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Development and Characterization of Palmyrah (*Borassus flabellifer* L.) Tuber Starch Incorporated Biodegradable Packaging Film

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ABSTRACT

Non-biodegradable synthetic packaging materials cause tremendous harmful effects on the ecosystem. As an alternative, biodegradable packaging materials have been introduced in food industries. Starch is one of the most important polysaccharides used in the formulation of biodegradable edible packaging films. Palmyrah (*Borassus flabellifer* L.) tuber is rich source of starch. The main objective of this research was the development and characterization of bio-degradable films for fresh cut fruits and vegetables. Five treatments with different ingredient formulations of palmyrah starch and gelatine mixed in distilled water and glycerol were experimented in forming packaging film solution and their physical parameters were assessed. Results revealed that T3 treatment containing 4% starch and 1% gelatine had the highest tensile strength with 10.0±0.00 MPa. Also it has favourable values such as 4.10±0.00 g/m^2.d of water vapour transmission rate, 0.14±0.01 mm of film thickness, 14.43±0.02 % of moisture content, 63.09±0.03 % of solubility and 41.00±1.41 mm of elongation at break parameters. Further, T3 film was enriched by incorporating clove oil, cinnamon oil, and the preservatives such as sodium metabisulphite, sodium benzoate and potassium sorbate to obtain antibacterial activity. The 1.5% concentration of clove oil or cinnamon oil exhibited antimicrobial activity against *Escherichia coli, Bacillus brevis* and *Pseudomonas aeruginosa* while other concentrations were less effective. The inhibitory zone was not observed in treatments with preservatives. In conclusion, the formulation comprised of 4% palmyrah tuber starch, 1% gelatine and 1.5% glycerol enriched with either 1.5% clove oil or cinnamon oil could be considered as the best formulation for development of biodegradable packaging material with antimicrobial activity.

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INTRODUCTION

Food packaging occupies a prominent place in the food supply chain as it ensures the quality and safe food product delivery to the consumer (Loo and Sarbon, 2020a). Novel approaches in storage techniques including active packaging, intelligent packaging, biodegradable packaging and edible packaging possess immense research potential and applicability (Fai *et al*., 2016). Nonbiodegradable nature of synthetic packaging materials affects the ecosystem negatively. Hence the Government is urged to reduce the usage of nonbiodegradable food packaging materials. As a result, there is an increasing demand to replace the synthetic packaging materials from biodegradable materials (Chisenga *et al*., 2020).

Proteins and polysaccharides are the main polymers used in making biodegradable films. Starch is the most abundant and important polysaccharide used in the formulation of biodegradable edible films (Chiumarelli and Hubinger, 2014). Incorporation of active ingredients into edible films provides added advantages (Fai *et al*., 2016). Accordingly, edible films have been treated with a wide range of antimicrobial agents. Organic acids, essential oils and extracts are released into food during storage thereby ensure the food safety and storability.

Palmyrah (*Borassus flabellifer* L.) tuber is a rich source starch and commonly used in food preparation (confectionaries, porridge and soups). Proximate composition, morphology, structure and physicochemical properties of palmyrah tuber starch were reported by Naguleswaran *et al*., (2010). Thus palmyrah tuber starch is a potential ingredient for the development of biodegradable packaging films. The main constrain was inadequate information in formulation of edible packaging films and applications of palmyrah tuber starch in food industry. Palmyrah tubers are seasonal and underutilized product. Hence this research was conducted to develop biodegradable packaging films for fresh cuts of fruits and vegetables.

MATERIALS AND METHODS

Materials

The Palmyrah tubers, gelatin, glycerol, cinnamon oil, clove oil, sodium metabisulphite, sodium benzoate and potassium sorbate were purchased from a food ingredient dealer, Jaffna, Sri Lanka. *Escherichia coli (*ATC35218), *Psuedomonas aeruginosa* (NCTC10662) and *Bacillus brevis* (Biochemically Identified) were used as the reference cultures.

Isolation of Starch

Isolation of starch was done using the method described by Osunsami and Oguntimein (1987) with some modifications. Palmyrah tubers were washed in distilled water to remove the traces of contaminants. Then, the shoots were sliced into pieces and subjected to wet grinding. The slurry obtained was filtered using a muslin cloth and kept 24 hours for starch sedimentation. The supernatant was decanted and tuber starch (the sediment) washed using distilled water to receive white colour starch suspension. Then, the sediment was dried at 60 ^{0}C for 3 hours and ground using the mortar and pestle. Finally, dried starch was packed in air tightly plastic bottles for further experiments. The yield of starch was calculated.

Analysis of Proximate Composition

Moisture, ash, acid insoluble ash contents of isolated starch were analysed (SLS 913: 1991) while protein and fat contents were analyzed using AOAC (2019). The starch content was measured using the method elaborated by Wijesinghe *et al*. (2015).

Development of Biodegradable Packaging Films

Palmyrah tuber starch was dissolved in 100 mL of distilled water and stirred for 30 min at 80 ^{0}C on a hot plate until fully gelatinized. Glycerol and gelatine were added to the film-forming solution. Then film forming solution was poured into 0.03 m^2 plastic trays, and allowed to dry at 40 ^{0}C for 24 hours. After that, the films were peeled for further analysis as described in Niu *et al.* (2021). Table 1 shows the formulations used for the film development.

Analysis of Mechanical and Physical properties of Films

Water Vapour Transmission Rate (WVTR)

The *WVTR* of each film was measured by a modified technique of wet cup method. The cup was filled with distilled water and sealed using the film. The cup was then placed in the desiccator to maintain the humidity and placed in the incubator to maintain the temperature. Silica gel was used to

Table 1. Ingredient formulations of the treatments used for film development.

Treatments	Starch (g)	Gelatin (g)	Glycerol (g)	Water (mL)
T1	3.0	2.0	1.5	100
T2	3.5	1.5	1.5	100
T3	4.0	1.0	1.5	100
T4	4.5	0.5	1.5	100
T5	5.0	0.0	1.5	100

control the relative humidity in the desiccator. Thermo couple and humidity meter were used to monitor the ambient temperature (23.7 ^{0}C) and relative humidity. This experiment was done at relative humidity of 48%. The cups were periodically removed and weighed. The mass of water loss from the cups was monitored as a function of time.

The WVTR was calculated using the following equation described in Basha *et al.* (2011),

$$WVTR = (\frac{W}{t})/A,$$

where,
$WVTR$: water vapour transmission rate (g/m^2. d),
w: weight change (g),
t: time (d) and
A: test area (m^2).

Water Solubility

WS was measured using the modified weight loss method. Briefly, films (2 cm × 2 cm) were weighed prior to dissolve in distilled water for 24 h and then removed and dried at 90 ^{0}C to a constant weight. WS was calculated by the following equation described by Niu *et al.* (2021),

$$WS = (M1 - M2)/M1,$$

where,
$M1$: Initial weight (dry basis) and
$M2$: Final weight (dry basis).

Moisture Content

Moisture content was estimated using cut pieces of packaging film (2.0 cm^2). The sample was placed in an oven at 110 ^{0}C until a constant dry weight was acquired (Marichelvam *et al.*, 2019) as follows, MC (%) = [(Wi - Wf)/Wi] 100,

where,
Wi : Initial weight and

Wf : Final weight.

Thickness

The thickness of the film was measured at five different points of the films using Micrometer (INGCO, HMM0025-ING, China) and the average thickness was calculated.

Biodegradability Test

Biodegradability test was performed with soil (500 g) as the method described in Marichelvam *et al.* (2019). The specimen was cut into pieces (4.0 cm^2). Near the roots of leguminous plants rich in nitrogenous bacteria, 500 g of soil with slight moisture content was collected. The sample was buried inside the soil at a depth of 3 cm. Buried film was taken from the soil and observed visually at the interval of 3 days for 15 days. Time taken for the complete biodegradation was observed.

Tensile Strength and Elongation at Break

The tensile strength and elongation at break of the films were measured using a Universal Testing Machine (WEW, Jinan Kason testing equipment Co. Ltd, China) according to method described in Tongdeesoontorn *et al.* (2012). The initial gauge length was 50 mm and crosshead speed was 50 mm/min.

Optimization of Formulations of the Packaging Film

The optimization of formulation was done considering higher tensile strength and lower water vapour transmission rate. The results were statistically evaluated by Analysis of variance (ANOVA) and Tukey's test at 95% confidence level using Minitab 17 statistical software.

Development of Antimicrobial Film with Essential Oil

Optimized ingredient formulation in the earlier step (Treatment, T3) was selected for further development of antimicrobial film with essential oil. The film forming solution was homogenized (10,000 rpm, 3 min) with essential oil. Then the film forming solution was poured into trays and allowed to dry in the hot air oven at 40 ^{0}C for 24 hours. The antimicrobial activity of packaging formulated films with different concentration (0.25, 0.5, 1.0, 1.5% w/w) of clove oil and cinnamon oil was determined.

Development of Antimicrobial Film with Preservatives

Optimized ingredient formulation in the earlier step (T3) was selected for further development of antimicrobial film with preservative. The pH of the film forming solution was adjusted by adding citric acid prior to adding the preservatives to get the optimum function of the particular preservative/s combinations (Table 2). Then the measured amount of preservative was added and stirred well. After that the film forming solution was poured into trays and allowed to dry in the hot air oven at 40 ^{0}C for 24 hours.

Disc Diffusion Test

The sterile plates containing solidified nutrient agar medium were inoculated homogeneously using a sterile swab containing bacterial count 10^5–10^6 CFU/ml (prepared using 0.5 M MacFarland standard). Antimicrobial activity of the films (control and treated) was determined using aseptically cut packaging film discs (7 mm diameter). The discs were placed on the surface of medium of the plates. The inhibition zone diameter surrounding the film discs (colony free perimeter) was measured after 24 hrs incubation at 37 ^{0}C.

Statistical Analysis

Statistical Analysis was performed using one-way ANOVA in Minitab 17 Statistical software.

Table 2. Ingredient formulations of the developed packaging films with preservatives

Concentration (w/w %)*	Adjusted pH
SMS: Sodium metabisulphite (%) 0.25, 0.5, 1.0,1.5	3.5
SB: Sodium Benzoate (%) 0.25, 0.5, 1.0, 1.5	4.5
PS: Potassium sorbate (%) 0.25, 0.5, 1.0, 1.5	4.5
SMS - 0.25% and SB - 0.25%	3.5
SMS - 0.5% and SB - 0.5%	3.5
SB - 0.25% and PS - 0.25%	4.5
SB - 0.5% and PS - 0.5%	4.5

**w/w = weight of preservative/weight of the film forming solution.*

RESULTS AND DISCUSSION

Analysis of Proximate Composition

The yield of starch (15.6%) and the purity of palmyrah tuber starch (93.05%) were determined. The yield and purity of dried tuber flour starch of palmyrah were 38.4% and 95.8%, respectively in the experiment conducted by Naguleswaran *et al.* (2010). The yield of the dried tuber flour starch was higher because of sieving through number of sieves with different pore size. In our experiment, wet milling and sedimentation of starch was done. Therefore, most of the cellular fragments were removed and not available in starch. Thus 93.05±0.36% pure starch was measured. The protein content (0.44%) of our study was less than that of dried tuber flour starch (2.5%) of Naguleswaran *et al.* (2010), the fat content of fresh tuber starch (0.14%) is greater than that of dried tuber flour starch (0.04%) of their study.

Analysis of Mechanical and Physical Properties of Film

Tensile strength and elongation at break of treatments are shown in Figure 1A and 1B. Tensile strength of the developed films increased by increasing the gelatine content. Film with the T3 treatment had significantly the highest ($P<0.05$) tensile strength, while films with T1, T2, and T3 were not significantly different from each other. The similar finding was reported in cassava starch and gelatine formulation by Tongdeesoontorn *et al*. (2012). There was no significant difference ($P>0.05$) in elongation at break of the films T2 (1.5 g gelatine) and T4 (0.5 g gelatine). The reason may be the inconsistency in formation of structure of the films.

The WVTR (Figure 1C) of the treatments T3 and T4 were reported as the lowest in the developed films. Film with higher amount of starch content possesses higher WVTR. Loo and Sarbon (2020b) stated that the addition of 5% tapioca starch decreased the WVTR value while a further increase in tapioca starch was significantly ($P<0.05$) increased in concentration of WVTR value. The WVTR of the films should be lower in films used in food packaging to prevent or reduce moisture migration in-between the food and the storage environment.

Solubility in water is an important property of starch based edible films. Most of the applications

Table 3. Proximate composition of palmyrah tuber starch.

Constituents	Value (%)
Starch yielded (wet milling)	15.60 ± 0.21
Moisture (wb)	10.10 ± 0.05
Pure starch (db)	93.05 ± 0.36
Fat (db)	0.14 ± 0.00
Protein (db)	0.44 ± 0.01
Ash (db)	0.001 ± 0.000
Acid Insoluble Ash (db)	0.000 ± 0.000

wb: wet basis, db: dry basis, ±SD calculated using three measurements (n= 3).

may require water insoluble films to resistance to enhance product integrity and water migration. However, water solubility of films might give advantageous for the encapsulation of food or additives.

Figure 1D shows solubility of the developed films. The solubility of the film decreases from 64.82% (0.5g gelatine, T4) to 43.28% (2 g gelatine,T1). The Solubility of cassava starch-gelatine composite films depends on the concentration of gelatine. Tongdeesoontorn *et al*. (2012), have stated that, water solubility in control film was 73%, and film solubility was decreased with increasing gelatine concentration.

Figure 1E shows the variation of moisture content at different starch concentration. Moisture content of packaging films of palmyrah tuber starch was varied from 14.43% to 18.14% and there was no significant difference among the treatments. A 10.30% moisture content was reported in cassava starch film by Qin *et al*. (2019).

Thickness (Figure 1E) of the palmyrah tuber starch film was varied from 0.11 - 0.16 mm. The average thickness of the bioplastics developed from potato starch, and composite of corn and rice starch were 0.07 mm and 0.25 mm, respectively (Marichelvam *et al*., 2019; Niu *et al*., 2021). Ghasemlou *et al* (2013) reported that 0.15 mm thickness value in corn starch packaging film. Hence their value was compatible with our findings.

Optimization of Formulation

The film developed from treatment; T3 possesses the highest tensile strength and lower WVTR (Figure 1A and Figure 1C). Therefore, Treatment T3 was selected for further development with antimicrobial activity of the film.

Antimicrobial Activity of the Film

Palmyrah tuber starch-based film formulation was enriched with antimicrobial agents, cinnamon oil, clove oil and preservatives as shown in Table 2, aiming at preventing the spoilage of fresh cut fruits and vegetables. The spoilage of vegetables is mainly caused by *E. carotovora* and occasionally by *Pseudomonas*, *Bacillus*, *Clostridium*, yeasts, and molds (Erkmen and Bozoglu, 2016). Tomato, chili, onion, lettuce, arugula, spinach, and cilantro, are incorporated in fresh dishes including ready-to-eat salads. The consumption of these fresh salads provides high nutritional value. However, the number of food borne outbreaks associated with fresh produce has been increasing, with *E. coli* being the most common pathogen associated with cut vegetables (Luna-Guevara *et al*., 2019). Based on the availability of the cultures, *E. coli*, *P. aeruginosa* and *B. brevis* were used as the reference cultures to determine the antimicrobial activity of the packaging films.

According to Table 4.0, cinnamon oil and clove oil with 1.5% concentration was evidenced the antimicrobial activity against *E. coli*, *B. brevis* and *P. aeruginosa.* Cinnamon oil and clove oil with 1% concentration was shown antimicrobial activity only against *E. Coli* and *B. brevis* (Table 4). Gelatine film prepared with incorporation of clove oil 0.75 ml/g of gelatine showed the inhibition zone of 9.95±0.72 mm against *E. coli* and 5.93±0.02 mm against *P. fluorescens* (Gómez-Estaca *et al*., 2009). Cinnamon oil (0.5%) incorporated apple puree film was shown an inhibitory zone of 1.1mm agazones *E. coli* O157:H7 (Rojas-Graü *et al*., 2006). None of the inhibition zone was observed from the film incorporated with SMS, SB, and PS with the concentration of 0.25, 0.5, 1.0, and 1.5%. Inhibition zone was not observed for the chitosan edible film containing potassium sorbate (200 mg/g chitosan) against *E. coli* (Pranoto *et al*., 2005). PS (0.05%) incorporated cassava starch–based edible coatings were not effective in controlling the growth of microbes (Garcia *et al*., 2012). This shows that the active compounds from the chemical preservatives are not penetrate through the film to activate against the microorganisms.

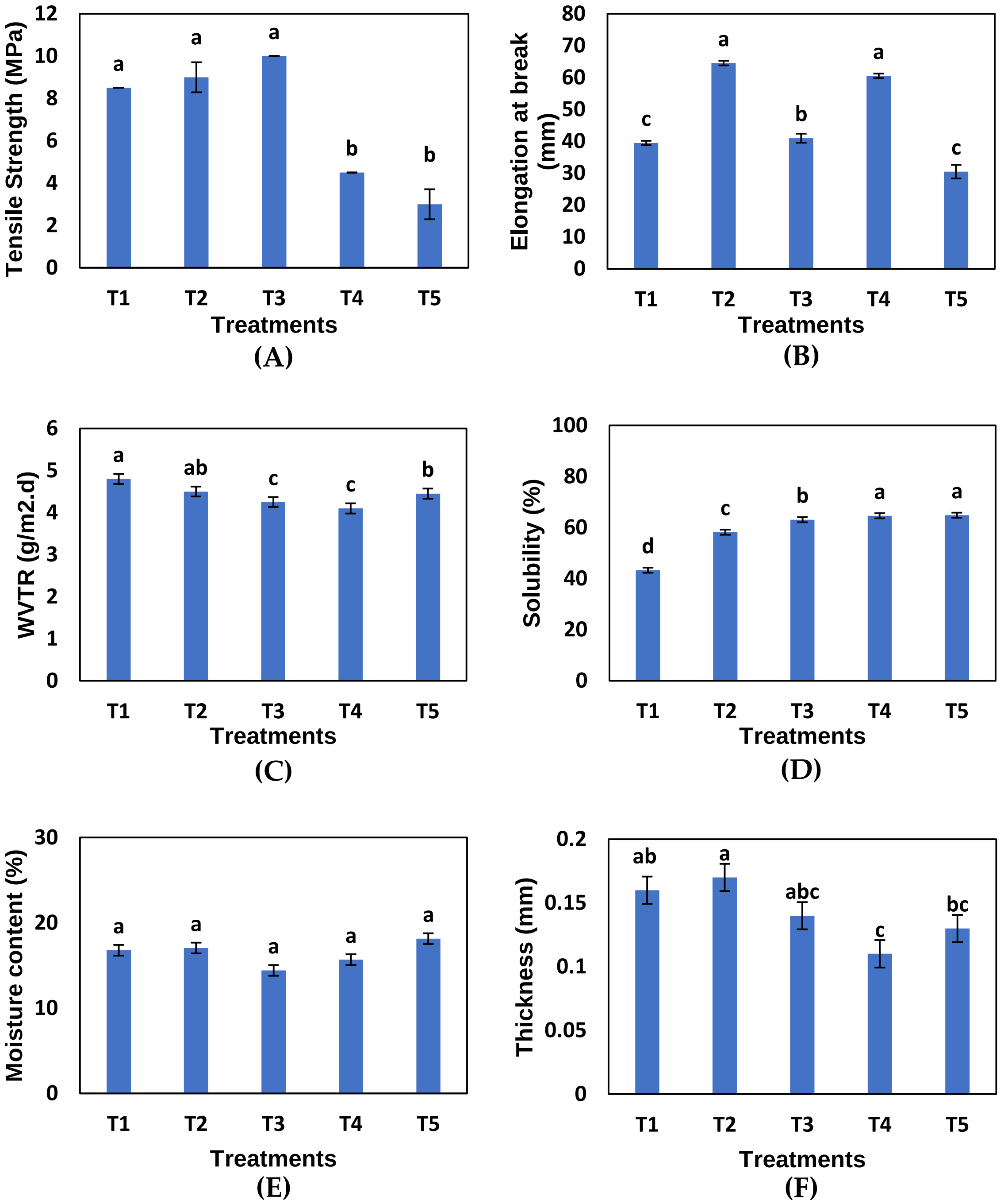

Figure 1. Physical and mechanical parameters of the packaging films developed using treatments T1, T2, T3, T4 and T5. (A) Tensile strength, (B) Elongation at break, (C) Water vapour transmission rate, (D) Solubility, (E) Moisture content and (F) Thickness. (Same letters indicated on the individual bars are not significantly different at $P<0.05$).

Table 4. Antimicrobial activity of the selected treatment (T3) with added preservatives and essential oil.

Concentration (% w/w)*	Diameter of the Inhibition zone (mm)		
	E. coli	*B. brevis*	*P. aeruginosa*
Cinnamon oil 0.25%	NCZ	NCZ	NCZ
Cinnamon oil 0.5%	NCZ	NCZ	NCZ
Cinnamon oil 1.0%	17mm	8mm	NCZ
Cinnamon oil 1.5%	24mm	17mm	8mm
Clove oil 0.25%	NCZ	NCZ	NCZ
Clove oil 0.5%	NCZ	NCZ	NCZ
Clove oil 1.0%	18 mm	8 mm	NCZ
Clove oil 1.5%	20 mm	20 mm	8 mm
SMS 0.25%	NCZ	NCZ	NCZ
SMS 0.5%	NCZ	NCZ	NCZ
SMS 1.0%	NCZ	NCZ	NCZ
SMS 1.5%	NCZ	NCZ	NCZ
SB 0.25%	NCZ	NCZ	NCZ
SB0.5%	NCZ	NCZ	NCZ
SB1.0%	NCZ	NCZ	NCZ
SB 1.5%	NCZ	NCZ	NCZ
PS0.25%	NCZ	NCZ	NCZ
PS 0.5%	NCZ	NCZ	NCZ
PS1.0%	NCZ	NCZ	NCZ
PS1.5%	NCZ	NCZ	NCZ
SMS 0.25% and SB 0.25%	NCZ	NCZ	NCZ
SMS 0.5% and SB 0.5%	NCZ	NCZ	NCZ
SB 0.25% and PS 0.25%	NCZ	NCZ	NCZ
SB 0.5% and PS 0.5%	NCZ	NCZ	NCZ

**w/w = weight of preservative/weight of the film forming solution. NCZ: No clear zone, SMS: Sodium metabisulphite, SB: Sodium benzoate, PS: Potassium sorbate*.

Biodegradability Test

The developed films with essential oil were delay in degradation compared to the control. Cinnamon oil (1.5%) and clove oil (1.5%) added packaging films were degraded after 15 days while the control film was degraded after 5 days. Films were broken into small pieces during biodegradation process.

CONCLUSION

The developed palmyrah tuber starch based packaging film using treatment, T3 was exhibited the highest tensile strength and lower water vapour transmission rate. The packaging film (T3) incorporated with cinnamon or clove oil with 1.5% w/w concentration possessed antimicrobial activity against *E. coli*, *B. brevis* and *P. aeruginosa*. Thus, T3 formulation with 1.5 % cinnamon oil or clove oil is recommended as the best formulation with antimicrobial properties. While 1.0% cinnamon oil or clove oil were not shown the inhibitory activity against *P. aeruginosa.* The inhibitory zones were not observed with sodium metabisulphite, sodium benzoate and potassium sorbate. Thus no effect on antimicrobial activity against tested microorganisms by the preservative concentrations. Further, there was no antimicrobial effect on adding 0.25% and 0.5% cinnamon oil or clove oils for the T3 formulation. Further investigation on characterization of antimicrobial films, application in fresh cut fruits and vegetables and shelf-life evaluation are recommended.

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