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Properties of composite film from tomato extracted pectin/ chitosan for use in sausage wrappingNatcharee Jirukkakul^{1,*}, Nachayut Chanshotikul¹¹School of Food Technology and Innovation, Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai, Thailand*Corresponding author: pntach@kku.ac.th

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Abstract

This study investigated the properties of pectin/chitosan film (2% w/v of the pectin and chitosan) in 5 pectin/chitosan ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. The findings suggest that decreasing the pectin proportion likely results in increases in certain properties of the film, including moisture content, water vapor permeability, and lightness (L^*). Meanwhile, tensile strength, the red/green coordinate (a^*), and the yellow/blue coordinate (b^*) tend to be lower in response to the soluble pectin/chitosan composite film relative to the single-composite film. Additionally, the pectin/chitosan film could preserve sausage freshness for 9 d at refrigerator temperatures (4-5 °C) without an alteration in the original color of the sausage, as well as slow down the decline in pH levels and the growth rate of microbes.

Keywords: Film, Pectin, Chitosan, Tomato extract**1. Introduction**

The tomato (*Lycopersicon esculentum* Mill.) is a nutritious plant. It is full of vitamins A and C, potassium, phosphorus, magnesium, and other minerals. There are two major uses of tomatoes in general: for cooking and for manufacturing processes. According to the Center for Agricultural Information Office of Agricultural Economics [1], there are approximately 11.6896 km² (7,306 rai) of tomato ranches in Thailand, able to produce 37,356 ton of tomatoes annually. Every year, about 10-15% of this produce, or about 3,735.6 - 5,603.4 ton, is wasted as manufacturing process by-product. According to Kusanteay and Uriyapongson [2], this tomato waste contains protein, fat, fiber and ash, accounting for 19.08%, 12.05%, 58.46% and 44.53% of dry matter, respectively. In addition to these substances, tomato waste is also full of pectin which is soluble fibers. There is a beneficial substance that helps to reduce blood sugar and cholesterol. Moreover, pectin can be preventive against many diseases. Pectin is a polysaccharide that cannot be digested by the small intestine. Properties and the quality of pectin can vary depending on species and plant ages, as well as fiber type which cannot be digested in the human body and depending on each plants [3].

Pectin is usually added into food as a gelling agent. It has special properties that cause it to form a soft gel-like compound when combined with sugar and fat in proper quantities. Pectin serves as a suitable thickening agent for jams and jellies, as well as a stabilizer for prevent sedimentation in acidified milk by stopping casein from settling. Moreover, pectin is an emulsifier that relieves surface tension between fat and water by stabilize the emulsion process. Additionally, pectin is a good source of food for probiotics which is the main ingredient in functional foods [3]. Due to its properties, pectin is also common in film production. Azeredo et al. [4] studied the properties of pectin film when mixed with pomegranate juice and citric acid. The findings showed that

pomegranate juice adds bright red color to the film. It is also a plasticizer that enhances the flexibility of the film, promoting elongation as well as water permeability; however, dissolution rate and tensile strength seemed to decline. On the other hand, adding citric acid into the process strengthened tensile strength and dissolution while lessening water permeability. A similar study on mechanical properties of pectin-chitosan film was conducted by Lorevice et al. [5]. The research showed that pectin-chitosan composite film, whether high or low in methoxy, had overall enhanced mechanical properties, ultimately resulting in a satisfactory film for food wrapping. A study on methylcellulose/pectin composite film by Saha et al. [6] found that at a 90:10 methylcellulose/pectin ratio the composite film was highly permeable, making it suitable for producing pharmaceutical products, particularly those with controlled drug release. Galus and Lenart [7] developed an alginate/pectin composite film in their study, resulting in a smooth and glossy film with relevant properties, including color, water permeability, tensile strength, and chemical compounds. Notably, an increased proportion of pectin was shown to result in higher levels of water permeability, as well as higher red/green and yellow/blue coordinates, resulting in decreased film clearness. Perez et al. [8] studied ascorbic acid stabilization and antioxidant properties in pectin/methylcellulose composite film, finding evidence of strong ascorbic acid stabilization. The highest stabilization occurred at the 50:50 composition ratio under vacuum conditions and 75.2% relative humidity. Also, when kept at 25 °C, the film was found to act as an antioxidant, preserving antioxidants and tocopherol in walnut oil. Eapitia et al. [9] studied film composed of apple peels and thyme oil, which was found to produce a smooth and consistent film. The study also showed that adding pectin increased tensile strength while tending to slow bacterial production rate, where optimal conditions were 6.07% apple peels and 3.1% thyme oil. A study by Khurana et al. [10] also suggested that pectin/chitosan (40:60) composite film from tamarind extract was suitable for spray coating pharmaceutical pills. The study showed a high adhesive force between the coating solution and the pills, with a low cohesive force between pills, providing further evidence that this type of composite film is suitable for pharmaceutical and food manufacturing. Moreover, a study conducted by Otoni et al. [11] provided further evidence of a composite film possessing antioxidant properties. Their pectin composite film from papaya and cinnamon had enhanced flexibility and water permeability due to the papaya, while tensile strength was lower and antimicrobial properties increased with cinnamon. This type of composite film is also suitable for food wrapping because it could against *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* and *Staphylococcus aureus*.

The gelling agent of pectin, antimicrobial properties of chitosan and the extensibility of previous pectin/chitosan film brings about to the experiment film which can be produced to casing for prolong sausage shelf-life. Therefore, the main objective of the present research was to study the properties of a pectin/chitosan composite film made from tomato waste extract in the context of sausage preservation without requiring added preservatives. Importantly, such a film could also result in value-added sausage products.

2. Materials and methods

2.1 Pectin extraction from tomato waste

Tomato waste was collected from tomato factories in Nong Khai province, Thailand. Five g of the tomato waste was put into a 250 ml Scott Duran bottle. Next, 0.0125 M hydrochloric acid was added into the bottle, resulting in 200 ml in total. Subsequently, the mixture was heated at 80 °C under high frequency using ultrasound bath (Elmasonic S70H, ElmaHans Schmidbauer GmbH & Co. KG, Singen, Germany) for 20 min before going straight to a spinner, where the matter underwent a speed of 8,000 rpm for 30 min in order to separate transparent matter. During the sedimentation process, 400 ml of 95% ethanol were added, and then the sample was stored in the chiller for 24 h. After that, the sediment was roasted for 1 h in a vacuum oven (model 23, Binder, Germany) at 60 °C before being stored in a zip bag in the chiller [12].

2.2 Pectin/chitosan film production

The process for producing the pectin/chitosan film was based on a method employed in a study by Saha et al. [6], where 2% pectin and chitosan (85% degree of deacetylation and 65 kDa average molecular weight from NNC Product Ltd., Thailand) in various ratios (100:0, 75:25, 50:50, 25:75, and 0:100) and 0.5% of acetic acid were added into distilled water. The solutions, each 50 ml in total, were mixed well at room temperature for 30 min before being poured on a plate (Paisan Superlene Co, Ltd., Bangkok, Thailand) and left to dry at 37 °C for 24 h. The thickness of films were 0.8-1.0 mm.

2.3 Analysis of physical properties of pectin/chitosan film

Moisture content [13] was determined by putting an aluminum bowl in 103±2 °C heat for an hour and then leaving it in a desiccator at room temperature. After accurately measuring and recording the film weight at 1 g

(A0), it was heated to 105 °C for 24 h, then left to cool in a desiccator. Weight after heating (A1) was then recorded. The formula used to determine moisture content was as follows:

$$\text{Percentage of moisture content} = (A0 - A1) \times 100/A0 \quad (1)$$

To determine water solubility, the study followed a method described by Su et al. [14]. Three pieces of 2×2 cm of film were roasted at 65 °C for 24 h before leaving them to cool in a desiccator. Their weight prior to liquidation (W0) was recorded, and then they were soaked in a beaker filled with 50 ml of water. The beaker was covered with parafilm and left at room temperature for 24 h. After that, the sample was filtrated through a Whatman No. 1 paper filter and then heated at 80 °C for 24 h before being left to cool in a desiccator. The weight after liquidation (W1) was recorded, and solubility was determined in terms of percentage using the equation:

$$\text{Percentage of solubility} = (W0 - W1) \times 100/ W0 \quad (2)$$

The water activity of films was examined using AquaLab (series 3, Decagon Devices, USA).

In accordance with ASTM E96 methods [15], water vapor permeability (WVP) was measured using a circular sample film six centimeters in diameter. The lid of a glass filled with distilled water was coated thinly with sealant, and the sample was placed on top of the glass to serve as a lid. The sample was tightened and the glass with sample was weighed, and placed in the WVP test unit. Weight measurements were taken every hour until a weight discrepancy of 1% was reached, where

$$\begin{aligned} \text{WVP} &= (G/t) \times T / (A \times (P_{A1} - P_{A2})) \quad (3) \\ G/t &= \text{Rate of weight change per day (g/d)} \\ T &= \text{Thickness (mm)} \\ A &= \text{Surface area of the samples (m}^2\text{)} \\ P_{A1} - P_{A2} &= \text{Differential pressure inside and outside the testing cup (kPa)} \\ \text{The } P_{A1} - P_{A2} &\text{ was equal to } (RH_1 - RH_2) \times P_{\text{atm}}/100 \end{aligned}$$

2.4 Color analysis of pectin/chitosan film

Color measurement was conducted using a color meter (model JS 555, Color Techno system.co.ltd, China) where 5×5 cm film samples were cut and tested repeatedly five times.

2.5 Determination of physical properties of pectin/chitosan film

The tensile strength (TS) of the film was tested according to ASTM D882 standards [16]. Film plates measuring 10×150 mm were kept at 23 °C with 50% relative humidity for 48 h. Then, the film plates were screwed to a texture analyzer (model TA.XT plus, by Micro Systems Ltd., UK). The analyzer was set to 50 mm/min and load cell = 0.5 kN, with the length between the two screws being 100 mm. Tensile strength was determined using the equation,

$$\text{TS} = \text{maximum load}/(\text{width} \times \text{thickness}) \quad (4)$$

2.6 Using pectin/chitosan film in sausage preservation

Sausage ingredients were pork mince (1 kg), accord (2.4 g), salt (8.5 g), pepper power (10.5 g), tapioca flour, phosphate and crushed ice. All ingredients were put in a chopper until the meat was tender. After that, the meat was wrapped with pectin/chitosan film. All samples were kept in refrigeration.

The pectin/chitosan film was applied toward sausage preservation using the common wrapping method. The fresh sausage product quality was in line with standards set by pork product quality control under the Thai Agricultural Commodity and Food Standards [17] and Thai Industrial Standards institute [18], which clearly limit the proper amount of living microorganisms (total plate count) in pork products at 10⁴ and 10⁵ colonies per g, respectively.

The 10 g of sausage was blended with 90 ml of de-ionized water for 1 min by Masticator Homogenizator (Compact, IUL Instruments, Spain). The evaluation of sample pH was conducted using pH meter (Seven compact, Mettler Toledo, Thailand). The mean value from 3 measurements were reported.

The evaluation of sausage color was conducted using a HunterLab spectrophotometer (model TC-P III A, Tokyo Denshoku Co., Ltd., Japan). The 20 g of the sausage was poured into the test cup, it was covered with a

lid, and the color value was read and recorded. The values were represented as L^* , a^* , and b^* . Then the color analysis was calculated into the total color difference according to the formula $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

The microorganisms (total plate count) in the produced sausage, sterilized plates with a nutrient agar (Biomark Laboratories, India) were prepared. Food solution with 10^{-6} , 10^{-5} and 10^{-4} dilution at 1 ml was collected by using a pipette and putting it on the melt nutrient agar which was on the plate. The plates were pored and left until they were harden. The plates then were inverted and incubated at 37 °C for 24 h. Then, the counting of the microorganisms was conducted which was 30-300 colonies per plate.

2.7 Statistical analysis

The experiment was completely randomized design (CRD). SPSS was employed to determine the statistical significance of mean differences using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT); statistical significance was identified at 95% confidence level ($p \leq 0.05$).

3 Results and discussion

3.1 Physical properties of pectin/chitosan composite film

The pectin/chitosan composite film in this study contained 23-28% moisture content, as shown in Table 1. The pectin-based film showed no statistical significance in terms of humidity levels, whereas the chitosan-based film was able to lock in higher levels of moisture due to its absorbent nature [19]. Water activity in both films was in the range of 0.45-0.47, putting them into the category of dry matter (DM) ($a_w < 0.6$). Water activity and moisture content in pectin/chitosan film were similar to those of high methoxy pectin (HMP): methylcellulose (MC) film, with 21.3% humidity and 0.3-0.6 water activity [8].

Water solubility of the film was a physical property that indicated how the film interacts with water. Similarly, water vapor permeability (WVP) was a property in food wrapping film that indicated whether it will prevent or allow contact between moisture and the product. In general, edible film or biodegradable film had low WVP. Water solubility and WVP were key properties when considering film applications. Pectin was highly soluble due to its distinct polymer matrix structure's ability to absorb moisture, leading to low WVP. When chitosan was added into the composition, the solubility rate lowered, leading directly to a rise in WVP. This phenomenon has been shown to take place due to the space in the pectin/chitosan structure allowing moisture to escape through the film [19]. The composition of these two combined substances allowed a more desirable WVP level than either pectin-based or chitosan-based film. Additionally, the pectin/chitosan film in this study showed a lower WVP rate than that of previously studied pectin-based film from papaya extract [11] or pectin-based film from pomegranate extract [4] which were 64.8-76.8 and 74.88-261.84, respectively.

Table 1 Physical properties of pectin/chitosan.

Pectin:Chitosan	Moisture content (%)	Water activity	Solubility (%)	WVP (g.mm/m ² .d.kPa)
100:0	23.25 ^b ± 1.54	0.46 ^a ± 0.01	84.71 ^a ± 1.52	0.03969 ^c ± 0.00018
75:25	22.79 ^b ± 1.08	0.46 ^a ± 0.01	26.22 ^{cd} ± 2.32	0.11769 ^b ± 0.00030
50:50	24.09 ^b ± 0.58	0.45 ^a ± 0.01	23.00 ^d ± 0.50	0.13286 ^a ± 0.00033
25:75	23.85 ^b ± 0.72	0.47 ^a ± 0.01	29.60 ^c ± 0.68	0.13823 ^a ± 0.00038
0:100	27.54 ^a ± 1.78	0.45 ^a ± 0.01	45.47 ^b ± 3.21	0.11366 ^b ± 0.00026

Letters indicate differences between average scores shown in the same column.

3.2 Pectin/chitosan film color

Packaging color was essential to the appearance of a product, as it speaks directly to consumers. Quality film was usually smooth and transparent, with no damage on the surface [7]. Pectin/chitosan composite film showed a higher degree of lightness (L^*). Meanwhile, the red/green coordinate (a^*) and yellow/blue coordinate (b^*) decreased as chitosan was increased and pectin was reduced. Pectin/chitosan film colors are shown in Figure 1. Chitosan enhances lightness of the film while pectin which was red by nature as it was a tomato extract, increases the concentration of the red/green coordinate (a^*) and the yellow/blue coordinate (b^*). Notably, L^* , a^* , and b^* values were similar to those of alginate/pectin [7]. Therefore, pectin/chitosan film from tomato waste extract was suitable for red/orange products.

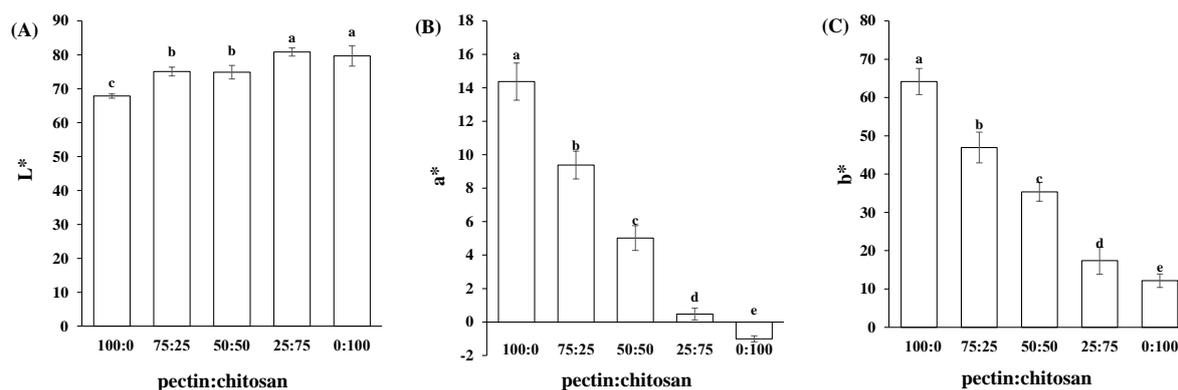


Figure 1 Pectin/chitosan (w/w) film. (A) = lightness (L^*), (B) = redness (a^*) and (C) = yellowness (b^*). The same lower case letter in each parameter did not differ significantly at $P < 0.05$.

3.3 Mechanical properties of pectin/chitosan film

The mechanical properties of a film depended on its chemical composition. The properties of degradable film were generally weaker than synthetic films in general. High strength films were more suitable in a variety of applications, while low strength films tended to have more limited applications. Mechanical properties should be considered in addition to other properties when deciding how to apply a film. Pectin composite film was high in methoxy, which contained a gel-like structure and was an acidic medium with the presence of co-solutes, and stabilization was achieved by cross-linking of polymeric chain aggregates of different sizes. In contrast, film low in methoxy contained hydrogen bonding and other Van der Waals interactions [5]. Nevertheless, both types of film caused to the high mechanical properties [11]. The pectin/chitosan film had a tensile strength in the 1-10 MPa range, which was low due to the brittle nature of pure pectin [6]. Tensile strength and elongation of the film fell with an increase in chitosan, as shown in Figure 2. This was due to weak intermolecular interactions between biopolymer chains. These results indicated a remarkable component effect of pectin extract from tomato pomace and chitosan which enhanced free volume and mobility, leading to decreased rigidity [11]. However, an increase in pectin can restore elongation to the film, as pectin has plasticizing effect on polysaccharide film [4].

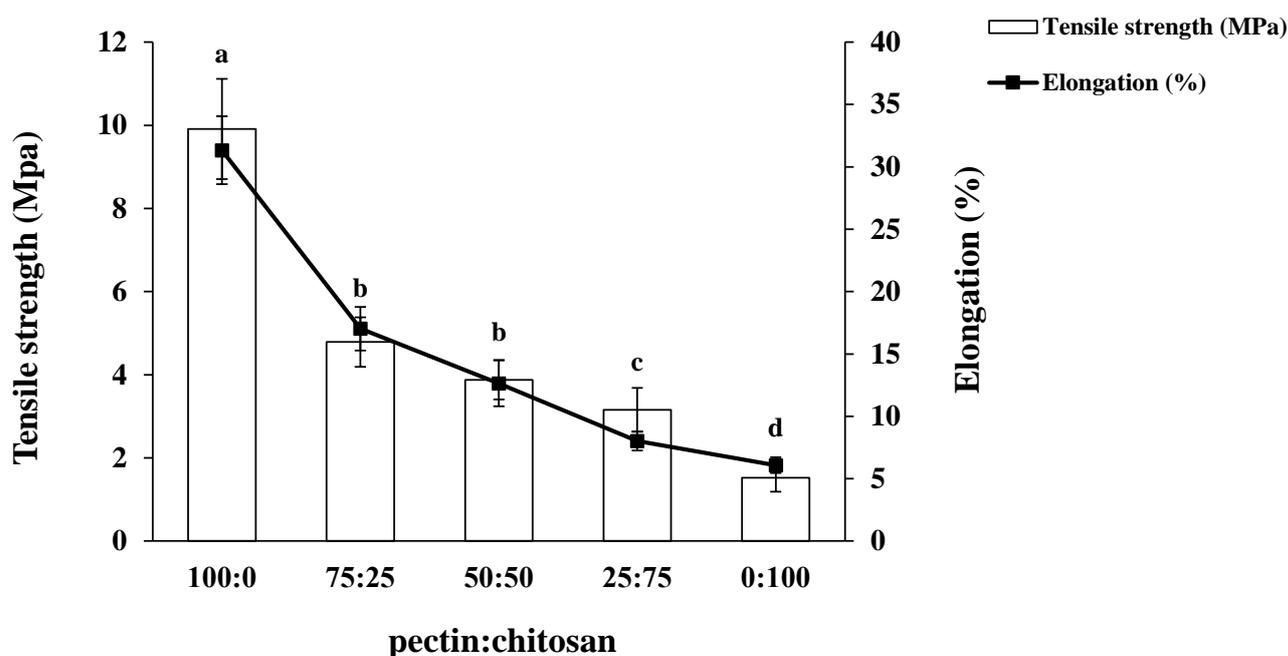


Figure 2 Mechanical properties of pectin/chitosan (w/w) film.

3.4 Application toward sausage product preservation

During storage of sausage products at refrigerated temperatures of 4-5 °C, the recommended temperature for storing pork products, the pH value fell in every sausage sample due to the fermentation process of bacteria producing lactic acid [20]. Values ranged from 6.4 initially to 5.0-5.4 on the 9th day. The decrease in pH in the chitosan-based film was lower than that which occurred in the pectin-based film, as shown in Figure 3. This can be explained by chitosan's antimicrobial properties [21].

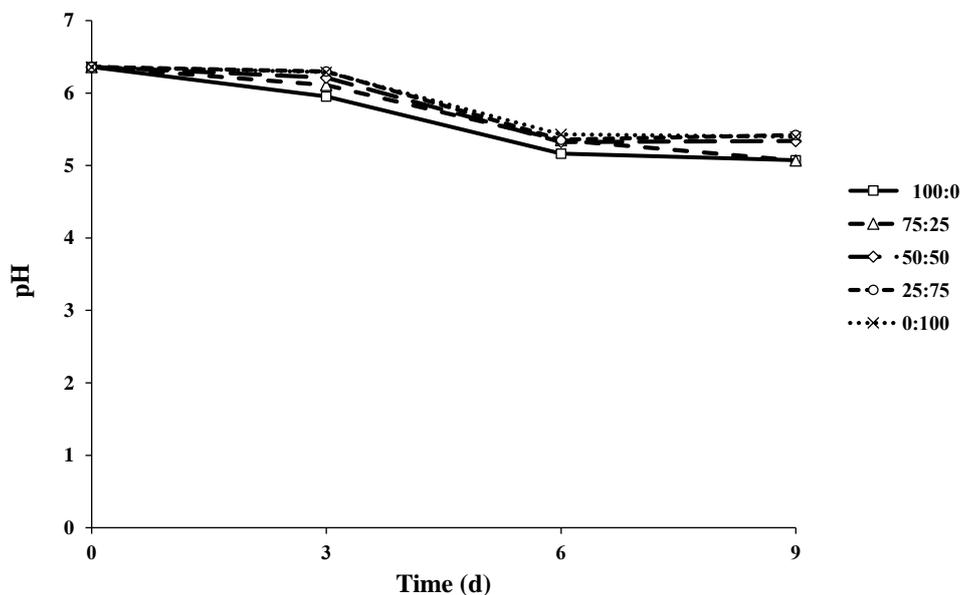


Figure 3 The pH levels of sausage samples during the preservation period; pectin:chitosan (w/w).

There was no significant difference in sausage total color difference among the samples. Thus, the film color did not alter or affect the color of the product wrapped with the composite film. Figure 4 shows that the highest total color difference occurred on days 6-9 of the preservation period.

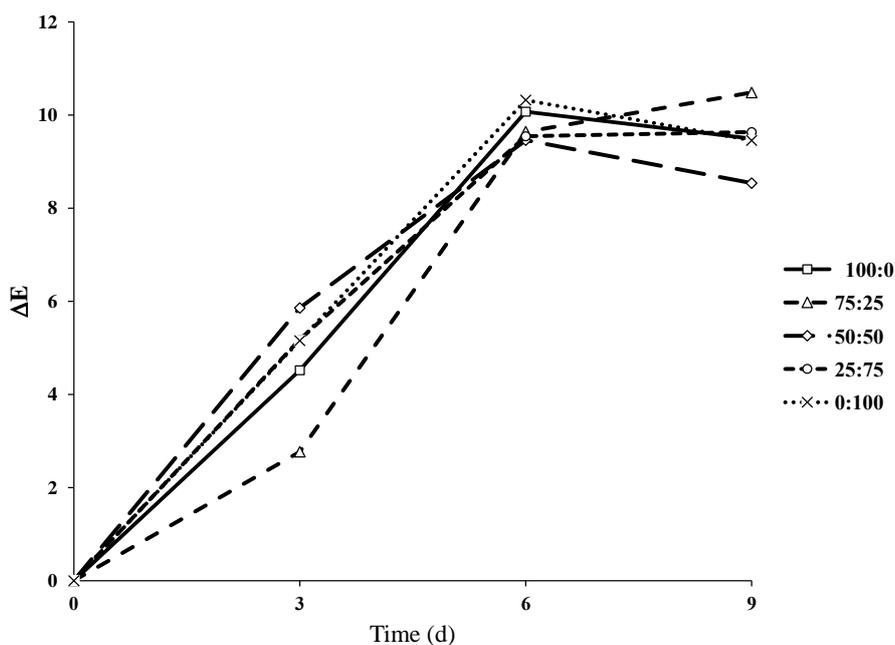


Figure 4 Total color difference of sausage during the preservation period; pectin:chitosan (w/w).

Generally, sausages can be stored in a refrigerator for about 7 to 10 d. The sausage wrapped with commercial wrapping film was expressed 10^7 of microorganism at 7 d of storage [20]. The sausages used in this study contained microorganisms at levels no higher than 10^3 colonies per gram as per the Agricultural Standards Act, which limits microorganisms in pork products to 10^4 colonies per gram [17] and pork sausages to 10^5 colonies per gram [18] (see Figure 5). The graph shows that the number of microorganisms (total plate count) in the sausages wrapped with the pectin/chitosan composite film was lower than that of the sausages wrapped with the pectin film. This finding is consistent with findings from a study on preservation of groomed vegetables using chitosan film conducted by Pitak and Rakshit [21], which revealed that chitosan film can decrease the growth rate of microorganisms in vegetables preserved in a chiller. The antimicrobial properties of chitosan affected the sausage wrapped with chitosan composition film which caused to reduce microorganism growth rate more than the sausage wrapped with the film without chitosan.

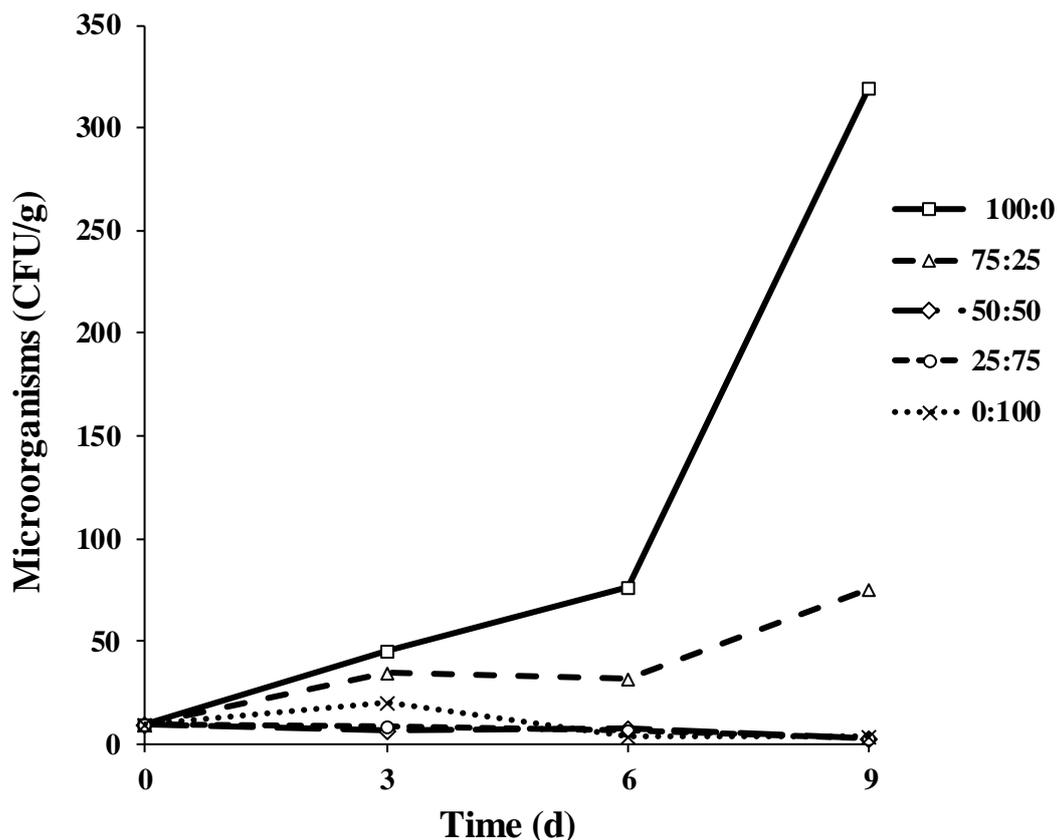


Figure 5 Total plate count in sausages during preservation period; pectin:chitosan (w/w).

4. Conclusion

The amount of pectin added into the composite film had a direct effect on its properties. That was, when pectin levels were lowered, the moisture content, permeability, and lightness (L^*) of the composite film increased. Meanwhile, tensile strength, the red/green coordinate (a^*), and the yellow/blue coordinate (b^*) decreased. The pectin/chitosan composite film was found to be less soluble than the single-component film. Due to its mechanical properties, the pectin/chitosan composite film can be applied as a film wrap in the sausage preservation process. It can prolong and preserve sausages stored at refrigerator temperatures in any proportion without altering the product's original color. Apart from that, the pectin/chitosan composite film tended to slow the decline of pH levels as well as the growth of microorganisms.

5. Acknowledgements

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