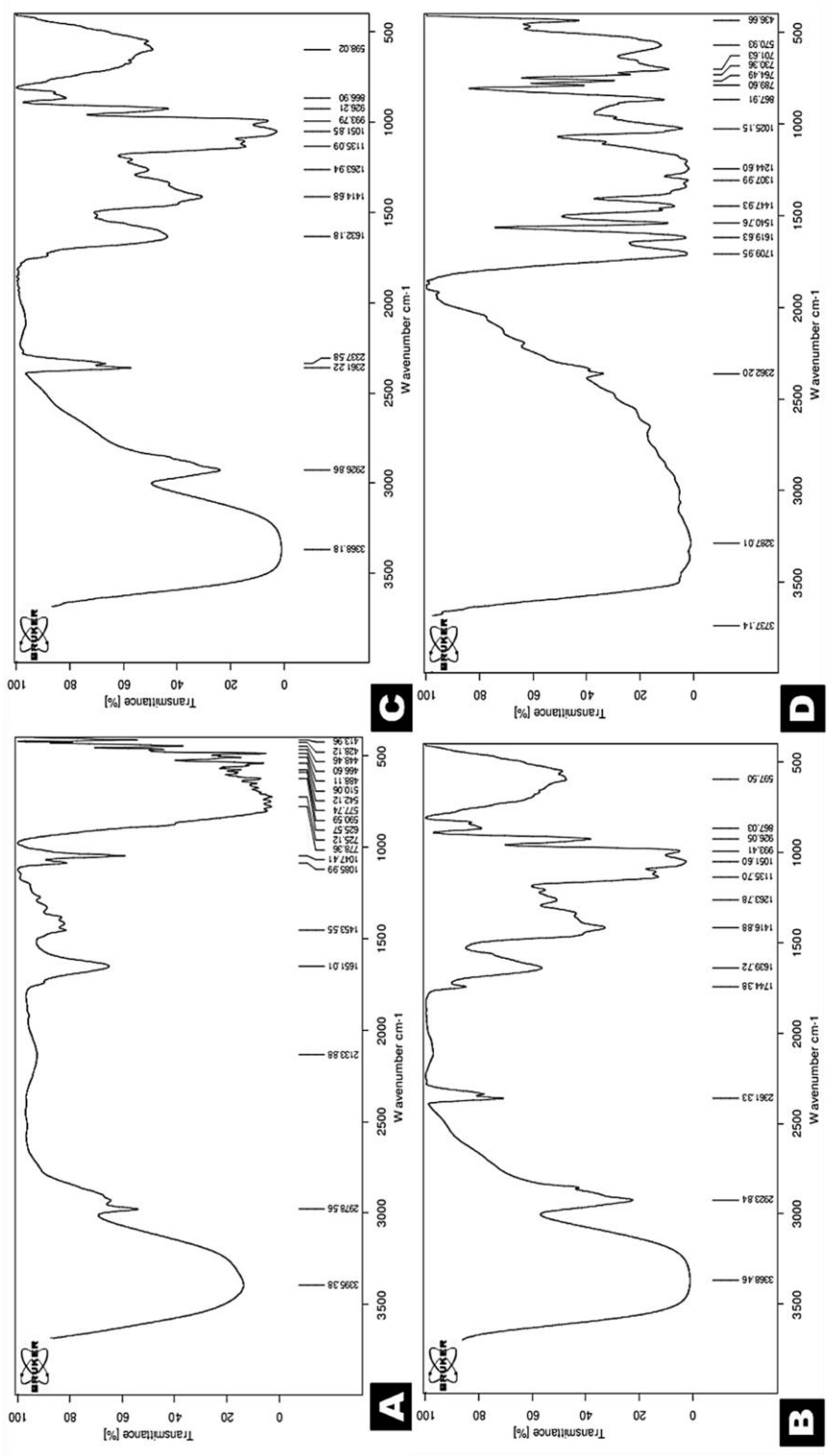

Figure 4. FTIR spectra of GRS wrapped in cornhusk soaked in hot water for 1 min (commercial packaging) **(A)**, GRS wrapped in cornhusk soaked in hot water for 1 min and PLE 75% for 15 min **(B)**, GRS wrapped in cornhusk soaked in hot water for 1 min and PLE 100% for 15 min **(C)**, and FTIR spectrum of gallic acid **(D)**.

3368, 2926, 1632, 1414, 1263, 1135, 1051, 926, and 866 cm^{-1}. The approach peaks and the experimental peaks were closely similar. The experimental peaks at 1632 and 1639 cm^{-1} were related to the stretching of aromatic C═C. The experimental peaks at 1414 and 1416 cm^{-1} were attributed to the C–C stretch (aromatic frame stretch vibrations). The experimental peak at 1051 cm^{-1} was related to C–O asymmetric stretch in the aromatic–OH group. The experimental peaks at 926, 867, and 866 cm^{-1} were mainly considered characteristic of tannin and were attributed to the C–H out of plane (aromatic).

Almutairi and Ali (2014) showed the saponin peaks at 3525 - 3281, 2932 - 2973, 1724, 1609, 1148, and 1074 cm^{-1}. These peaks were quite similar to the experimental peaks. Saponin characteristic peaks could be at 1744 cm^{-1} (Figure 4B) and 1051 cm^{-1} (Figures 4B and 4C). The peak at 1744 cm^{-1} was related to the stretching vibration of C=O, which was one of the functional groups for triterpenoid and steroid backbone. The peak at 1051 cm^{-1} was assigned to its sugar chains, and attributed to the stretching vibrations of C–O and C–C. These peaks confirmed that the saponin structure consisted of a triterpene or steroid and one or more sugar chains. However, quantitative studies can be further performed to evaluate the bioactive compounds in GRS accurately, and their migration behaviour.

Conclusion

PLE at concentrations of 75 and 100% boosted the antimicrobial activity of cornhusk in retarding the increase in TPC, YMC, and *Aspergillus flavus-A. parasiticus* counts during storage. At the same concentration, PLE improved the WVTR, elongation, and tensile strength of the cornhusk. The use of those concentrations lowered TPC, YMC, and *Aspergillus flavus-A. parasiticus* counts of GRS during storage, as well as yielding the least FFA content of GRS. The particle of the extract that adhered to the cornhusk surface was clearly seen under SEM observation. FTIR was able to identify the characteristic bands of saponin and tannin present on the surface of GRS, in which those compounds have previously been quantified in the PLE. PLE applied in the present work could serve as a promising agent for controlling microbial contamination of the packaging material (cornhusk) and packaged food (GRS), as well as retarding the rancidity of GRS.

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