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The effect of eel's protein extract on the characteristics of edible film from crosslinked modified canna starch

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Abstract

The present work aimed to investigate the effect of eel's protein extract on characteristics of modified canna's starch edible film. The eel's protein extract at concentrations of 2, 4 and 6% (v/v) were added into the formulation of modified Canna's starch edible film. The observed parameters were elongation percentage, compressive strength and water vapour transmission rate. The addition of eel's protein extracts increased the elongation percentage and decreased water vapour transmission rate of edible film. The edible compressive strength of the film decreased after the addition of eel's protein extract, but addition of higher concentration of eel's protein extracts increased the compressive strength of edible film.

Introduction

The use of canna's starch as raw material for edible film is still limited due to low values of elongation percentage, compressive strength and water vapour transmission rate. A strategy that can be applied is by using modified canna's starch through crosslinking of $POCl_3$ because the amylose and amylopectin compositions are relatively balanced with magnitude of 25% and 75%, respectively.

The use of modified starch is very important to produce edible film matrix because $POCl_3$ compound creates a cross link between one amylose chain to another through phosphate link. This phosphate link can produce three dimensional continuous net that can trap water through reactive OH which is not bound to $POCl_3$. The cross link of starch polymer chains occurred at groups containing more OH reactive, especially at OH group numbers 2, 3 and 6 (Yoshida *et al*., 2002). The numbers of reactive OH group are affected by cross-linking degree and starch concentration. Granular size and ratio of amylose and amylopectin numbers of starch may also affect the numbers of reactive OH group. The characteristics of these starches may have effect on the production of modified starch. According to Santoso *et al*. (2011) the modified starch through cross linking by $POCl_3$ may produce good compressive strength but low elongation percentage and water vapour transmission rate edible film. This low elongation percentage and water vapour transmission rate characteristics can be improved by the addition of other compound such as glycerol and beeswax.

Glycerol and bees wax not only have positive effect, but also negative effect such as increasing water vapour transmission rate and decreasing elongation degree of edible film respectively. The addition of eel's extract protein is important to solve this problem. According to Artharn *et al*. (2008), fish protein based edible film which consisted of myofibrillar protein or sarcoplasm generally have better mechanical properties, especially in term of flexibility property. Nakai and Modler (1999) had added that myofibrillar and sarcoplasmic proteins are found in abundant quantity in eels. Amino acid types and pH isoelectric points also have profound effect on the edible film matrix (Prodpran *et al*., 2007). Were *et al*. (1999) described that amino acids containing sulphur have important role in edible film formation through disulfide bonding. Poeloengasih and Marseno (2003) had added that in addition to disulfide bonding, hydrogen bonding and hydrophobic interaction also determine edible film properties, especially in term of amino acids having hydrophobic characteristic such as alanine, valine, leucine, tryptophan and phenylalanine.

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The present work was therefore aimed to investigate the effect of eel's protein extract on characteristics of modified canna's starch edible film such as elongation percentage, compressive strength and water vapour transmission rate of the edible film.

Materials and methods

Materials and equipment

Materials used in the present work were canna's starch (white colour) from Pagaralam City, and eels from Perumnas Market of Palembang. Eel was processed into surimi to obtain protein extract. Chemicals used were alcohol, distilled water, ether, HCl, NaOH, Fehling solution, ethanol, methylene blue, iodine solution, acetic acid, glycerol and trisodium citrate. Chemicals for the production of modified starch and edible film were sodium sulphate, $POCl_3$, glycerol, CMC, beeswax, pp indicator, HCl and ammonium molybdate. Equipment used were hot plate, magnetic stirrer, vortex, oven, desiccator, texture analyser, testing Machine MPY (Type: PA-104-30, Ltd Tokyo, Japan), and water vapour transmission rate tester.

Methods

Modified starch processing from canna's starch with $POCl_3$ by using cross linking method

The procedure for modified starch processing with cross-linking method by using multi-functional reagent of $POCl_3$ was according to Wattanachant *et al*. (2003) with slight modifications as follows: 30 g sodium sulphate (Na_2SO_4) was added with 300 mL distilled water and magnetically stirred while adding 200 g starch. Next, 5% NaOH was added while stirring to prevent starch gelatinisation. The pH was adjusted to 10.5. The solution was incubated using a shaker incubator at 40±2°C (200 rpm, 24 h). Next, $POCl_3$ was added 0.08% (w/w) while stirring and incubated at 40+2°C (200 rpm, 2 h). The pH was adjusted to 5.5 using 10% HCl solution to stop the reaction. Starch was sieved using Whatman paper no. 4 and washed with distilled water for 5 min. Starch drying was done at 45°C for 6 h to get starch with water content of 10-12%.

Protein extract preparation

The procedure for protein extraction from eel and its preparation for the addition into edible film was based on the modified method described by Heruwati and Jav (1995). Briefly, the eel was cleaned, beheaded, gutted, and filleted. Eel fillets were then mashed and stirred with cold water until becoming homogenous. Floating impurities and lipid were discarded. Water was separated from cleaned mashed fillet by pressing. Next, 0.3% salt (w/v) was added and followed by recompressing to discard excess water. Next, 2% sorbitol (w/v) was added and mixed until becoming homogenous. Mashed fillet was frozen for 1 w. Frozen mashed fillet was thawed for 30 min followed by weighing of 2% (w/v), 4% (w/v) and 6% (w/v) from total distilled water volume. Then, 100 mL distilled water and NaOH 1 M were added to achieve pH 11, stirred and heated at 55°C for 30 min. Heating and screening of suspension were done to produce protein extract.

Edible film preparation.

The canna starch edible film with and without the addition of protein extract of eel was performed. The addition of distilled water was followed by stirring and sieving. The heating of starch suspension at gelatinisation temperature of 65°C using hot plate was followed by stirring with magnetic stirrer. The slow addition of glycerol 3% (v/v) into the gelatinised starch suspension was followed by heating for 10 min. The addition of eel extract protein according to treatments with concentrations of S_1 (2%), S_2 (4%), and S_3 (6% v/v) was then performed. Next, the CMC surfactant was added according to treatments with concentration of 2% (b/v). The suspension was stirred until becoming homogenous and the bees wax was added with concentration of 1% (b/v). Gas was removed by using vacuum pump for 1 h. Next, 40 mL suspension was poured onto Petri dish (15 cm in diameter) followed by moulding and heating at 70°C for 12 h in the oven. After cooling to room temperature, the edible film was removed and wrapped in plastics before being dried in a desiccator for 24 h.

Statistical analysis

Statistical analysis on completely randomised experiment was done by using SAS computer Program. One-way Analyses of Variance (ANOVA) were carried out and mean comparisons were processed by Duncan test. Significance was defined as $p < 0.05$.

Results and discussion

The addition of eel's protein extract was done to improve the elongation percentage of canna starch edible film. Protein molecules of eel within edible film matrix were bound with hydrophilic components such as starch, glycerol and CMC. Astiana (2012) had explained that eels contain 15.90% essential and

non-essential hydrophobic proteins which consisted of lysine and glutamic acid at 7.13 g/100 g and 12.89 g/100 g, respectively.

Table 1. Results of Duncan test for the effect of eel's protein extract on elongation percentage, compressive strength and water vapour transmission rate of canna's starch edible film.

Treatment	Elongation percentage (%)	Compressive Strength (Newton)	Water vapour transmission rate ($g.m^{-2}.d^{-1}$)
S_1	47.60[a]	51.69[a]	22.60[a]
S_2	51.67[b]	58.17[b]	21.68[b]
S_3	58.84[b]	63.87[c]	18.85[c]

Remarks: Numbers followed by the same letter in the same column are not significantly different at 5% level of Duncan test

Analysis of variance showed that the eel's protein extract had significant effect on the elongation percentage of edible film but their interaction did not. Results of Duncan test (Table 1) showed that the elongation percentage of modified canna starch edible film was significantly different than that of untreated canna starch edible film. The treatment at 2% eel's protein extract was significantly different than that of 4% and 6%. However, 4% eel's protein extract was not significantly different than that of the 6% in term of edible film elongation percentage.

The elongation percentage of modified canna starch edible film (57.33%) was higher than that of untreated canna starch edible film (48.00%). This was due to the fact that modified canna starch had more open starch molecules structure and low retrogradation characteristics. This starch structure might have caused the protein molecules of eel to easily enter the edible film matrix and bind to the hydrophilic components such as starch, glycerol and CMC. Low retrogradation characteristics of modified canna starch also might have trapped the protein molecules of eel and within the edible film matrix.

According to Nakai and Modler (1999), protein extract of eel contained similar protein component such as that found in fish i.e., myofibrillar and sarcoplasmic at 65 to 75% and 20 to 30%, respectively. Weng *et al*. (2007) had stated that myofibrillar protein has fibrous form and elastic whereas sarcoplasmic protein is globular. Myofibrillar protein affects the edible film's elasticity and sarcoplasmic protein decreases the polymer interaction and cohesive power of the edible film matrix as well as produces more flexible edible film. Fish protein based edible film consisting of myofibrillar or sarcoplasmic protein generally have better mechanical properties, especially flexibility property, but has low barrier to water vapour transmission rate (Hamaguchi *et al*., 2007)

Table 1 shows that higher protein extract of eel resulted in higher elongation percentage of the edible film. This might be explained by the fact that higher protein extract of eel had higher quantity of myofibrillar and sarcoplasmic proteins which were trapped within the edible film matrix. The mean value of elongation percentage of canna starch edible film without the addition of eel's protein extract was lower than that of edible film with the addition of eel's protein extract (Figure 1). However, this edible film elongation percentage did not fulfil the stated standard of JIS 1975, i.e. minimum of 70% for all addition levels of protein extract.

Analysis of variance results showed that the eel's protein extract had significant effect on edible film compressive strength value but with insignificant interaction. Compressive strength value of modified canna starch edible film was significantly different to the untreated starch edible film. The addition of eel's protein extract at 2, 4 and 6% w/w gave significantly different values of edible film compressive strength (Table 1). This was caused by stronger molecular

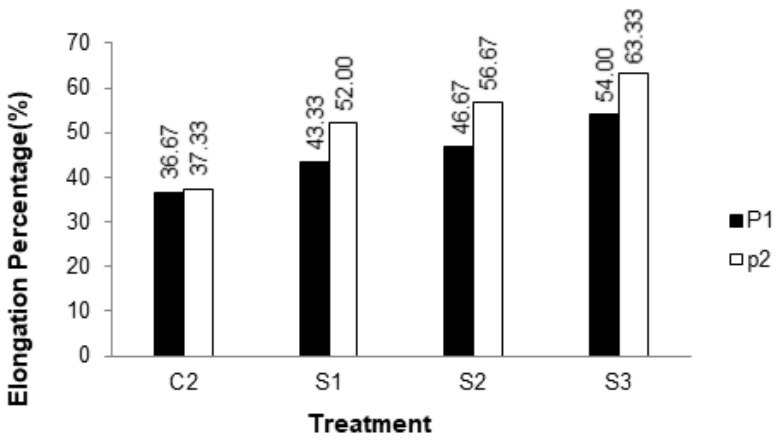

Figure 1. Mean values of elongation percentage of canna's starch edible film prior to and after addition protein extract of paddy field's eel.

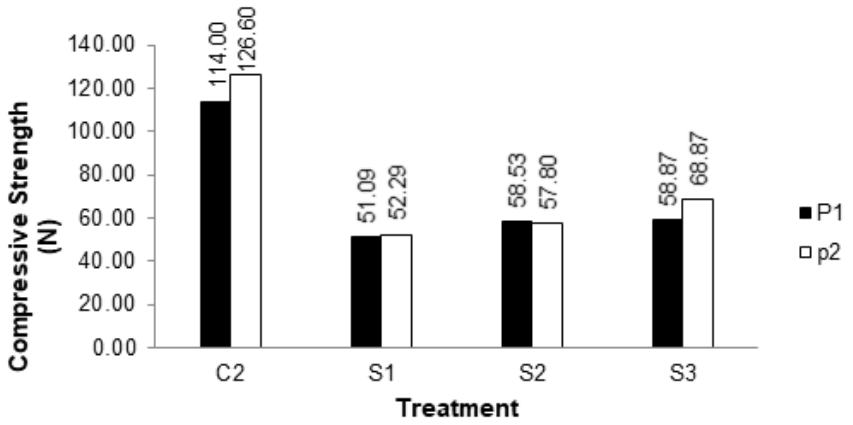

Figure 2. Mean values of edible film's pressure strength prior to and after protein extract addition of paddy field's eel

structure of canna starch added with eel's protein extract. $POCl_3$ cross linked canna starch had some substituted OH groups by phosphate. This substitution increased the structural strength of starch molecules which resulted in stronger starch molecules. The degree of edible film matrix strength increased by increasing the starch strength.

Table 1 also shows that the higher the protein extract concentration of eel, the higher the compressive strength of edible film. This was due to the fact that protein extract of eel contains myofibrillar protein. These protein molecular chains have fibrous form and length. Higher myofibrillar protein content results in more compact edible film and higher resistance power to pressure. This result is in accordance to a study reported by Sobral *et al*. (2005) which showed that the use of 2 g myofibrillar protein in 100 g film solution had higher pressure strength than that of 1 g myofibrillar protein in similar film solution. Compressive strength value of canna starch edible film without the addition of protein extract was higher than that of edible film added with protein extract (Figure 2). Sarcoplasmic protein is globular and dispersed amongst edible film matrix which lowers the interaction with film matrix polymers and also lowers the compactness which subsequently decreases the edible film resistance power to pressure. Artharn *et al*. (2008) had reported that compressive strength of protein-based edible film will decrease when concentration of sarcoplasmic protein is increased.

Analysis of variance showed that the treatments of eel's protein extract had significant effect on water vapour transmission rate of edible film in which the higher the protein concentration the lower the water vapour transmission rate observed. This might be due to protein extract of eel which had myofibrillar and sarcoplasmic proteins. Molecular structure of myofibrillar protein is consisted of fibrous molecular chains. The higher the concentration of protein extract of eel, the higher the quantity of myofibrillar protein which in turn produce more solid and compact film matrix structure. This condition impedes the water vapour from penetrating the edible film matrix. Shiku

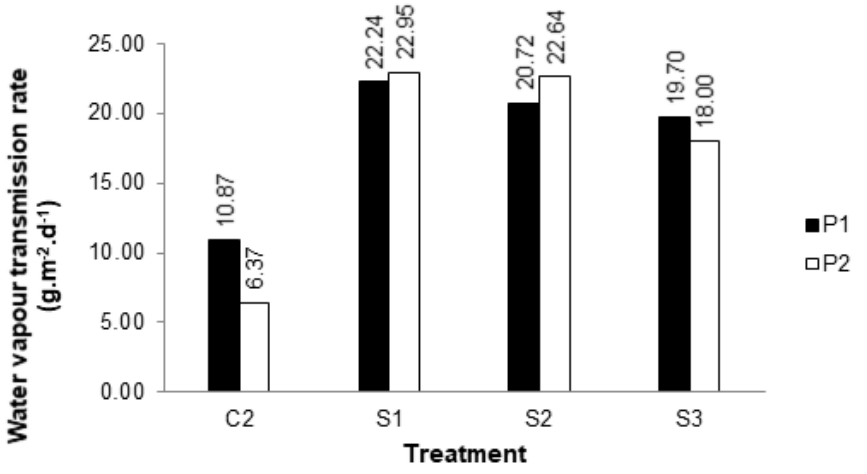

Figure 3. Mean values of water vapour transmission rate of canna's starch edible film prior to and after protein extract addition of paddy field's eel

et al. (2003) had added that water vapour transmission rate of edible film produced from myofibrillar protein was relatively lower than that of edible films produced from other proteins. Sarcoplasmic proteins are globular proteins containing most of the hydrophobic and SH groups hidden in the interior of molecules.

Figure 3 shows that the water vapour transmission rate of canna starch edible film without the addition of protein extract was lower than that of edible film added with protein extract. This could be due to protein molecules as structural components of edible film matrix which had hydrophilic characteristics. The protein addition resulted in the increase of hydrophilic components within the edible film matrix. Therefore, the higher the hydrophilic components, the easier the water vapour penetrates the edible film. Yoshida *et al*. (2002) described that natural hydrophilic property of protein in edible film formulation facilitated the interaction with water which lowered the edible film resistance to water vapour. Nayak *et al*. (2008) described that protein-based edible film was very effective as barrier to oxygen gas and aroma, but this edible film showed relatively high value of water vapour transmission rate.

Conclusion

The addition of eel's protein extract had increased elongation percentage and decreased water vapour transmission rate of the edible film. Compressive strength of edible film had decreased with eel's protein extract addition, but addition of higher concentration of eel protein extracts had increased its compressive strength.

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