Research Paper

Future of Food: Journal on Food, Agriculture and Society
11 (5) December 2023

The effect of wuluh starfruit (*Averrhoa bilimbi* L.) added on the physicochemical and antimicrobial characteristics of chitosan-PVA edible film

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Data of the article

First received : 23 September 2023 | Last revision received : 04 November 2023
Accepted : 17 December 2023| Published online :28 December 2023
DOI : 10.17170/kobra-202307218416

Keywords

antimicrobial; edible film; physicochemical properties of wuluh starfruit

Edible film is an eco-friendly packaging innovation that can be a solution in dealing with the plastic waste problem. Apart from that, edible film is expected to have antimicrobial properties that can extend packaged food products' shelf life. Wuluh starfruit (*Averrhoa bilimbi* L.), a tropical plant from Southeast Asia that contains organic acid compounds, functions as an antimicrobial that can be added to edible films. This research aims to determine the effect of wuluh starfruit juice addition in making the edible film in terms of its micro-bacterial and physicochemical properties. A Completely Randomized Design (CRD) with seven treatment levels and five replications was used as the experimental design. The results showed that the treatment with the addition of 4% wuluh starfruit juice (Treatment C) with an antibacterial value of the disc method of 1.28 mm was the most optimal treatment because the bacterial inhibitory power at this concentration increased the most optimally compared to other treatments. The physicochemical characteristics of the treatment C edible file are moisture content of 17.29 %; thickness of 0.55 mm; water vapour transmission rate of 60.01 g/m2/day; solubility of 42.79 %; tensile strength of 22.29 kgf/cm2; elongation of 44.47 %; and pH of 6.23.

1. Introduction

Plastic waste is one of the problems faced by many countries in the world because it has properties that are difficult to break down naturally, and the amount increases along with the rate of population growth. Based on Global Plastics Outlook: Economic Drivers (OECD) data, world plastic production is estimated to reach 250 million tons, and more than 250,000 tons of this waste pollute the sea. Based on data from the Indonesian Plastic Industry Association (Inaplas) and the Central Statistics Agency (BPS), plastic waste in Indonesia reaches 64 million tons per year, and 3.2 million tons of plastic waste is thrown into the sea.

Food packaging is the most common type of plastic waste (Desy *et al*., 2018; Salim *et al*., 2020). Plastic is one of the most widely used types of packaging. Besides its relatively cheap price, plastic is flexible and does not break easily. The development of alternative packaging technologies is necessary to reduce plastic use, one of which is active packaging.

Active packaging is one of the developments in packaging innovation that has the potential to reduce plastic use. Active packaging is designed to extend shelf-life by preserving the packaged food condition. The concept of this technology is to add specific components to the packaging matrix that can absorb or release certain substances from the packaged food or vice versa (Flórez *et al.*, 2022; Song *et al.*, 2022).

Active ingredients (active agents) can be added to packaging materials or packaging surfaces such as labels, sachets, or bottle caps. Active ingredients that can be added include organic acids, fungicides, bacteriocins, enzymes, ions, natural extracts, and so on. Active packaging can remove unwanted compounds such as excess water, oxygen, ethylene, carbon dioxide, odours, and other unnecessary compounds. Several types of active packaging are oxygen or moisture absorbers, carbon dioxide emitters, self-heating packaging, and antimicrobial packaging (Widiastuti, 2016). These types of active packaging work by preventing compounds that can cause the shelf life of packaged food to be short. One example of active packaging is edible film.

Edible film is an eco-friendly alternative packaging because it is biodegradable and does not pollute the environment. The application and development of edible film in food applications help minimize the use of plastic and environmental pollution (R & Hema, 2021). Other advantages of the edible film are that it is biocompatible, non-toxic, does not cause pollution, has properties as a mass transfer inhibitor (water vapour, oxygen, and dissolved substances), and is cheap (Aripin *et al.*, 2017; Sutra *et al.*, 2020). Adding active ingredients to maintain the packaged product is necessary to increase the effectiveness of edible film, one of which is antimicrobial agents.

Antimicrobial packaging is divided into two groups, namely packaging with antimicrobial materials that can migrate to the surface of the food so that they make contact with the food and antimicrobials that effectively inhibit the growth of microbes on the surface of the food without migration. This packaging is very effective for food products where microbial contamination occurs on the product's surface. Antimicrobial packaging can extend shelf-life and maintain food quality and safety by inhibiting the growth of spoilage microbes (Soltani Firouz *et al.*, 2021; Yin *et al.*, 2023). To obtain antimicrobial properties, edible films can be made by adding natural ingredients that have antimicrobial properties, one of which is wuluh starfruit (*Averrhoa bilimbi* L.).

Wuluh starfruit is often called vegetable or sour starfruit because it has a sour taste and is widely used as a cooking spice or herbal medicine and contains lots of tannins, saponins, glucose, sulphur, formic acid, peroxide, flavonoids and triterpenoids (Suryaningsih, 2016). Wuluh starfruit is a source of organic acids such as citric acid, formic acid, and other active substances such as flavonoids, polyphenols, tannins, and saponins (Datu *et al.*, 2015). These compounds are pharmaceutical components and have antimicrobial properties (Yuliansyah *et al.*, 2014). Wuluh starfruit is a plant that contains flavonoid compounds. The mechanism of action of flavonoids as antibacterial in starfruit is by inhibiting the function of cell membranes so that they form complex compounds that can damage bacterial cell membranes (Abdullah & Munadirah, 2021).

This research aims to determine and study the effect of wuluh starfruit juice addition in edible films with chitosan-PVA as the main ingredient in terms of physicochemical and antimicrobial properties.

2. Materials and Methods

2.1. Materials and tools

Fresh wuluh starfruit harvested from our garden in Padang Pasir Village, West Padang District, Padang City, West Sumatra Province, Indonesia. The other ingredients used in making edible film, the chemicals and the instruments used in parameter testing are presented in Table 1.

2.2. Research procedure

2.2.1. Preparation of wuluh starfruit juice

1 kg of star fruit was weighed and washed thoroughly, then mashed and separated from the pulp using a juicer. Pure starfruit juice was obtained.

2.2.2. Modified edible film preparation (Warsiki *et al.*, 2013)

a) Preparation of 3% PVA solution

PVA powder was weighed at 7.5 grams, and mineralized water was added to a volume of 250 ml and homogenized for 20 minutes at 80oC. PVA functions as a plasticizer. A PVA concentration that is too high causes a decrease in the tensile strength value of the

ISSN-Internet 2197-411x OLCL 862804632

Table 1. Materials and tools

Materials and tools	Specifications
Polyvinyl alcohol/pva	Sigma-Aldrich; 99,0%
Chitosan	Sigma-Aldrich; $\geq$75,0%
Glyserol	Bratachem; 98,0%
Acetic acid glacial	Smart Lab; 99,7%
Demineralized water	Bratachem; 100%
Silica blue	Bratachem; >98%
Digital screw micrometer	Mitutoyo 293 Series
Tensile strength test equipment	Instron 3400 Series
Analytical balance	Shimadzu ATX224
Desiccator	Iwaki Pyrex
pH meter	Hanna HI2211

resulting edible film.

b) Preparation of 3% chitosan solution

Chitosan powder was weighed at 7.5 grams, and 125 ml of 1% acetic acid was added and filled to 250 ml with mineralized water, then homogenized for 20 minutes at a temperature of 80oC. Chitosan functions as a gel former. Chitosan concentrations that are too high will cause the edible film formed to become thicker. 1% acetic acid was obtained by diluting concentrated acetic acid with water in a fume hood.

c) Making edible film with the addition of wuluh starfruit juice

100 ml of 3% chitosan solution was pipetted and mixed with 150 ml of 3% PVA solution and 2.5 ml of 98% glycerol. Wuluh starfruit juice was added to the mixture according to the treatment. The mixture was homogenized for 2 minutes and poured into glass moulds. The glass mould was inserted into the oven at 50oC for 24 hours. The dry edible film was removed from the glass mould.

2.3. Experimental Design

A Completely Randomized Design (CRD) with seven treatment levels and five replications was used as the experimental design in this study. The data were analysed using analysis of diversity (ANOVA) with the F test and Duncan's New Multiple Range Test (DNMRT) with a significance level of 1 %. Data were processed and analysed with IBM SPSS Statistics version 29.0.1 software.

The treatment of wuluh starfruit juice addition used in this research was as follows:

A: 0 % wuluh starfruit juice
B: 2 % wuluh starfruit juice
C: 4 % wuluh starfruit juice
D: 6 % wuluh starfruit juice
E: 8 % wuluh starfruit juice
F: 10 % wuluh starfruit juice
G: 12 % wuluh starfruit juice

Determination of variations in adding wuluh starfruit juice was based on preliminary experiments. The concentration of starfruit juice that is too high causes an increase in the moisture content of the edible film, which affects other physicochemical parameters and results in the colour of the edible film becoming increasingly yellow. Edible film products from seven treatments can be observed in Figure 1.

2.4. Edible film characterization

2.4.1. Moisture content (AOAC, 2005)

The moisture content of edible film affects the stability and shelf life of packaged food products. Moisture content was tested and measured using the air oven method. Drying was carried out at 105oC for 5 hours or until the final weight was constant. This method was based on measuring the lost weight of the sample during the drying in a laboratory oven. The formula for calculating water content was as follows:

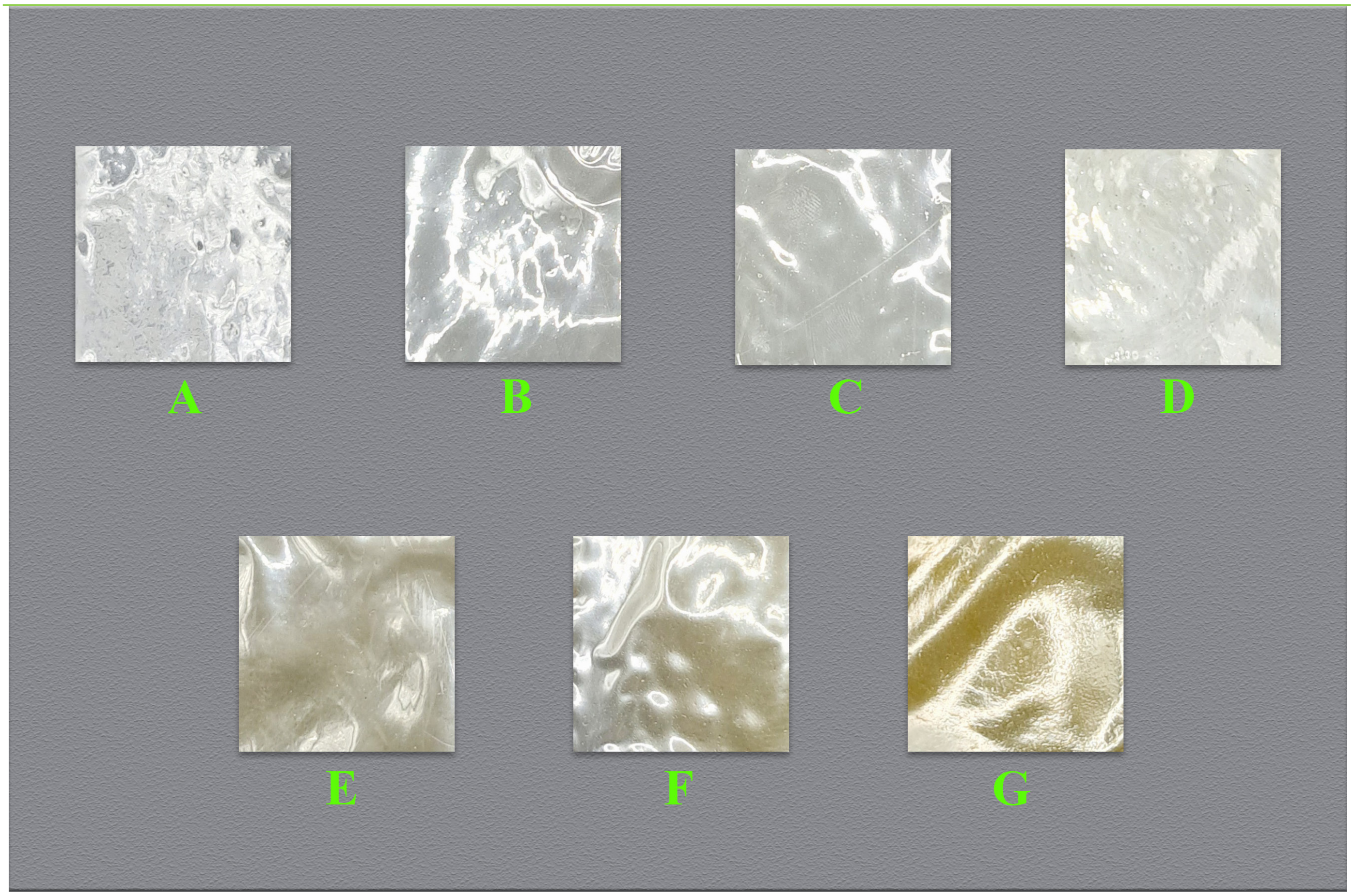

Figure 1. Edible film with the addition of wuluh starfruit juice

$$Moisture\ content = \frac{b-(c-a)}{c-a} x\ 100\%$$

A is the weight of the empty cup (g), b is the weight of the initial sample (g), and c is the weight of the final sample + cup (g).

2.4.2. Solubility (Gontard *et al*., 1993)

Solubility measurements were carried out to study the solubility of edible film in water. Solubility also influences the water vapour transmission rate and other physicochemical parameters. Samples were cut to size 2 x 2 cm. Samples and filter paper were dried at 105oC for 24 hours. Then, the sample was weighed, and the weight was determined as the initial weight. The sample was put into 50 mL of mineralized water and then immersed for 6 hours while being stirred. The sample was filtered using filter paper and then dried at 105oC for 24 hours. The remaining sample and the filter paper were weighed (final weight). Percent solubility was determined using the following equation:

$$Solubility = \frac{w1-w2}{w1} x\ 100\%$$

w1 is the initial weight (g) and w2 is the final weight (g).

2.4.3. pH value

pH is an essential parameter in edible film. The pH of edible film must be adjusted to the pH of the mouth so that it does not cause irritation when consumed. A total of 10 g of edible film was weighed, then put into a 100 mL measuring flask and filled with demineralized water to the limit mark. The solution was homogenized at 25oC and transferred into a beaker. The pH was measured using a pH meter calibrated using pH buffer solutions.

2.4.4. Thickness (Alemán *et al*., 2016).

Thickness is a parameter that influences the tensile strength and elongation of edible film. Thickness also impacts the appearance of the packaged food. A micrometre was used to measure the film thickness at six different positions on the surface. The thickness value was determined from the average measurement of the six positions. The thickness unit used is millimetres (mm).

2.4.5. Water vapour transmission rate (WVTR) (Gontard *et al.*, 1992).

The water vapour transmission rate was measured to determine the water absorption capacity of the edible film. This parameter influences the water content of food products during the storage period. The edible film was placed on a container filled with blue silica gel; then, it was tightened with an airtight lid. After being weighed, the container was placed in a demineralized water-containing desiccator. The container was weighed at three-hour intervals within a 24-hour interval. The calculation for the water vapour transmission rate using the following equation:

$$WVTR = \frac{w}{A \; x \; t}$$

WVTR is the water vapour transmission rate (g/m2/24 hours), w is the weight increase of silica gel (g), A is the edible film surface area (m2), and t is the interval of storage time (hours).

2.4.6. Tensile strength and elongation

Tensile strength and elongation are mechanical parameters that are quality standard requirements for edible films. A tensile strength and elongation testing machine were used to measure these parameters. The calculation for tensile strength and elongation using the following equation:

$$Tensile\ strength = \frac{F}{A}$$

F is the tensile strength force (kgf) and A is the edible film surface area (cm2). The tensile strength unit used is kgf/cm2.

$$Elongation = \frac{B - A}{A} \; x \; 100\%$$

A is the initial length (mm) and B is the length at break (mm).

2.4.7. Antibacterial activity (Ariyani *et al.*, 2018; Mehdizadeh *et al.*, 2012)

Antibacterial activity testing was carried out to study the inhibitory power of the growth of spoilage bacteria, which affects the shelf life of food products. The determination of antibacterial activity was based on the disc method. The bacteria used in this test are *Escherichia coli* and *Staphylococcus* aureus, which are the bacteria that most often cause disease in the human body through microbial contamination in food. In the disc method, a paper disc was used as a container for antimicrobial substances. The filter paper was placed on an agar plate inoculated with *Escherichia coli* or *Staphylococcus aureus*and then incubated at 37oC for 24 hours. The radical zone was an area around the disk without the growth of bacteria. The diameter of the radical zone was measured to determine antibacterial potential.

3. Results and discussion

3.1. Moisture content

Moisture content showed the amount of water contained in a material in percent units. The results of the diversity analysis showed that the different treatments for the addition of wuluh starfruit juice in the process of the chitosan-PVA edible film had significantly different effects on moisture content. The data are presented in Table 2.

The data above shows that the more wuluh starfruit juice is added, the higher the moisture content of the edible film. Treatment A (0% wuluh starfruit juice) has a moisture content value of 14.20%, which is the control. The more starfruit juice added, the moisture content increased to 26.52% (Treatment G, 12% wuluh starfruit juice).

Edible film is expected to have low moisture content because the stability of coated food products is greatly influenced by moisture content. Moisture content influences the resistance of edible film to water, one of the most essential properties of food products' stability (Dey & Neogi, 2019; Kumari *et al.*, 2021). Packaging materials with low moisture content tend

Table 2. Chemical characteristics of edible film from seven treatments of the wuluh starfruit juice addition

Treatments	Parameters		
	Moisture (%)	**Solubilty (%)**	**pH**
A	14,20 a ± 0,01	29,13 a ± 1,59	6,58 a ± 0,08
B	15,97 ab ± 0,60	37,22 b ± 2,40	6,33 b ± 0,02
C	17,29 b ± 0,94	42,79 c ± 1,84	6,23 bc ± 0,04
D	18,27 b ± 1,41	51,64 d ± 1,52	5,84 c ± 0,09
E	22,43 c ± 1,33	55,76 de ± 2,65	5,69 d ± 0,09
F	25,59 d ± 0,29	58,39 e ± 2,20	5,62 d ± 0,12
G	26,52 d ± 0,89	67,21 f ± 1,30	5,40 e ± 0,07

^{a,b,c}Means within a row with different superscript letters are significantly different between treatments

to absorb high amounts of water, which will change the appearance, shelf life, and taste of the packaged food. On the other hand, edible film, which has a high moisture content, causes the texture of the packaged food to become soft and susceptible to the growth of microorganisms.

Adding wuluh starfruit juice to make the edible film increased the moisture content in each treatment. Because starfruit has a high moisture content, reaching 94.78% (Eren *et al*., 2015). With different amounts of wuluh starfruit juice added to the edible film, there was an increase in each treatment. In treatment B (2% wuluh starfruit juice), the moisture content increased in value compared to treatment A (0% wuluh starfruit juice).

Moisture content affects the quality of edible film when stored or applied as packaging for a product. Also, moisture content is directly related to the film solubility. Moisture content is also affected by the type and amount of ingredients that make up the edible film (Diova *et al*., 2013). It is expected that this increase in water content will not occur because a value that is too high will negatively impact the texture of the edible film, which will become too soft and disrupt the stability of the product to be packaged. On the other hand, the water content of edible film that is too low will cause the texture of the edible film to become stiff and brittle, making it unsuitable for packaging.

3.2. Solubility

The diversity analysis results showed that the different treatments of wuluh starfruit juice in making the chitosan-PVA edible film had a very significantly different effect on solubility. The solubility is presented in Table 2. From Table 2, it can be seen that the solubility increased along with the addition of wuluh starfruit juice. As a control, Treatment A had a solubility value of 29.13 %. In the subsequent treatment, the solubility value increased to 37.22-67.21 %.

The edible film solubility is a significant indicator affecting biodegradable film's value. The source of the primary ingredients largely determines the solubility of edible film. Wuluh starfruit juice, which has a high moisture content, greatly affects the solubility value of edible film. The increase in solubility is directly proportional to the moisture content of the edible film in each treatment.

Plasticizers also affect the solubility of edible film, whereas hydrophilic plasticizers can increase the film's solubility in water. A glycerol plasticizer can increase the solubility. Apart from its hydrophilic nature, glycerol also has a small molecular weight, allowing for easier interaction with polymer chains, which causes an increase in affinity for water (Mali *et al*., 2005; Tong *et al*., 2013). The lower hydrophilic groups in the constituent materials can cause a decrease in solubility (Warkoyo *et al*., 2014).

Edible film with high solubility indicates that the edible film is easy to consume. However, solubility that is too high will disrupt the stability of the packaged food product. In addition, the high solubility of edible film in water causes the shelf life of packaged food to be shorter. Therefore, treatments B, C, and D are considered to have optimal solubility values.

3.3. pH

The degree of acidity, or pH, is the acidity value of a compound or the hydrogen value of that compound (Luthfiati *et al*., 2023). The diversity analysis results indicated that the different treatments of wuluh starfruit juice had a very significantly different effect on the pH value. The pH values of edible film are presented in Table 2.

pH measurements were carried out to determine the pH of edible film, which was formulated according to the physiological pH of the mouth so that it did not cause an irritation reaction when consuming edible film. From Table 2, the edible film's pH ranged from 5.40-6.58. This range met the physiological pH range of the human's mouth, between 5.5-7.9 (Zhai *et al*., 2018).

The more wuluh starfruit juice is added, the lower the pH value. This was because unripe wuluh starfruit juice's pH was 1.99. The decrease in pH value in edible film is in line with the increase in moisture content, which is also caused by the high moisture content of wuluh starfruit juice. The lower the pH, the higher the moisture content. Another cause is that more acidic conditions have high reactivity, and the affinity for other compounds also increases, especially compounds with hydroxyl groups such as H2O. The more water that is bound in the matrix system, this has an impact on the water activity of the edible film (Santoso *et al*., 2014).

3.4. Thickness

The diversity analysis indicated that the different treatments of wuluh starfruit juice in making the chitosan-PVA edible film had significantly different effects on thickness. The thickness of the edible film ranges from 0.22 to 1.62 mm, as presented in Table 3. Table 3 shows that the more wuluh starfruit juice is added, the more thickness increases. The thickness of treatment A was 0.22 mm. After the addition of wuluh starfruit juice in treatment B, the thickness decreased to 0.46 mm. The more wuluh starfruit juice is added, the higher the thickness value to 1.62 mm (Treatment G).

The thickness of edible film is a crucial factor because it can impact the durability of a food product. The thickness is also related to water absorption or water vapour transmission rate (Sinaga *et al*., 2013). The transmission rate of water vapour, gases, and other physical characteristics, such as tensile strength and elongation, can be influenced by the thickness. The more wuluh starfruit juice was added, made the film thicker. This occurs due to the high moisture content in wuluh starfruit, which causes an increase in the thickness.

Thickness is the physical property of the film, which determines other properties such as water vapour transmission rate, tensile strength, and elongation (Fera & Nurkholik, 2018). The thicker the edible film, the stronger the barrier properties or ability to withstand water migration because the structure is denser. The optimal edible film for the application is expected to have a thickness of 0-5 mm. The edible film that is too thin will cause lower tensile strength, elongation, and water vapour transmission rate, while the edible film that is too thick will disrupt the appearance of the packaged food product.

Table 3. Physical characteristics of edible film from several treatments with the wuluh starfruit juice addition

Treatments	Parameters			
	Thickness (mm)	WVTR (g/m^2/day)	Tensile Strength (kgf/cm^2)	Elongation (%)
A	0,22 a ± 0,02	89,29 a ± 1,62	29,39 a ± 0,58	49,26 a ± 2,33
B	0,46 b ± 0,10	82,63 b ± 1,80	26,06 ab ± 0,40	44,66 b ± 6,02
C	0,55 bc ± 0,17	60,01 c ± 2,28	22,29 b ± 1,16	44,47 b ± 4,76
D	0,65 c ± 0,01	48,62 d ± 3,10	18,47 c ± 1,13	42,15 c ± 3,08
E	0,92 d ± 0,02	38,99 e ± 1,84	12,96 d ± 0,21	40,92 d ± 3,34
F	1,14 e ± 0,05	31,75 f ± 1,50	11,38 e ± 0,39	38,89 e ± 4,76
G	1,62 f ± 0,04	24,63 g ± 1,12	10.72 f ± 0,26	35,80 f ± 2,13

^{a,b,c}Means within a row with different superscript letters are significantly different between treatments

3.5. Water vapour transmission rate

The diversity result analysis displayed that the different treatments of wuluh starfruit juice in making the edible film had very significantly different effects on the water vapour transmission rate. The water vapour transmission values of edible films are presented in Table 3.

Table 3 shows that the more wuluh starfruit juice is added, the more water vapour transmission decreases. Treatment A's water vapour transmission was 89.29 g/m2/day. After the addition of wuluh starfruit juice in treatment B, the water vapour transmission decreased to 82.63 g/m2/day. The water vapour transmission value further decreased as the amount of starfruit juice was increased to 24.63 g/m2/day (Treatment G).

One of the requirements for good packaging is a low water vapour transmission rate (Cheng & Cui, 2021; Li *et al.*, 2022). Low water vapour transfer value makes the product more stable and has a longer shelf life. With a higher water vapour transmission rate, the film matrix can be more easily penetrated by water vapour from outside. This will interfere with preservation due to the addition of water to the packaged food.

The smaller the water vapour transmission rate in products packaged with edible film, the lower the humidity on the surface of the packaged food product. So, the product became more resistant to the growth of microbacteria. The water vapour absorption by the film is also in line with the thickness (Ghoshal & Kaur, 2023). The thicker the film, the lower the water vapour transmission rate. This statement was proven from the data in Tables 1 and 2.

3.6. Tensile strength and elongation

The diversity results analysis indicated that the different treatments of wuluh starfruit juice in making the film had a very significantly different effect on the tensile strength values. The tensile strength of edible films is shown in Table 3.

Table 3 shows that the tensile strength decreases when more wuluh starfruit juice is added. As a control, treatment A had a tensile strength value of 29.39 kgf/cm2. In treatment B, the tensile strength decreased to 26.06 kgf/cm2 and further decreased to 10.72 kgf/cm2 in treatment F.

Based on Table 3, the tensile strength test results of chitosan-PVA edible were inversely proportional to the addition of wuluh starfruit juice. This statement was inversely proportional to research which experienced an increase in the tensile strength of chitosan edible film with avocado seed starch (Susilowati & Lestari, 2019). The more chitosan added, the tensile strength value increased. As a blending biopolymer, chitosan increases tensile strength in specific formulations by forming hydrogen bonds, making the edible film tighter. Tensile strength was directly proportional to the chitosan added. The greater the percentage of chitosan, the tensile strength value would tend to increase (Setiani *et al.*, 2013; Widodo *et al.*, 2019).

In this study, the treatment was the addition of wuluh starfruit juice as an antimicrobial, not the primary raw material such as chitosan. The addition of wuluh starfruit juice, which contained high moisture content (reaching 94.78 %), decreased tensile strength. The presence of large amounts of water caused the texture of the edible film to become softer. This tensile strength decrease is supported by research which states that the antimicrobials addition causes a decrease in the tensile strength compared to control treatment (Syaichurrozi *et al.*, 2012). The added antimicrobials make the film matrix inhomogeneous and unstable (Kechichian *et al.*, 2010). This inhomogeneity causes the film to be less able to accept the forces imposed on the film.

Elongation means the change of percentage in length when the film is pulled until it breaks or its ability to elongate before breaking (Rusli *et al.*, 2017). The diversity analysis showed that the different treatments of wuluh starfruit juice in making the chitosan-PVA edible film had a very significantly different effect on elongation. Edible film elongation is presented in Table 3.

From Table 3, it can be observed that increasing the wuluh starfruit juice addition causes the elongation of edible film to decrease. For treatment A, the elongation value was 49.26 %. The more wuluh starfruit juice was added, the elongation value of the chitosan-PVA edible film decreased to 35.80 % in treatment G.

Based on Table 3, the elongation decreased with the increase in wuluh starfruit juice added to the edible film. This decrease in elongation was in line with the tensile strength decrease. This decrease was because of the citric acid compound in wuluh starfruit. The increase in the concentration of citric acid made the hydrophilic nature of the edible film increase and had an impact on reducing the elongation.

Films made from pure chitosan had a higher tensile strength than chitosan mixed with other ingredients, but the elongation was lower (Purwanti, 2010). This statement aligns with the results obtained in this study, where treatment A obtained the highest elongation, which was a control treatment without the addition of wuluh starfruit juice.

Tensile strength and elongation are mechanical properties that play a role in determining the strength and flexibility of the film (Rahmadi Putri *et al.*, 2023). The choice of film-forming material determines the balance of these two characteristics to maintain food integrity during storage (Farajpour *et al.*, 2020; Malik *et al.*, 2022). Therefore, treatments A, B, and C were optimal treatments observed from the perspective of tensile strength and elongation values.

3.7. Antimicrobial activity

The antimicrobial activity test on chitosan-PVA edible film was performed to determine the addition of wuluh starfruit juice effect in inhibiting the spoilage microbes' growth (*Escherichia coli* and *Staphylococcus* aureus). Table 4 shows the effect of wuluh starfruit juice addition on the antimicrobial activity of chitosan-PVA film.

From the table above, it can be seen that the average antimicrobial activity of chitosan-PVA edible film against *Escherichia coli* and *Staphylococcus aureus*-bacteria in various treatments of wuluh starfruit juice ranged from 0.33-1.52 mm. Adding 2% wuluh starfruit juice (Treatment B) increased the antimicrobial activity compared to the control (Treatment A). In the table, it can also be seen that the highest antimicrobial activity was in treatment G at 1.52 mm, and the lowest antimicrobial activity was in treatment A at 0.14 mm.

The greater the antibacterial activity, the stronger the antibacterial ability of a material. The antimicrobial activity value of the chitosan-PVA edible film against spoilage microbes such as *Escherichia coli* and *Staphylococcus aureus*was higher as the concentration of wuluh starfruit juice increased. Wuluh starfruit contains saponins, flavonoids, and tannins as antibacterial (Kambaya *et al.*, 2021).

Wuluh starfruit plant can inhibit the development of *Staphylococcus aureus*on leaves and fruit (Liantari, 2014). It also contains tannins, which can damage bacterial cell membranes and cause intracellular leakage (Smullen *et al.*, 2007). In addition, certain chemicals, especially triterpenoids, have the ability to interact with porins (transmembrane proteins) in bacterial cell walls, producing strong polymer bonds that damage the porins. By reducing the permeability of bacterial cell walls and removing nutrients, damage to porins, which serve as entry and exit points for chemicals, will cause bacteria to stop growing or die.
High antibacterial activity will make it difficult for spoilage microbes, in this study *Escherichia coli* and *Staphylococcus aureus*bacteria, to grow and develop. Thus, the preservation and safety of food packaged

Table 4. Antibacterial activity of edible film from several additions of wuluh starfruit juice

Treatments	**Antibacterial activity (mm)**
A (0% wuluh starfruit)	0,14
B (2% wuluh starfruit)	0,33
C (4% wuluh starfruit)	1,28
D (6% wuluh starfruit)	1,31
E (8% wuluh starfruit)	1,39
F (10% wuluh starfruit)	1,45
G (12% wuluh starfruit)	1,52

with edible film is better.

4. Conclusions

Differences in the addition of wuluh starfruit juice had an influence on the physicochemical and antimicrobial characteristics of the chitosan-PVA edible film. In addition, a concentration of 2 to 12 % wuluh starfruit juice could inhibit the growth of *Escherichia coli* and *Staphylococcus* bacteria. The concentration of 4 % wuluh starfruit juice (Treatment C) with a value of 1.28 mm was the most optimal treatment because the antimicrobial activity in this treatment increased most significantly compared to other treatments. The physicochemical characteristic values of treatment C were moisture content 17.29 %, pH 6.23, solubility 42.79 %, thickness 0.55 mm, water vapour transmission 60.01 g/m2/day, tensile strength 22.29 kgf/cm2, and elongation 44.47 %.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the research; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgment

We would like to thank the Directorate of Research, Technology and Community Service (DRTPM), Ministry of Education, Culture, Research and Technology of the Republic of Indonesia as the funder based on contract numbers: 002/LL10/PG.LJT/2023 and KPt/001/ LPPM-UNES/IV/2023. We would also like to thank the Chancellor of Ekasakti University, the Chair of the Institute for Research and Community Service at Ekasakti University, the Dean of the Faculty of Agriculture, Ekasakti University, our team and the students who contributed to this research.

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