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Effect of edible coating of sago starch-gelatine incorporated with papaya seed extract on the storage stability of Malaysian fish sausage (*keropok lekor*)

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Abstract

The quality change of fish sausage (*keropok lekor*) coated in sago starch-gelatine coating with papaya seed extract (PSE) during chill storage (7°C) was determined. During storage, pH, thiobarbituric acid value (TBA), colour, moisture, and the total plate count were evaluated. pH of samples significantly dropped ($p < 0.05$) during storage, and the highest decrease was in control sample. The moisture content in control sample had an increasing trend while that of samples with 5 and 7% PSE coatings significantly decreased, and only a slight change for samples with 0% PSE coating. All samples had significant increase in their TBA values during storage. The presence of the coating provided a positive effect on the colour of the fish sausages since no significant colour changes were observed during storage. TPC of control and coated sausage in 0, 5, and 7% PSE exceeded the recommended microbial standard after 2, 6, 8, and 4 d of storage, respectively. Overall, coating with 5% of PSE was the most effective in retarding the quality deterioration of the fish sausages.

Introduction

Malaysian fish sausages come in different sizes, and are locally called *keropok lekor*. It is a popular fish-based snack in Malaysia and many South-East Asian countries. Unlike conventional sausages of the West, this sausage does not have fat incorporated in the formulation. It has a high content of fish meat ranging from 60 - 30% by weight, and starch as the next major ingredient, which is a mixture of tapioca, sago or other starches that make up to approximately 40 - 50% by weight. The fish-starch dough is shaped by rollers, boiled or steamed, air-cooled, and packed. The post-cooling stage had been identified as the greatest point for cross-contamination (Nor-Khaizura *et al.,* 2009; 2010; Hamat *et al.,* 2019). *Escherichia coli* and *Vibrio* spp. were predominant bacteria isolated from raw materials of the sausage, whereas *Staphylococcus* spp. were the predominant bacteria on food contact surfaces (Lani *et al.,* 2017). Fish sausages spoil very rapidly due to the high water activity of about 0.98 a_w (Nor-Khaizura *et al.,* 2009). The total viable count could increase to 1.5×10^8 CFU/g within 2 d from the initial count of 1×10^2 CFU/g at room temperature (28ºC) (Che Rohani and Mat Arup, 1992). The practice of dipping freshly cooked sausages in cooking oil to prevent slime formation is a common practice among the processors. Hence, an effective barrier such as an antibacterial coating to reduce surface contamination could be an alternative technique to enhance the storage life of the product.

Edible coating which encases the food product can act as a protective outer skin layer against spoilage agents such as microbial contamination, gas diffusion, and moisture migration. Incorporation of functional ingredients such as antioxidant and antimicrobial agents into the coating material may allow a slow release of the additive onto the surface of the product to afford enhanced barrier property (Sanchez-Ortega *et al.,* 2014). The tightly packed and ordered hydrogen-bonded network structure of starch films have resulted in excellent gas barrier properties and good mechanical properties of the films (Poeloengasih and Anggraeni, 2014). Edible films or coatings have been mainly reported for the application for whole or minimally processed fruits, meats, and seafoods. Scarce report on the application of these films or coatings on fish-based products could be found. Tannic acid incorporated into rice starch-gelatine films were reported to have moderate antibacterial properties against *E. coli* and *Salmonella* Typhi, although it was added as a cross-linking agent in the film (Bakar *et al.,* 2017). The rice starch in the edible coating could be substituted with sago starch since it is a cheaper starch. An edible coating based on starch or a combination of protein-starch could be a potential coating for fish cracker since it contains high content of starch.

Peter *et al.* (2014) reported that the methanolic

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and aqueous extract of the *Carica papaya* var Pusa dwarf showed inhibition against both Gram-positive and Gram-negative bacteria, which indicated a broad-spectrum effect. *Staphylococcus aureus, Bacillus cereus, E. coli, Pseudomonas aeruginosa,* and *Shigella flexneri* were sensitive towards the extract (Ojagh *et al.,* 2010). Besides the methanolic extract, the acetonitrile extract of *C. papaya* seed also had significant antibacterial activity against *Shigella sonnei, Salmonella* Typhimurium, *E. coli, Salmonella* Enteritidis, *Vibrio vulnificus*, and *Proteus mirabilis* (Muhamad *et al.*, 2017).

Therefore, the present work was conducted to determine the effect of sago starch-gelatine-tannic acid based coating incorporated with papaya seed extract (0, 5, and 7%) on the quality attributes of Malaysian fish sausages during chill storage at 7°C; which is the most common storage temperature used by retailers and distributors in the region.

Materials and methods

Materials

Carica papaya cv. Sekaki fruits in their third maturity stage (26 - 50% of yellow skin), flour, and other ingredients for the fish sausage formulations were purchased from the local supermarket. Fresh fish was purchased from the local wet market, and chemicals used were purchased from the local chemical suppliers.

Methods

Extraction of papaya seed extract

Carica papaya cv. Sekaki fruits were split into halves. Seeds were removed, washed, and oven dried at 45°C for about 3 - 4 h. Active compounds in the papaya seed powder was extracted using methanol (MeOH) according to Muhamad *et al.* (2017). Extracts were then concentrated using rotary vacuum evaporator (Laborota 4000 Efficient, Heidolph, Germany) at 40°C. The concentrated papaya seed extract was kept at 4°C for further use. The extract contained negligible amount of methanol (Muhamad *et al.,* 2017).

Preparation of coating mixture

Preparation of coating mixtures was modified from Rozhin (2015), where the content of tannic acid was partially replaced by citric acid. Briefly, 1.5 g of sago starch was dissolved into 90 mL of distilled water. The sago starch solution was heated at 85°C for 30 min in a shaking water bath to gelatinise the starch. It was then cooled down to 60°C. Separately, 2.10 g of fish gelatine (240-260 Bloom) and of 20 - 40 mesh (Custom Collagen, Adison, USA) was dissolved in 10 mL of distilled water and heated for 30 min at 50°C. Both starch and gelatine solutions were mixed at 60°C with slow stirring followed by the addition of sodium carbonate (1.0 g), tannic acid (0.21 g), citric acid (0.35 g), and papaya seed extracts. The solution was then homogenised using a homogeniser (WiseTis HG-15D*;* Witeg, Germany) for 5 min at 3500 rpm. Three different formulations were evaluated with and without papaya seed extract (PSE) (A = 0% PSE, B = 5% PSE, and C = 7% PSE).

Preparation of boiled fish sausages

Fresh threadfin breams were degutted, beheaded, and washed under running tap water before being deboned using a fish deboner (Assasemarak (M) Sdn. Bhd., Malaysia) to produce minced fish meat. Ingredients were intermittently mixed according to Murad *et al.* (2017) for 6 min in a bowl cutter (CM-21, Benua Sains Sdn. Bhd., Malaysia). The dough was portioned into approximately 15 g each, and rolled manually into 2 × 5 cm sausage-like dough. The rolled dough was boiled in boiling water for 10 min or until they float. Following this, they were removed and placed on a cooling rack before the coating process.

pH of fish sausages

About 10 g of sample was homogenised in 100 mL of distilled water, and the pH readings were directly measured using a pH meter (Oakton PC2700, USA). Readings were taken in triplicates, and means of samples were determined.

Moisture content of fish sausages

The moisture content of samples was determined using a moisture analyser (MA 35, Sartorius). Readings were taken in triplicates, and means of samples were determined.

Thiobarbituric acid value (TBA) of fish sausages

The thiobarbituric acid value (TBA) was determined following AOAC (1995) with slight modification. Briefly, about 10 g of sample was weighed in a beaker, and 100 mL of distilled water was added. The mixture was homogenised for 3 min, and a few drops of 6 M hydrochloric acid were added to adjust the pH of the mixture to about 1.5. The mixture was transferred to a round bottom flask and steam distillated until 50 mL of distillate was collected. An aliquot of 5.0 mL of the distillate was pipetted into a dry stoppered test tube, and 5 mL of TBA reagent (prepared by dissolving 0.288 g of 2-TBA in 90% of acetic acid) was added. The test tube was vortexed and placed in boiling water bath for 35 min, then cooled. The absorbance of the reacted solution was measured at 538 nm

using a spectrometer (GENESYS 20, Thermo Scientific, USA) against TBA blank. The absorbance of the reagent blank was recorded. TBA values were expressed as mg malonaldehyde (MA)/kg of sample after calculated by using Eq. 1:

TBA value = 7.8 × absorbance of sample (Eq. 1)

Colour values of fish sausages

A CR-400 chroma meter (Konica Minolta, US) was used to determine the colour values [L^*, a^*, and b^*, where L^* = black (-) to white (+), a^* = red (-) to yellow (+), and b^* = blue (-) to greenish (+)] of fish sausages at three difference locations. The device was calibrated using a white calibration plate. Fish sausages were cut into pieces of 4 × 4 cm and placed on the white plate background, and the colour values were determined, respectively. Readings were taken in triplicates, and means of samples were determined

Total plate count (TPC) of fish sausages

Total plate count of samples was determined using the standard procedures (Downes and Ito, 2001). A 1:10 dilution of sample was aseptically prepared by homogenising 10 g of sample with 90 mL of 0.1% sterile peptone water (Oxoid, England) in a stomacher bag, and mixed for 3 min using a stomacher (Bagmixer 400, Interscience, France). The sample was diluted to 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 using the same diluent. Approximately 0.05 mL aliquot was transferred onto sterile Plate Count Agar (PCA) (Oxoid, England), and spread by using hockey stick. The plate was then incubated at 37°C for 48 h, and colonies were then counted. Results were expressed as log CFU/g samples.

Coating and storage study of fish sausages

The cooked sausages were coated by dipping in the coating mixture, drip-dried a few seconds to remove excess coating and oven dried at 80°C for 15 min. They were removed from the oven and allowed to cool on trays at room temperature (28°C) prior to packing in polyethylene bags for storage study. Packed samples were kept at 7°C for 10 d. Sampling was carried out at a two days interval for the determination of pH, moisture content, colour, TBA values, and TPC as in the methods described earlier.

Statistical analysis

Data were presented as a mean ± standard deviation for all analyses. One-way analysis of variance (ANOVA) with Tukey's test was conducted using Minitab Software (Minitab 18.0 for Windows, Minitab, USA) to determine the significant difference between the means at 95% confidence level ($p < 0.05$).

Results and discussion

Storage stability of coated fish sausages

pH values

The pH of food is an important property of food since the extent of microbial spoilage is highly dependent on this. The initial pH value (day 0) for fresh uncoated sausage was approximately 6.5, which is similar to that reported by Nor-Khaizura *et al.* (2009), while all coated fish sausages had significantly more alkaline pH as compared to control. pH for samples coated with formulations A, B, and C were 7.58 ± 0.01, 7.77 ± 0.02, and 7.64 ± 0.01, respectively (Figure 1); which could be influenced by the alkaline pH (≈9.75) of the coatings. A decreasing trend in pH was observed in all samples during the 10-d storage, which may indicate that some form of fermentation had taken place. Lowest pH (4.69) was obtained for the uncoated fish sausages, which was a significant drop ($p < 0.05$) from the initial value. Although during

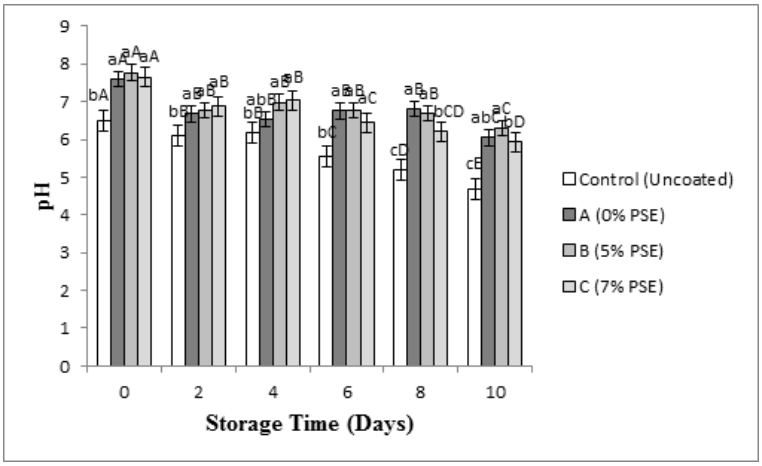

Figure 1. Effect of coating on pH of fish sausages (*keropok lekor*) during storage at 7°C for 10 d.

storage, a general significant decreasing trend in the pH values of all coated samples were observed, nonetheless, they were still within the acceptable pH range of 6.0 to 6.30 (Jamilah, 1983).

Moisture content

The coated food can have an extended shelf-life due to the ability of the coating material to restrict moisture migration inward and outward of the food. The initial moisture content of uncoated samples (~46.85%) was significantly lower ($p < 0.05$) than the coated ones (Figure 2). The higher initial moisture contents in the coated samples could be due to the slight moisture pick-up by the outer layer of the samples from the coating mixtures during coating process. During storage, the uncoated samples had an increasing trend in the moisture content even though statistically this was not significant. Unlike coated samples, the opposite was observed in coated samples. The least change in the moisture content of samples during storage was obtained from samples coated with formulation A (0% PSE), while the biggest decrease was obtained in samples coated with formulation C (7% PSE), followed by those samples coated in formulation B (5% PSE). Increasing the percentage of PSE incorporation in the coating, may have resulted in a higher surface dehydration of the sample due to higher rate of moisture lost. PSE contained some fatty acids which could decrease the compactness of the coating matrix and hence contributed to the higher water vapour permeability of the coatings with increasing incorporation of the extract.

Thiobarbituric acid value (TBA)

Edible coatings and films had shown to be effective in delaying lipid oxidation in many food products (Ambardekar, 2007; Amiza and Kang, 2013; Khan *et al.,* 2015). The initial TBA values among samples were significantly different ($p < 0.05$) with control having the highest TBA value at 2.16 mg MA/kg sample (Table 1). The initial TBA values of samples coated in formulation A (0 % PSE) > formulation C (7% PSE) > formulation B (5% PSE). Throughout storage, control (uncoated) samples showed the most rapid increase in TBA values, and the final value on day 10 was more than doubled as compared to day 0. Samples coated with PSE also showed an increasing TBA trend during storage though not as rapid as control. However, after day 8 of storage, the TBA values in all samples with PSE incorporated were observed to escalate and those coated with formulation C had similar final TBA values as control. This could be due to higher surface hydration of samples coated in formulation C as observed in the moisture content of samples. The cut-off point for acceptable TBA values in *keropok lekor* should be around 4.50 mg Ma/kg sample (Jamilah, 1983). Based on the TBA values, it could be concluded that the incorporation of PSE in the coating material at less than 7% could delay rancidity development in the sausages.

Colour values

Table 2 shows the '*L*', '*a*' and '*b*' values of all samples during storage. The '*L*' value of control (uncoated) sausages was 52.24 ± 0.68. There were no significant differences in the '*L*' values among samples on Day 0 thus indicating that the coatings had not noticeably influenced the surface lightness of the cracker. The '*L*' values of all samples had slight changes during storage, however they were not significant. The '*a*' value of all coated samples was higher than that of control; especially those coated in 0 and 5% PSE incorporated coatings. In general, the '*a*' values of samples during storage were signifi

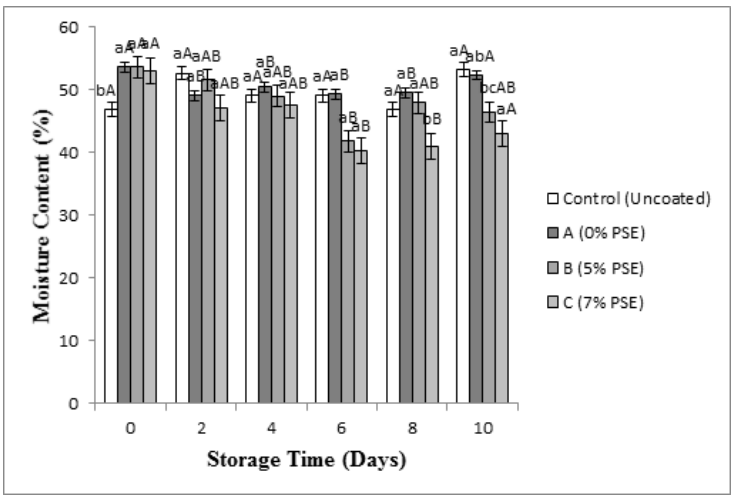

Figure 2. Effect of coating on moisture content of fish sausages (*keropok lekor*) during storage at 7°C for 10 d.

Table 1. Changes in thiobarbituric acid value (TBA) of fish sausages (*keropok lekor*) during storage at 7°C for 10 d.

Days of storage at 7°C	Thiobarbituric acid value (mg MA/kg sample)			
	Control	A	B	C
0	2.16 ± 0.01^{aD}	1.48 ± 0.06^{bE}	0.77 ± 0.02^{cD}	0.90 ± 0.02^{dD}
2	2.30 ± 0.07^{cD}	2.49 ± 0.07^{bD}	2.03 ± 0.02^{cC}	2.89 ± 0.05^{bB}
4	3.40 ± 0.07^{aCD}	2.83 ± 0.07^{bCD}	2.34 ± 0.03^{bcC}	2.19 ± 0.17^{cC}
6	4.08 ± 0.04^{aC}	3.36 ± 0.17^{aB}	2.45 ± 0.13^{bcC}	2.86 ± 0.08^{bB}
8	4.78 ± 0.07^{aB}	3.16 ± 0.16^{bBC}	3.23 ± 0.17^{bB}	2.89 ± 0.05^{cB}
10	5.31 ± 0.13^{aA}	4.30 ± 0.28^{bA}	4.19 ± 0.39^{bA}	5.32 ± 0.03^{aA}

Control = uncoated; A = 0% PSE; B = 5% PSE; and C = 7% PSE. Means ± standard deviations with different uppercase superscripts within the column and lowercase superscript within rows are significantly different ($p < 0.05$).

Table 2. Colour values of the surface of fish sausages (*keropok lekor*) during storage at 7°C for 10 d.

Days of storage	Control	A	B	C
L* **value**				
D0	52.24 ± 0.68^{aAB}	51.62 ± 1.24^{aA}	53.56 ± 0.68^{aA}	53.70 ± 0.41^{aA}
D2	53.26 ± 0.93^{aA}	53.08 ± 1.19^{aA}	50.49 ± 2.23^{aA}	50.57 ± 1.78^{aAB}
D4	53.30 ± 3.11^{aA}	51.02 ± 2.43^{aA}	51.79 ± 1.18^{aA}	50.07 ± 0.19^{aB}
D6	53.36 ± 0.46^{aA}	53.52 ± 1.03^{Ab}	50.28 ± 0.23^{cA}	51.39 ± 0.84^{bcC}
D8	51.20 ± 1.65^{aAB}	53.31 ± 0.58^{aA}	51.69 ± 1.88^{aA}	50.05 ± 1.86^{aB}
D10	53.1 ± 0.60^{abA}	53.89 ± 1.42^{aA}	50.08 ± 0.21^{cA}	51.44 ± 0.79^{bcAB}
a* **value**				
D0	2.73 ± 0.07^{bcCD}	4.22 ± 0.19^{aAB}	3.48 ± 0.27^{bAB}	2.95 ± 0.02^{bC}
D2	2.56 ± 0.19^{bD}	3.37 ± 0.08^{Ab}	3.29 ± 0.29^{aB}	3.01 ± 0.07^{abC}
D4	3.58 ± 0.11^{cB}	4.51 ± 0.34^{Aa}	4.18 ± 0.05^{abA}	3.94 ± 0.01^{bcAB}
D6	2.97 ± 0.04^{bC}	3.71 ± 0.39^{abAB}	4.19 ± 0.36^{aA}	3.68 ± 0.03^{abB}
D8	4.53 ± 0.01^{aA}	3.81 ± 0.18^{bAB}	4.17 ± 0.27^{abA}	4.11 ± 0.17^{abA}
D10	2.94 ± 0.08^{aC}	4.08 ± 0.67^{aAB}	4.20 ± 0.37^{aA}	3.84 ± 0.23^{aAB}
b* **value**				
D0	8.04 ± 0.08^{bC}	14.76 ± 1.82^{aA}	14.27 ± 1.33^{aA}	10.12 ± 1.04^{bAB}
D2	7.99 ± 0.00^{aC}	14.44 ± 0.18^{aA}	13.22 ± 0.13^{abAB}	10.01 ± 0.66^{bAB}
D4	9.11 ± 0.74^{aA}	13.76 ± 1.12^{abA}	12.11 ± 0.50^{abAB}	10.77 ± 0.09^{bAB}
D6	8.56 ± 0.35^{bcC}	9.83 ± 0.59^{abC}	10.39 ± 0.53^{aB}	11.12 ± 0.11^{bAB}
D8	8.56 ± 0.34^{bC}	10.3 ± 0.21^{Abc}	10.74 ± 1.00^{aB}	11.78 ± 0.11^{bA}
D10	11.48 ± 1.31^{aB}	9.04 ± 1.68^{aC}	9.84 ± 1.02^{aB}	11.44 ± 0.35^{aA}

Control = uncoated; A = 0% PSE; B = 5% PSE; and C = 7% PSE. Means ± standard deviations with different uppercase superscripts within the column and lowercase superscript within rows are significantly different ($p < 0.05$). *L** = index of lightness/darkness; *a** = index of redness; and *b** = index of yellowness.

cantly affected except for samples of A and B. The fluctuations in the readings could be due to the uneven surface nature of the sausages. After 10 d of storage, uncoated and coated samples with 7% PSE added showed an increasing trend in the '*b*' value.

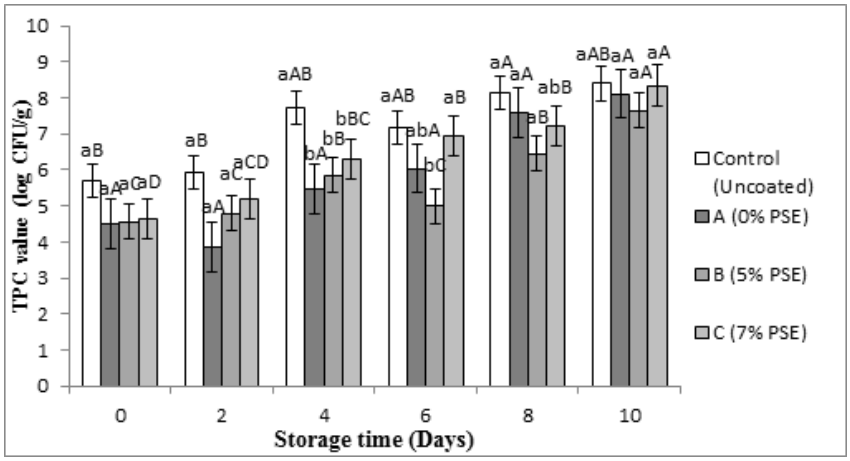

Figure 3. Effect of coating on total plate count (TPC) of fish sausages (*keropok lekor*) during storage at 7°C for 10 d.

Total plate count (TPC)

Total plate count (TPC) of control and coated sausages during storage is shown in Figure 3. Based on Laws of Malaysia (2018), microbial standard for fish and fish product ready for consumption should not exceed 10^6 CFU/g (log 6 CFU/g).

At day 0, the TPC value of control was significantly higher than coated samples; however, no significant differences ($p > 0.05$) were noted among coated samples. The TPC values of samples were log 5.91 ± 0.75, 4.47 ± 0.09, 4.66 ± 0.17, and 4.64 ± 0.06 CFU/g for control, and coated sausages of A, B, and C, respectively. All samples showed an increasing trend in their TPC values over time with the final values of log 8.39 ± 0.26 (Control), 8.11 ± 0.05 (A), 7.65 ± 0.71(B), and 8.34 ± 0.06 (C) CFU/g.

The incorporation of 5% PSE in the coating material exhibited a stronger positive effect in suppressing the microbial growth as compared to that of 7% PSE. Although PSE has been indicated to have an antimicrobial effect, one would assume at higher incorporation, the effectiveness against microbial growth should be enhanced. Perhaps this property is offset by higher permeability to oxygen as seen in the slightly higher TBA values in samples coated in formulation C as compared to those coated in formulation B. An overall lower TPC value for all coated samples, especially at the beginning of the storage period, is also indicative that tannic acid and PSE could be an additive to coatings to provide an additional antimicrobial effect. Peter *et al.* (2014) reported that papaya seed contains carpaine, benzyle isothiocynate, caricin, and myrosin enzyme which could act as antimicrobial agents against *S. aureus*, *B. cereus*, and *E. coli* that are abundant in fish sausages. The present of tannic acid in the formulation also help to lengthen the shelf life of the fish sausages. Tannic acid incorporation in biodegradable films had been proven to be an effective antimicrobial agent (Bakar *et al.,* 2017). The TPC results of control and coated fish sausages in 0, 5, and 7% PSE exceeded the recommended microbial standard after 2, 6, 8, and 4 d of storage at 7ºC, respectively.

Conclusion

The incorporation of papaya seed extract (PSE) in coatings reduced the quality changes of fish sausages during storage. However, the incorporation of PSE in coatings at 7% did not produce more positive outcome as compared to lower concentration. The incorporation of PSE at 5% showed the best protective effect against quality deterioration of the fish sausages by extending the shelf-life of the fish sausages for an additional six days.

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