Malaysian Applied Biology (2023) 52(5): 29-34
https://doi.org/10.55230/mabjournal.v52i5.cp18

Research

Physico-Chemical Characteristics of Crosslinked-Biofilm Made From *Passiflora edulis* Waste

Nozieana Khairuddin[1]*, Sheena John[2], Suguna Selvakumaran[2] and Shiamala Ramaiya Devi[3]

1. Department of Science and Technology, Faculty of Humanities, Management, and Science, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu Sarawak, Malaysia
2. Department of Biotechnology, School of Biotechnology, Manipal International University, 71800, Nilai, Negeri Sembilan, Malaysia
3. Department of Crop Science, Faculty of Agriculture and Forestry Science, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu Sarawak, Malaysia
*Corresponding author: nozieana@upm.edu.my

ABSTRACT

In numerous tropical countries, *Passiflora Edulis* which is also known as passion fruit is grown largely for domestic consumption in both the fresh and processed form. Generally, the sweeter purple passion fruit referred to as the granadilla is preferred for consumption as fresh fruit, while the yellow passion fruit is grown mainly to produce juice concentrate or single-strength juice, fruit preserves, and jams, and as a flavoring agent. Passion fruit peel was used to extract pectin to produce biofilm because of its gelling properties and chemical composition to avoid wastage in the juice industry. The objective of this study is to characterize the physical and chemical properties of the pectin-based biofilm. The films were prepared using a casting technique where pectin acts as biopolymer, starch as the base, and glycerol as the plasticizer. Calcium chloride and citric acid were used as cross-linking agents. The results of the solubility test showed that pectin-based biofilms made from passion fruit are more hydrophilic compared to starch, but there was no significant difference in moisture content between the control and film containing 5 and 7 w/v% of crosslinking agent added. The film formed with calcium chloride showed better physical and chemical properties in terms of thickness, solubility, and moisture content. The formulation based on starch and pectin mixture was less rigid and had better elasticity compared to the control film. Therefore, producing films from passion fruit is a new alternative by taking waste from the juice industry.

Key words: Cross-linking agents, food packaging, passion fruit, pectin-based film, and physical and chemical characteristics

Article History
Accepted: 8 November 2023
First version online: 15 December 2023

Cite This Article:
Khairuddin, N., John, S., Selvakumaran, S. & Devi, S.R. 2023. Physico-chemical characteristics of crosslinked-biofilm made from *Passiflora edulis* waste. Malaysian Applied Biology, 52(5): 29-34. https://doi.org/10.55230/mabjournal.v52i5.cp18

INTRODUCTION

In the past 100 years, the production of plastics has increased enormously, and Statista accounted for a global production of 322 million tonnes in 2015. At this point, this enormous number of plastic products causes extreme plastic pollution and are usually manufactured using non-inexhaustible sources. Biofilm is plastics obtained from inexhaustible sources of biomass, and it is assumed that its demand is developing at a rate of 20 - 25% per year (Ezgi Bezirhan & Havva Duygu, 2015).

Passiflora Edulis which also known as passion fruit is grown largely for domestic consumption in both the fresh and processed form. Passion fruit peel was used to extract pectin to produce biofilm because of its gelling properties and chemical composition to avoid wastage in the juice industries (Batori, 2018). The non-toxic, odorless, and biodegradable qualities of pectin films are noteworthy (Espitia *et al*., 2014)

The development of biofilms from polysaccharide-based build-ups of soil venture products has become the latest trend in the exploration of the production of bioplastics from the auxiliary feedstock. The combination of components of the various wellsprings of foods grown from the earth, such as gelatin, starch, lining, cellulose, and hemicelluloses, makes these lignocellulosic feedstocks interesting and

promising for the development of bioplastic films (Bioplastics, 2015). The objective of this study is to characterize the physical and chemical properties of the pectin-based biofilm.

MATERIALS AND METHODS

Materials

Purple passion fruit *(Passiflora Edulis)* was collected from UPM Bintulu Sarawak Campus. Corn starch was purchased from a local market. The chemicals used were citric acid-2-hydrate (Bendosen, Malaysia), calcium chloride-2-hydrate (Bendosen, Malaysia), and glycerol (Chem Soln, Malaysia).

Preparation of films

Dried passion fruit mesocarp (PFM) was turned into powder by using a dry blender and stored in a jar at room temperature until further usage of the sample. Five percent (w/v of film solution) of the passion fruit peel was measured and heated on a stirring hot plate till it reached 80 °C for 30 min. Then, using a coffee filter the filtrate was filtered, and the precipitate was discarded. 50 mL of filtrate was centrifuged twice at 6000 r.p.m. for 15 min. The pellet was discarded. Using a 0.45 µm membrane syringe filter, the supernatant was filtered into falcon tubes.

Next, 0.7 g/10 mL of corn starch was heated on a hot plate with continuous stirring using a glass rod until a gel-like texture was formed. Next, the filtered PFM extract solution was added to hot starch and stirred well using a stirrer. Then, the mixture of the starch-PFM solution was added to 1 g of glycerol and stirred well for 20 min. 25 mL of the film solution was then cast into glass plates and left to dry in an oven at 40 °C for 18 - 20 h. The film was transferred into the desiccator for three days. Finally, when the film was completely dried, it was peeled off, and cut into strips (2 cm by 2 cm) in size for further analysis.

Cross-linking the films

Three different concentrations (3%, 5%, & 7% w/v) of calcium chloride ($CaCl_2$) were used as crosslinking agents.

Once the films were completely dried, the films were soaked in 50 mL of the three different concentrations of $CaCl_2$ (3, 5, & 7% w/v) each for 30 min. Then, the films were left to dry in the 40 °C oven for 24 h. Finally, the films were transferred into the desiccator until the films were completely dried. Table 1 summarizes the concentration and symbol representing each of the films.

Table 1. Amount of crosslinking agent added to each biofilm

Film	Amount of crosslinking agent added (% w/v)
Control	0
CL-3	3
CL-5	5
CL-7	7

Physical characterization of films

Film thickness measurement

The thickness of the film was measured using a micrometer screw gauge that has a sensitivity of 0.001 mm. The measurements were taken at 5 points which were one at the centre and all four opposite sides. This measurement was performed in triplicates.

Moisture content (MC)

The mass of the film was measured on a weighing scale and placed in a falcon tube. The falcon tube was placed in an oven without the lids at the temperature of 60 °C for 24 h. Next, the final mass was weighed. This was performed in triplicates. The moisture content was calculated using the formula:

$$\text{MC} = \frac{(W_o - W_i)}{W_o} \times 100$$

Where,
W_o is the mass of the sample after dried in the oven whereas W_i is the mass of the sample before dried in the oven.

Solubility test

The mass of the film was measured on a weighing scale and placed in a falcon tube. 50 mL of distilled water was added to the falcon tube. Then, the film was placed in an incubator shaker at 150 with a temperature of 28.6 °C for 24 h. Next, the distilled water was drawn out and the films were placed in an oven of 105 °C without the lids for the next 24 h to dry. Finally, the final mass of the film was weighed. This was performed in triplicates. The solubility of the film was calculated using the formula:

$$\text{Solubility} = \frac{(IM - FM)}{FM} \times 100$$

Where;
IM is the initial mass while the *FM* is the final mass after the solubility test.

Surface micrograph by using Scanning Electron Microscopy (SEM)

The surface of the biofilms formed was studied using scanning electron microscopy (SEM). All of the biofilms were dried for 24 h in a vacuum oven at 70 °C, then mounted on stubs, sputter-coated with gold in a vacuum chamber, and photographed using a scanning electron microscope operating at 5 kV under magnifications of 100x, 500x, 1000x, 2000x, 5000x.

Chemical characterization by using Fourier transform Infrared Spectroscopy (FTIR)

ATR-FTIR was used to obtain information about the interactions between components in films. The FTIR spectra of the films were recorded with a spectrometer (Thermo Scientific, Boston, MA, USA) using attenuated total reflectance mode (ATR). Each spectrum resulted from 32 scans at 4.000 cm^{-1} resolution, for a spectral range of 400 – 4000 cm^{-1}. All the readings were performed at room temperature (20 °C).

Statistical analysis

The statistical analysis of the data was performed through one-way analysis of variance (ANOVA) using Microsoft EXCEL 365. The data were ranked, and statistical differences were evaluated on the ranks with a one-way analysis of variance (ANOVA). In all cases, a value of $p<0.05$ was significant.

RESULTS AND DISCUSSION

Physical characterization

Figure 1 shows a film from PSM that has been dried. All films are yellowish and translucent including the control films which have no crosslinking agent being added.

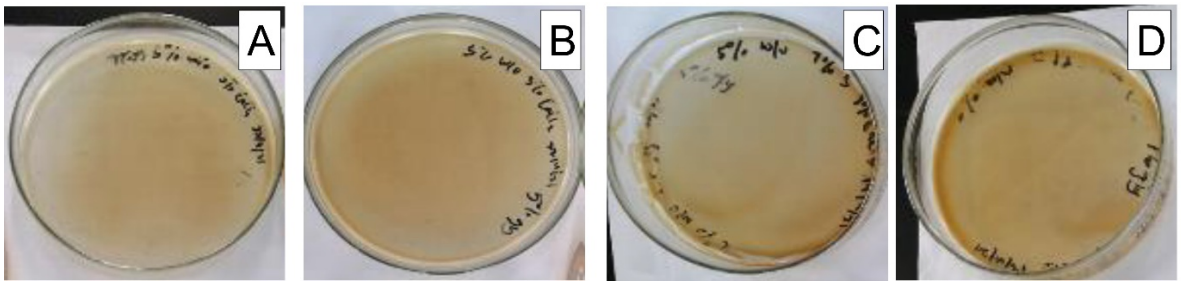

Fig. 1. Appearance of dried films containing (a) 0%, (b) 3%, (c) 5%, and (d) 7% w/v of CaCl2 with respective labels as CONTROL, CL-3, CL-5 and CL-7.

The physical parameters of the control and the crosslinked films are summarized in Table 2. The thickness of the Passiflora Edulis films crosslinked with $CaCl_2$ was significantly lower compared to the control film ($p<0.05$) whereas there is no significant difference in thickness among three concentrations of crosslinker added to the films.

No significant difference in moisture content between the control and film containing 5 and 7 w/v% of crosslinking agent. However, the film with 3% of $CaCl_2$ exhibited a significantly higher percentage of moisture content.

The results of the solubility test showed that pectin-based biofilms made from passion fruit are more hydrophilic compared to starch.

Table 2. Physical parameters of crosslinked PFM films

Film	Thickness (mm)	Moisture content (%)	Solubility (percentage of weight loss)
Control	0.43±0.02[a]	18.18±0.01[a]	60.00±0.01[a]
CL-3	0.15±0.04[b]	83.33±0.01[b]	72.73±0.01[b]
CL-5	0.18±0.04[b]	30.00±0.01[a]	75.00±0.01[b]
CL-7	0.16±0.04[b]	42.86±0.01[a]	81.82±0.01[b]

Surface micrograph by using Scanning Electron Microscopy (SEM)

The SEM images of the different biofilms were obtained. SEM images give an overview of the morphology of the different biofilms. The SEM image of the control film (Figure 2) shows a visible pore on the structure of the film as well as rough surfaces. The optical micrographs reveal that crosslinked film (CL3) has a spherical shape and a broad distribution of oil drops spread throughout the matrix. This can be caused by the rough surface and gelling of pectin (Aguilar *et al*., 2015). The morphology of the biofilm could also be affected by the large particles of fibers from the mesocarp of the passion fruit present in the film. Similar results were shown by Fang *et al*. (2002) where greater protein aggregation in the film was caused by the addition of Ca^{2+} leaving the irregular but continuous surface.

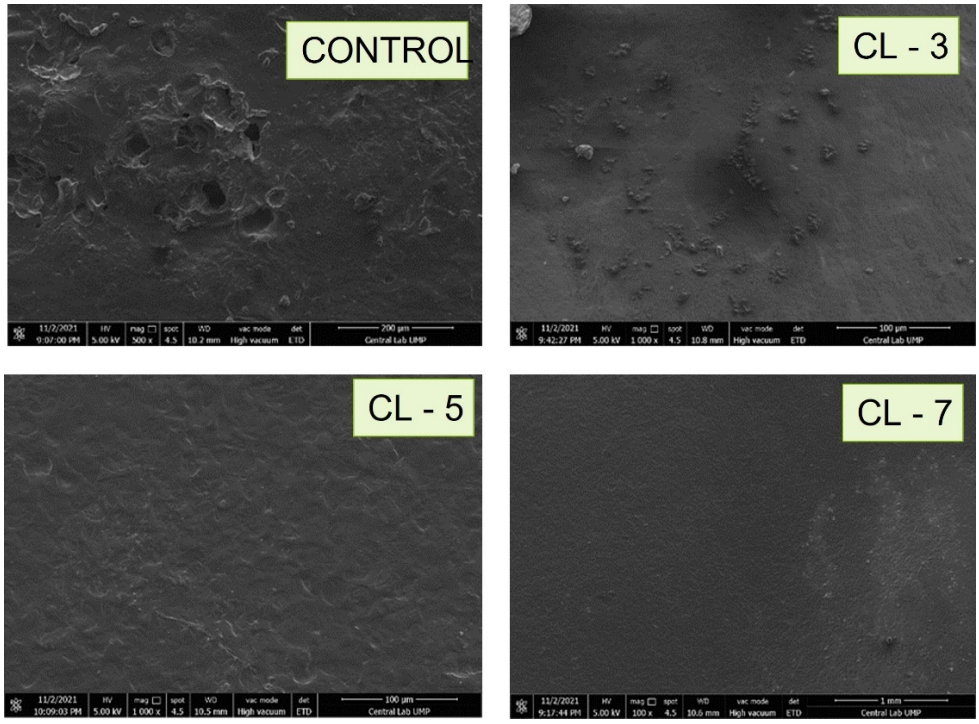

Fig. 2. SEM images of CONTROL, CL-3, CL-5, and CL-7 with 0%, 3%, 5% and 7% w/v of $CaCl_2$ respectively.

Chemical characterization by using Fourier transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the different biofilms were taken and can be seen in Figure 3. The IR-spectrum of all four biofilms is almost similar to each other. According to Nisar *et al*. (2019), the pectin structure's inter- and intramolecular hydrogen bonding is responsible for the large absorption region between 3600 and 3000 cm^{-1}. Meanwhile, the absorption band at 2925 cm^{-1} is related to the stretching of C–H bonds, which are involved in CH, CH_2, and CH_3 groups of stretching and bending vibrations (Lorevice *et al*., 2016).

Whereas the carbonyl groups (COOH) and acetyl groups ($COOCH_3$) of pectin are confirmed by the bands about 1616 cm^{-1} and 1734 cm^{-1}. The strong peak at 1061 cm^{-1} suggests the strain vibration band of the symmetric COC group, which also confirms the high degree of esterification and the presence of high methoxyl pectin (Henao-Díaz *et al*., 2021).

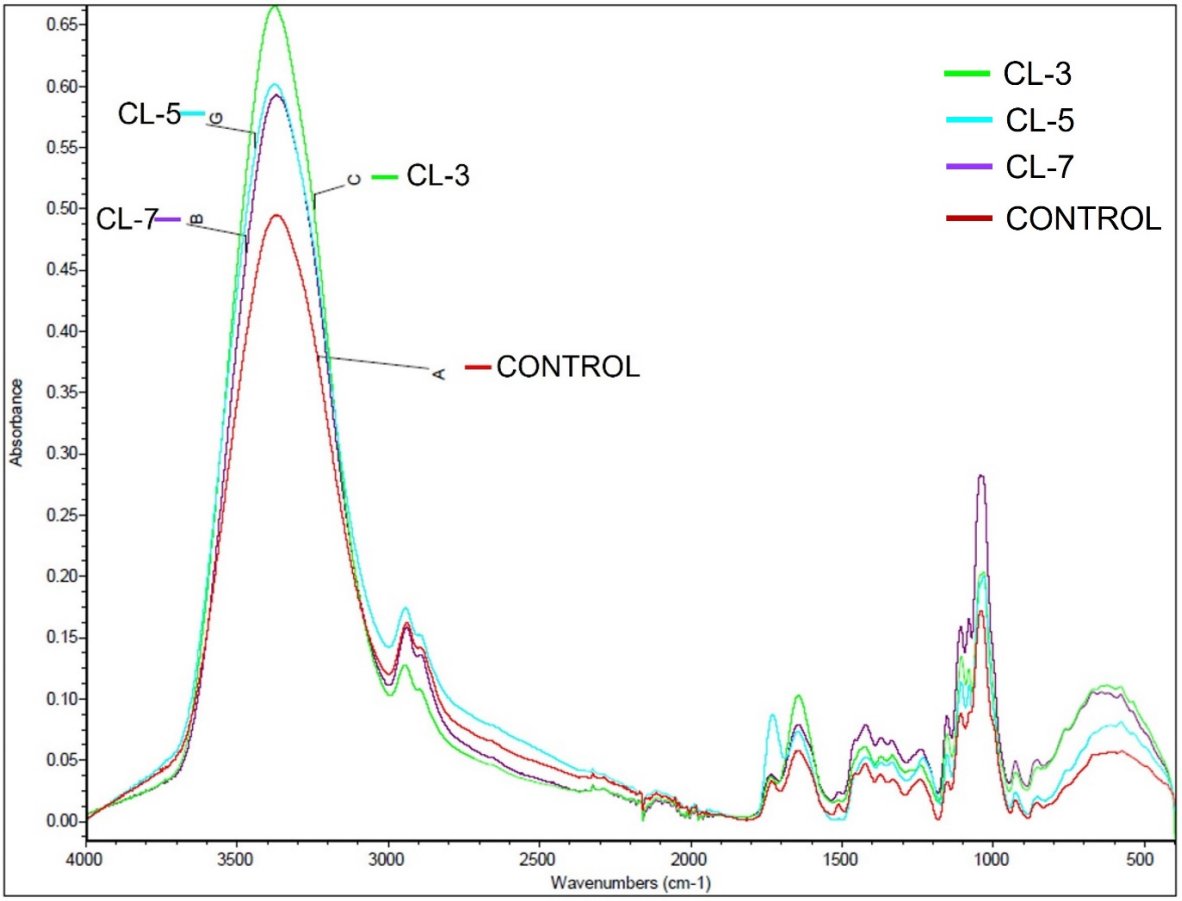

Fig. 3. FTIR spectrum indicating the presence of different functional groups in CONTROL, CL-3, CL-5, and CL-7 biofilms.

The amount of available OH in different films was compared by the difference of ratio between the intensity peak which was previously used by Shi *et al*. (2007) which is associated with the vibration stretching of 'C=O' in the 'C=O=H' group. The esterification process between $CaCl_2$ and the OH groups of glycerol causes a decrease in the quantity of OH. Table 3 depicts the matching compounds validating the material incorporated in the control and crosslinked films. The presence of $CaCl_2$ can be observed in the crosslinked films which confirmed the success of the crosslinking process.

Table 3. Matching compounds in ATR-FTIR validating the material incorporated in the control and crosslinked biofilms

Match	Compound Name	Library Name	Sample
79.06	L-Glucose	Sigma Sugars and Carbohydrates	Control and CL films
73.68	D-Glucose	Sigma Sugars and Carbohydrates	
71.16	D-Idose	Sigma Sugars and Carbohydrates	
68.83	3-Deoxy-D-ribose	Sigma Sugars and Carbohydrates	
68.81	Glycerol, 99.5+%, Spectrophotometric Grade	HR Aldrich Alcohols and Phenols	
68.16	Glycerol	HR Aldrich Solvents	
68.14	Glycerol; 1,2,3-Propanetriol; Glycerine	HR Food Additives	
67.69	Hydroxypropyl-Beta-Cyclodex, MS-0.8	HR Aldrich Alcohols and Phenols	
67.63	Hydroxypropyl-Beta-Cyclodex, MS-0.6	HR Aldrich Alcohols and Phenols	
64.64	D-Eryhrose	Sigma Sugars and Carbohydrates	CL films
63.31	D-Idoes	Sigma Sugars and Carbohydrates	
61.12	L-Glyceraldehyde	Sigma Sugars and Carbohydrates	
60.48	Chloride; Nickel(II); Hexahydrate	HR Inorganics	
60.44	D-Ribulose	Sigma Sugars and Carbohydrates	

CONCLUSION

Biofilms obtained from the passion fruit mesocarp, have been elaborated with different concentrations of crosslinker CL3, CL5, and CL7. The film formed with the crosslinker showed better physical and chemical properties in terms of thickness, solubility, and moisture content. SEM analysis revealed

that, as the crosslinker content increases, the surface becomes more irregular, granular, and folded. Besides, the crosslinked film does not change the chemical composition as compared to the control film as revealed from the FTIR results.

ACKNOWLEDGEMENTS
This work was supported/funded by the Ministry of Higher Education under the Fundamental Research Grant Scheme (FRGS/1/2018/TK05/UPM/02/8). The authors would also like to thank the Financial Department of Universiti Putra Malaysia Bintulu Sarawak Campus and Research Management Centre, UPM for their support of this study for the IPM-Putra Grant (9653300). We would like to express our great appreciation to FoBerg Laboratory at Universiti Teknologi Malaysia and Manipal International University for the facilities.

ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

REFERENCES
Aguilar, K.C., Tello, F., Andrea, C.K., Bierhalz, M.G., Romo, G., Héctor, E.M.F., & Carlos. 2015. Protein adsorption onto alginate-pectin microparticles and films produced by ionic gelation. Journal of Food Engineering, 154: 17-24. https://doi.org/10.1016/j.jfoodeng.2014.12.020

Arikan., E.B. & Ozsoy, H.D. 2015. A Review: Investigation of Bioplastics, Journal of Civil Engineering and Architecture, 9(1): 188-192. https://doi.org/10.17265/1934-7359/2015.02.007

Bátori, V. 2018. Fruit wastes to biomaterials: Development of Biofilms and 3D Objects in a Circular Economy System (Ph.D). University of Borås.

Bioplastics, E. 2015. Biobased plastics - Fostering a resource efficient circular economy; Benefits, feedstock types, sustainable sourcing, land use. In: Proceedings of the 10th European Bioplastics Conference, Berlin, Germany.

Espitia, P.J.P., Du, W-X., Avena-Bustillos, Jesús Avena-Bustillos, R., Soares, N.D.F.F. & McHugh., T.H. 2014. Edible films from pectin: Physical-mechanical and antimicrobial properties - A review. Food Hydrocolloids, 35: 287-296. https://doi.org/10.1016/j.foodhyd.2013.06.005

Fang, Y., Tung, M.A., Britt, I.J., Yada, S. & Dalgleish, D.G. 2002. Tensile and barrier properties of edible films made from whey proteins. Journal of Food Science, 67(1): 188-193. https://doi.org/10.1111/j.1365-2621.2002.tb11381.x

Henao-Díaz, L.S., Cadena-Casanova, C.L., Bolio-López, G.I., Veleva, L., Azamar-Barrios, J.A., Hernández-Villegas, M. M. & Córdova-Sánchez, S. 2021. Obtaining and characterizing bioplastic films obtained from passion fruit (*Passiflora edulis* Sims) waste. Agro Productividad, 14(7). https://doi.org/10.32854/agrop.v14i7.2010

Lorevice, M.V., Otoni, C.G., Moura, M.R.D. & Mattoso, L.H.C. 2016. Chitosan nanoparticles on the improvement of thermal, barrier, and mechanical properties of high- and low-methyl pectin films. Food Hydrocolloids, 52: 732-740. https://doi.org/10.1016/j.foodhyd.2015.08.003

Nisar, T., Wang, Z.C., Alim, A., Iqbal, M., Yang, X., Sun, L. & Guo, Y. 2019. Citrus pectin films enriched with thinned young apple polyphenols for potential use as bio-based active packaging, CyTA - Journal of Food, 17(1): 695-705. https://doi.org/10.1080/19476337.2019.1640798

Shi, R., Zhang, Z., Liu, Q., Han, Y., Zhang, L. & Chen, D., & Tian, W. 2007. Characterization of citric acid/glycerol co-plasticized thermoplastic starch prepared by melt blending. Carbohydrates Polymers, 69(4): 748-755. https://doi.org/10.1016/j.carbpol.2007.02.010