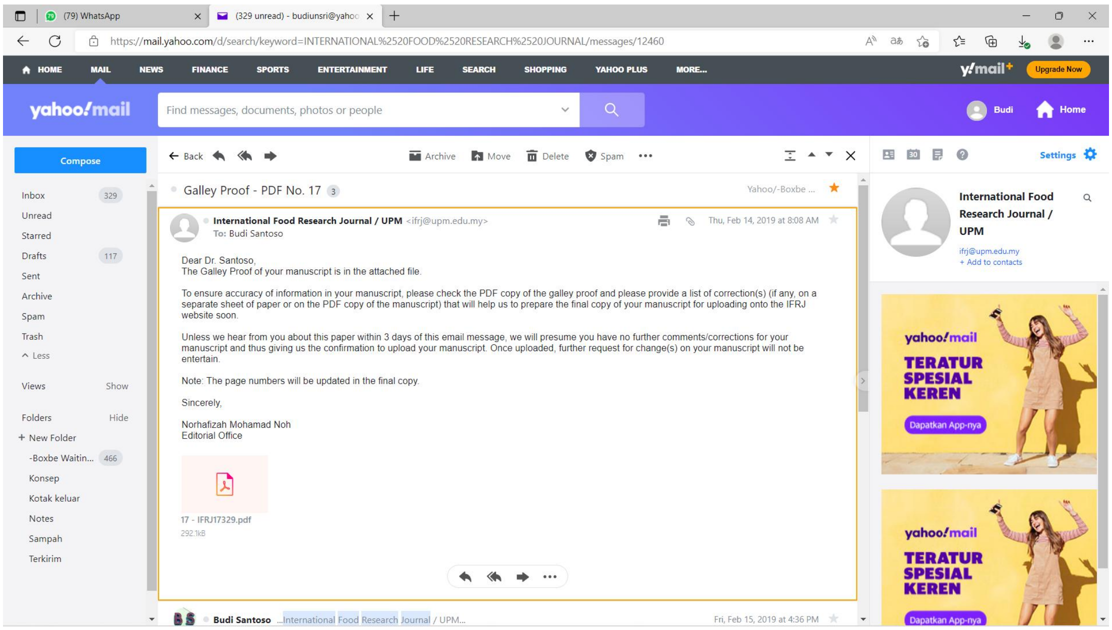

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International Food Research Journal / UPM <ifrj@upm.edu.my> Thu, Feb 14, 2019 at 8:08 AM
To: Budi Santoso

Dear Dr. Santoso,
The Galley Proof of your manuscript is in the attached file.

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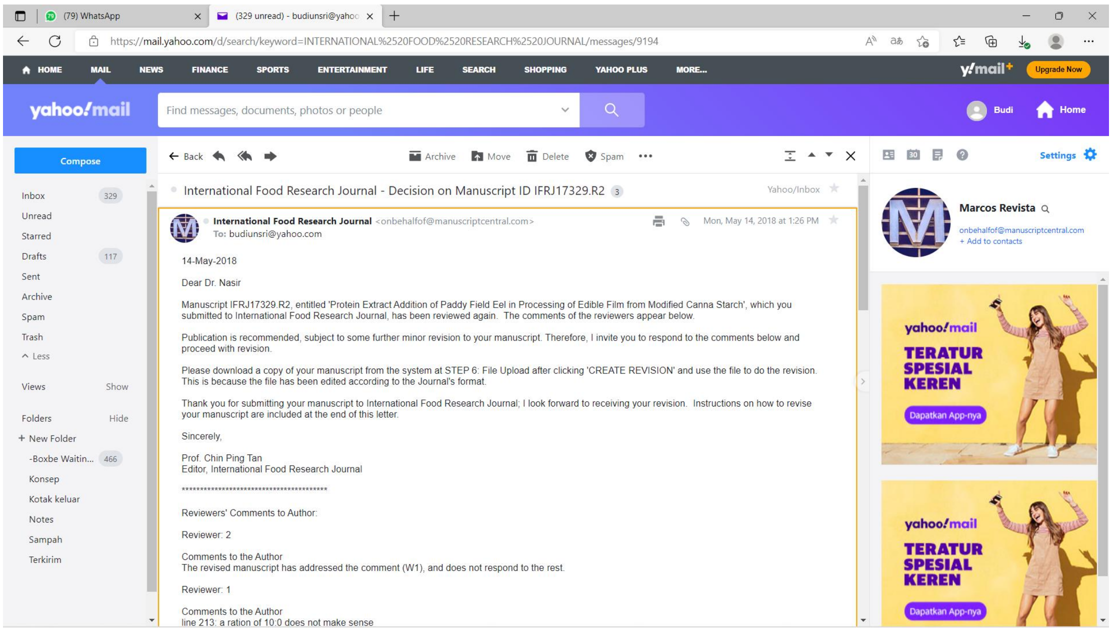


International Food Research Journal - Decision on Manuscript ID IFRJ17329.R2

International Food Research Journal <onbehalfof@manuscriptcentral.com> Mon, May 14, 2018 at 1:26 PM
To: budiunsri@yahoo.com

14-May-2018

Dear Dr. Nasir

Manuscript IFRJ17329.R2, entitled 'Protein Extract Addition of Paddy Field Eel in Processing of Edible Film from Modified Canna Starch', which you submitted to International Food Research Journal, has been reviewed again. The comments of the reviewers appear below.

Publication is recommended, subject to some further minor revision to your manuscript. Therefore, I invite you to respond to the comments below and proceed with revision.

Please download a copy of your manuscript from the system at STEP 6: File Upload after clicking 'CREATE REVISION' and use the file to do the revision. This is because the file has been edited according to the Journal's format.

Thank you for submitting your manuscript to International Food Research Journal; I look forward to receiving your revision. Instructions on how to revise your manuscript are included at the end of this letter.

Sincerely,

Prof. Chin Ping Tan
Editor, International Food Research Journal

****

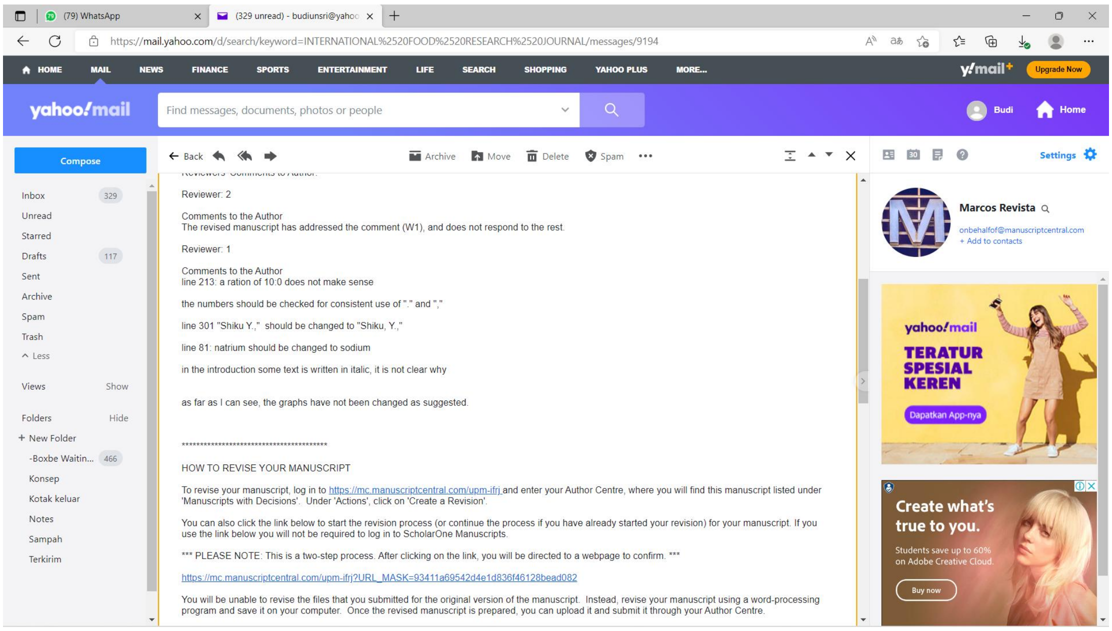
Reviewers' Comments to Author:

Reviewer: 2

Comments to the Author
The revised manuscript has addressed the comment (W1), and does not respond to the rest.

Reviewer: 1

Comments to the Author
line 213: a ration of 10:0 does not make sense



Reviewer: 2

Comments to the Author
The revised manuscript has addressed the comment (W1), and does not respond to the rest.

Reviewer: 1

Comments to the Author
line 213: a ration of 10:0 does not make sense

the numbers should be checked for consistent use of "." and ","

line 301 "Shiku Y.," should be changed to "Shiku, Y.,"

line 81: natrium should be changed to sodium

in the introduction some text is written in italic, it is not clear why

as far as I can see, the graphs have not been changed as suggested.

****

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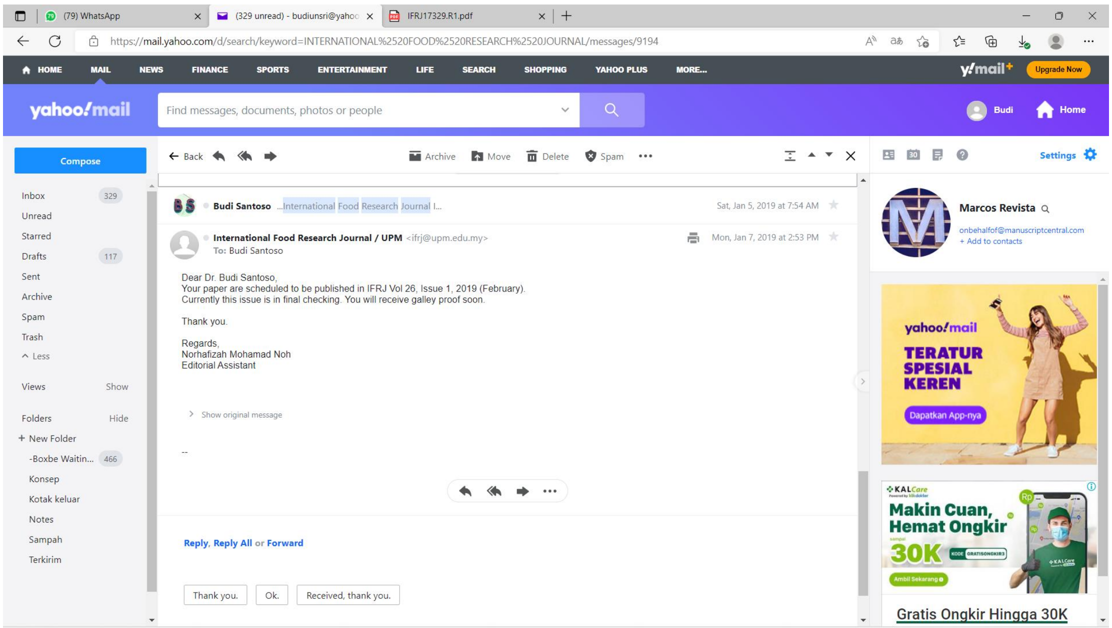

Budi Santoso ...International Food Research Journal I... Sat, Jan 5, 2019 at 7:54 AM

International Food Research Journal / UPM <ifrj@upm.edu.my> Mon, Jan 7, 2019 at 2:53 PM
To: Budi Santoso

Dear Dr. Budi Santoso,
Your paper are scheduled to be published in IFRJ Vol 26, Issue 1, 2019 (February).
Currently this issue is in final checking. You will receive galley proof soon.

Thank you.

Regards,
Norhafizah Mohamad Noh
Editorial Assistant

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

Abstract

This study aimedtostudy theeffect of eel's proteinextractoncharacteristics ofmodified Canna's starch ediblefilm.Research method was addition of eel's protein extract at concentrations of 2%(v/v), 4%(v/v), and 6%(v/v) in the formulation of modified Canna's starch ediblefilm.The observed parameters were percentelongationpercentage, compressive strength, water vapor transmission rateand themicrostructure. The addition of eel's protein extracts increases the elongation percentage and decreases water vapor transmission rate of edible flim. The edible compressive strength of the film decreases after the addition of eel's protein extract, but addition of higher concentration of eel's protein extracts had increased compressive strength of edible film.

Comment [W1]: There is no discussion of microstructure in the manuscript.

Keywords: edible film, Canna's starch, eel's extract protein, modified,$POCl_3$.

Introduction

The use of Canna's starch as raw material for edible flim is still limited due to low values of elongation percentage, compressive strength and water vapor transmission rate. One of effort that can be done is by using modified Canna's starch through crosslinking of $POCl_3$because the amylose and amylopectin compositions are relatively balance 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 anothers through phosphate link. This phosphate link can produce three dimension continous net and this net can trap water through reactive OH which not bound with $POCl_3$. The cross link of starch polymer chains is 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 is affected by cross-linking degree and starch concentration. Granulair size and ratio of amylose and amylopectin numbers of starch may also affect the numbers of reactive OH group. The characterisics of these starches may have effect on the producing of modified starch. According toSantoso*et al*.(2011)the modified starch through cross linking by $POCl_3$ may produce good compressivestrengthbut low percent elongation and water vapor transmission rate edible film. This low percent elongation and water vapor transmission rate characteristics can be improve by the addition of other compound such as glycerol and beeswax.

Glycerol and bees wax not only has positive effect, but also negative effect such as increasing water vapor transmission rate anddecreasing elongation degree of *edible film respectively.* The addition of eel's extract protein is important to solve this problem. According toArtharn*et al*. (2008),fish protein based *edible film* which consisted or myofibrillar protein or sarcoplasm generally have better mechanical properties, especially in term of flexibility property. Nakai and Modler (1999) had added that protein types of myofibrillar and sarcoplasmic are found in abundant quantity on eels. Amino acid types and pH isoelectric point 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. PoeloengasihandMarseno (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 hydophobic charateristic such as alanine, valine, leusin, triptophane and phenylalanine.

This research aimedtostudy theeffect of eel's proteinextractoncharacteristics ofmodified Canna's starch ediblefilm and eel' protein extract which in turn can improve elongation percentage, compressive strength, and water vapour transmission rate of edible film.

Material and method

Materials and equipments

Material used in this study were canna's starch (white color) from Pagaralam City, eel from Perumnas Market of Palembang. Eel is processed into surimi to obtain protein extract. Chemicals used for preliminary study were alcohol, distilate water, ether, HCl, NaOH, Fehling solution, ethanol, methylene blue, iod solution, acetate acid, glycerol and trisodium citrate having technical quality. Chemicals for production of modified starch and edible film were natrium sulphate, $POCl_3$, glycerol,

Comment [W2]: acetic

Comment [W3]: technical grade

CMC, *beeswax,* pp indicator, HCl and ammonium molybdate.Equipments used in this study were *hot plate*, *magnetic stirrer*, *vortex*, oven, desicator, texture analyzer, testing Machine MPY (Type: PA-104-30, Ltd Tokyo, Japan), and water vapor transmission rate tester Bergerlahr.

Method

Modified Starch Processing from Canna's Starch with $POCl_3$by Using Cross Linking Method (Modified method of Wattanachantet al., 2003)

Procedure for modified starch processing with cross-linking method by using multi functional reagent of $POCl_3$is as follows:natrium sulphate (Na_2SO_4) with magnitude of 30g (15% dry weight of starch) is prepared and added with 300mL distilate water and it is stirred by using *magnetic stirrer*at 3 scale; 200g starch is added while stirred; 5% NaOHis added while stirred byusing magnetic stirrer at 8 scale to prevent starch gelatinization and pH level of solution is set at 10.5 and stirred for 30 minutes at room temperature;The solution is incubated using shaker incubator at temperature of 40$\pm$2°C (200rpm, 24 hours); $POCl_3$is added 0.08%(w/w) while stirred byusing magnetic stirrer at 8 scale for 30 minutes and then incubated at temperature of 40$\pm$2°C (200rpm, 2 hours); pH of solution is set to 5.5 using 10% HCl solution to stop the reaction; Starch is sieved by using Whatman paper no. 4 and washed with distilate water for 5 minutes. Starch drying is done at temperature of 45°Cfor 6 hours to get starch with water content of 10-12%.

Protein extract preparation

Procedure for protein extract from eel and its preparation for addition into edible film is as follows (modified method ofHeruwati and Jav, 1995): Eel is cleaned by discharging its head part and stomach content followed by cleaning with clean water; Cutting operation is done to separate flesh portion from bone and skin (*fillet)*followed by smashing of eel flesh;Smash flesh is cleaned with cold water at temperature of about 1 to 5°Cusing water having volume 5 times of smash flesh volume for 10 minutes; Smash flesh is stirred within cold water until homogenous and is stopped to precipitate smah flesh, whereas impurities and lipid are floating in water surface, followed by discharging the impurities; Water is separated from cleaned smash flesh by using pressing equipment; Smash flesh is recleaned within cold water and added with 0.3% salt (w/v) at the third cleaning and followed by recompressing to discharge water as much as possible; 2% of sorbitol (w/v)is added and stirred until homogenous; Smash flesh is freezed within *freezer*at temperature of about -15°C for 1 week; Frozen smash flesh is thawed for 30 minutes followed by weighing with magnitude of 2% (w/v), 4% (w/v) and 6% (w/v) from total aquadest volume; 100ml aquadest and NaOH 1 M are added to achieve pH 11 and stirred, heated at temperature of 55°C for 30 minutes; Heating and screening of suspension are done to produce protein extract; Reheated is done at temperature 60°C; andsolution (suspension) is ready to be used as edible film material.

Edible film preparation.

Procedure for processing of canna starch edible film without and with the addition of protein extract of eel is as follows: Preparation of canna starch native (P_1) and (P_2) modified using $POCl_3$ 0,08% with magnitude of 4% (w/v); The addition of aquadest followed by stirring and sieving; Starch suspension heating at gelatinization temperature of 65°Cusing *hot plate*followed by stirring with *magnetic stirrer*; The slowly addition of glycerol 3% (v/v) to starch suspension that had fully gelatinized and then followed by heating for about 10 minutes;Addition of eel extract protein according to treatments with concentration of S_1 (2%), S_2(4%), andS_3(6%) v/v; Addition of CMC surfactan according to treatments with concentration of 2% (b/v); Suspension is stirred until homogenous and addition of bees wax with concentration of 1% (b/v); Removing dissolve gas (*degassing)*by using vacuum pump for 1 hour; Pouring the 40mL of suspension to petri dish 15cm in diameter followed by moulding and heating at 70°C for 12 hours using oven;andcooling at room temperature followed by edible film removal from moulder and wrapped with plastics, put it into desicator for 24 hours.

Statistical analysis

Statistical analysis on completely randomized experimental was done by using SAS computer Program of Window 6.12 version. 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

Addition of protein extract of eel has an objective to improve elongation degree of canna starch edible film. Protein molecules of eel within edible film matrix is bound with hydrophilic components such as starch, glycerol and CMC. Astiana (2012) had explained that eels contain 15.90% essential and non-essential hydrophobic proteinswhich consisted of lysine and glutamic acid with magnitudes of 7.13g / 100g and 12.89g/100g, respectively.

Analysis of variance results showed that extract protein of eel treatments had significant effect, whereas their interaction had no significant effect on elongation percentage value of edible film (α=0.05). Results of Duncan test (Table 1) showed that elongation percentage value of modified canna starch edible film was significantly different than that of native canna starch edible film.Treatment 2% protein extract of eel was significantly different than that of 4% and 6%, but treatment 4% protein extract of eel was not significantly different than that of 6% in term of edible film elongation percentage.

Elongation degree value of modified canna starch edible film (57.33%) was higher than that of native canna starch edible film (48.00%). This was due to the fact that modified canna startch had more open starch molecules structure and low retrogradation characteristics. This starch structure cause protein molecules of eel is easily enter to edible film matrix and boundwith hydrophilic components such as starch, glycerol and CMC.

Low retrogradation characteristics of modified canna starch cause protein molecules of eel trapped and stable within edible film matrix.

According toNakai and Modler (1999),protein extract of eel contain the same protein such as found in fish, i.e. myofibrillar and sarcoplasmic with magnitude of 65 to 75% and 20 to 30%, respectively. Weng *et al*. (2007)had stated that myofibrillar protein has fibrous form and elastics whereas sarcoplasmic protein is globular. Myofibrillar protein affect edible film elasticity increment and sarcoplasmic protein decrease polymers interaction and cohesive power of edible film matrix as well as produce more flexible edible film. Fish protein based *edible film* consisting of myofibrillaror sarcoplasmicprotein generally have better mechanical properties, especially flexibility property, but has low barrier to water vapour transmission rate (Hamaguchi*et al*., 2007)

Table 1 showed that higher protein extract of eel results in higher elongation degree of *edible film*. Higher protein extract of eel results in higher quantity of myofibrillar and sarcoplasmic protein molecules which are trappedwithin edible film matrix.

Average value of elongation percentage of canna starch edible film with protein extract of eel was higher than that of edible film without protein extract(Figure 1). However, this edible fim elongation percentage had not fulfilled the stated standard of JIS 1975, i.e minimum of 70% for all addition levels of protein extract.

Comment [W4]: Please make it clear. The figure 1 can not confirm this statement. The treatmen C2 (in figure 1) needs to be clearly defined in edible film preparation whether it is a control (treatment without addition of protein extract).

Analysis of variance results showed that protein extract of eel had significant effect on edible film compressive strength value, whereas their interaction was not significant (α=0.05). Compressive strength value of modified canna starch edible film was significantly different than to native starch edible film. The use of protein extract of eel with concentrations of 2%, 4% and 6%gave significantly different values of edible film compressive strength (Table 1).

Compressive strength value of modified canna starch edible film was higher than native starch film. This was caused by stronger molecular structure of canna starch modified than native. $POCl_3$ cross linked canna starch had some substituted OH groups by phosphate. This subtitution increase the structural strength of starch molecules that resulted the stronger starch molecules. Degree of edible film matrix strength is increased by increasing starch strength.

Table 1 showed 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. This protein moleculair chains have fibrous form and length. Higher myofibrillar protein content results in more compact edible film and higher resistance power to pressure. This results was in accordance to study that reported bySobral*et al.*(2005) which showed that the use of 2g of myofibrillar protein in 100g film solution had higher pressure strength than that of 1g myofibrillar protein in 100g film solution.Artharn *et al.* (2008) had stated that edible film formulation with ratio myofibrillar protein and sarcoplasmic protein of 10:0 had produced the highest value of compressive strength.

Compressive strength value of canna starch edible film with protein extract of eel was lower than that without protein extract addition (Figure 2). Sarcoplasmic protein is globulair form protein. This protein is dispersed amongst edible film matrix which lower interaction with film matrix polymers and lower compactness which subsequently decrease the edible film resistance power to pressure.Artharn *et al*. (2008)had reported that compressive strength of protein-based edible filmwill decrease when concentration of sarcoplasmic protein is increase.

Comment [W5]: Please make it clear. The figure 2 can not confirm this statement. The treatmen C2 (in figure 2) needs to be clearly difined in edible film preparation whether it is a control (treatment without addition of protein extract).

Analysis of variance results showed that treatments of protein extract of eel had significant effect on water vapour transmission rate of edible film (α=0,05). Results of Duncan test (Table 1) showed that treatments of 2%, 4% and 6% protein extract of eel had significant effect on water vapour transmission rate of edible film.

Table 1 showed that higher protein concentration had produced lower water vapour transmission rate. This was due to protein extract of eel which had myofibrillar and sarcoplasmic proteins.Molecular structure of myofibrillarprotein 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 of film matrix structure. This condition impede water vapour to penetrate edible film matrix. Shiku *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. Formation of sarcoplasmic protein films prepared from blue marlin (*Makairamazara*) has mainly involved thermal treatment of film-forming solutions at temperature ranging between 55°C and 90°C (Iwata *et al.,* 2000).

Figure 3 showed that water vapour transmission rate of canna starch edible film with protein extract of eel was higher than that without protein extract of eel.This was due to protein molecules as structural components of edible film matrix which has hydrophilic characteristics. Protein addition results in increase of hydrophilic components within edible film matrix. Therefore, the higher the hydrophilc components, the easier the water vapour to penetrate edible film. Yoshida *et al*. (2002)described that natural hydrophilic property of protein in edible film formulation facilitate the interaction with water which lower edible film resistance power 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 relativey high value of water vapour transmission rate.

Comment [W6]: See w5

Conclusion

Addition of eel's protein extract had increased elongation percentage and decreased water vapour transmission rate of edible film. Compressive strengthof 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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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

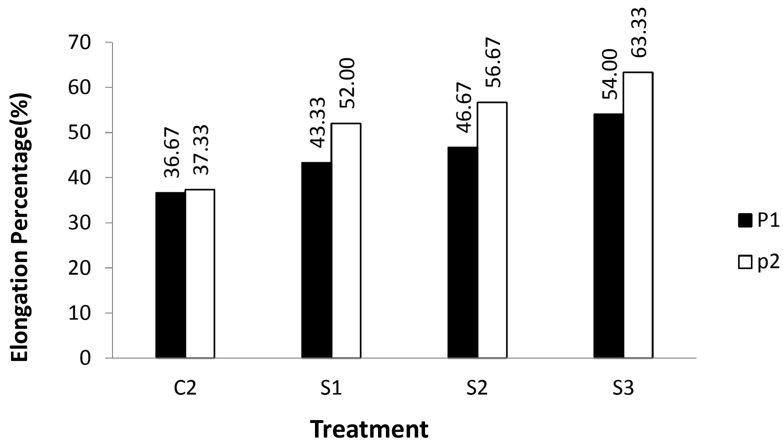

Figure 1. Average value of elongation percentage of canna's starch edible film prior to and after addition protein extract of paddy field's eel.

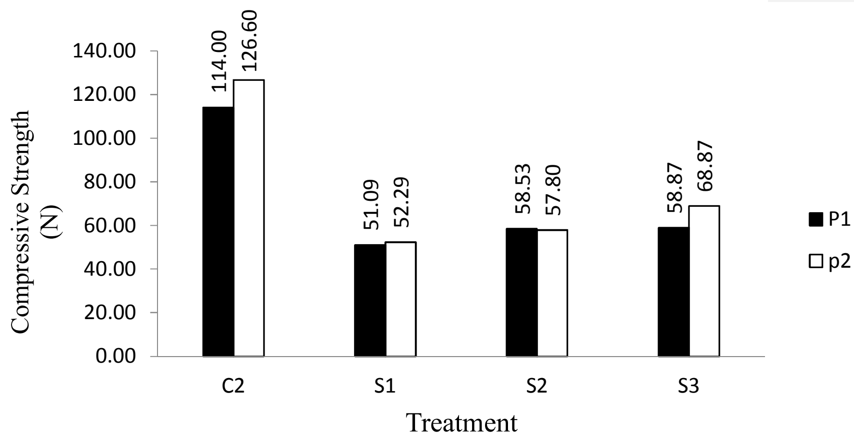

Figure 2. Average value of edible film’s pressure strength prior to and after protein extract addition of paddy field’s eel

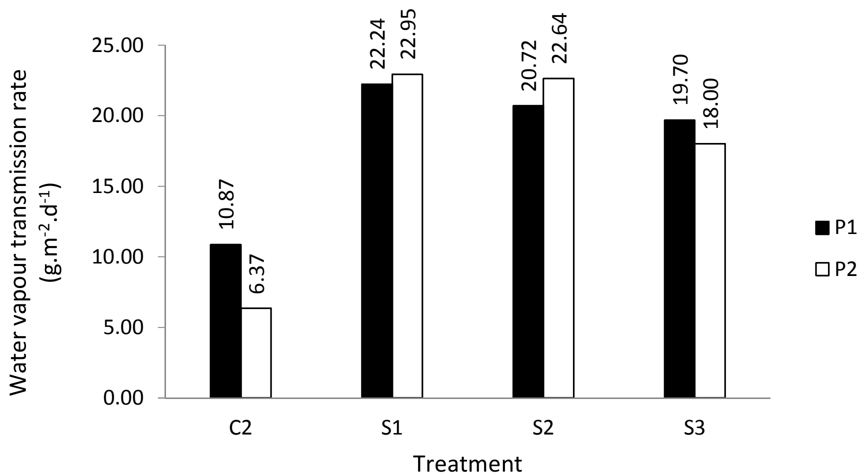

Figure 3. Average value of water vapour transmission rate of canna's starch edible film prior to and after protein extractaddition of paddy field's eel