



Antimicrobial Efficacy of Xylitol-Incorporated Gelatin Edible Films Against *Streptococcus mutans*: A Novel Approach for Healthy Gummy Products

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Abstract. This study aimed to develop a healthy xylitol-based edible film for gummy products with optimal antibacterial properties. Using a Completely Randomized Design (CRD), the experiment tested four xylitol concentrations (3.6%, 3.9%, 4.2%, and 4.5% w/v) with three replications per treatment. Data were analyzed using analysis of variance (ANOVA) with significant differences ($P < 0.05$ or $P < 0.01$) further examined through Duncan's Multiple Range Test. The films were evaluated for antibacterial activity (inhibition zone), water content, water activity, and microstructural properties via optical microscopy. Results revealed that the 4.2% (w/v) xylitol concentration yielded optimal properties, demonstrating an inhibition zone of 12.87 mm, water content of 2.31%, and water activity of 0.87. Optical microscopy confirmed superior structural integrity, showing a smooth surface with minimal bubble formation and clear appearance. These findings suggest that 4.2% xylitol concentration produces edible films with ideal physicochemical and antibacterial properties for application in healthy gummy product manufacturing.

Keywords: Betalain, Gelatin, Healthy Gummy, Xylitol.

1 Introduction

Streptococcus mutans bacteria are flora that are commonly found in the oral cavity. *Streptococcus mutans* causes dental caries. Dental caries is defined as an infectious disease of the oral cavity that occurs due to infection by microorganisms and results in demineralization, resulting in dissolution, and damage. The initial process of dental caries is the appearance of signs of increased activity of microorganisms in the oral cavity [1]. In addition to the increased activity of *Streptococcus mutans*, dental caries can arise from sweet foods. People who consume sweet foods need to brush their teeth regularly because if they do not brush their teeth regularly, sticky glycoproteins will stick, and nest on the teeth. This will form dental plaque and at the same time there are millions of *Streptococcus mutans* bacteria attached to the glycoprotein [2]. Other causes of tooth decay can also occur because someone likes to consume cariogenic foods [3]. Some foods that are included in the cariogenic category include added sugar, candy, chips, chocolate, chocolate milk, cakes, fruit-flavored drinks, honey,

ice cream, biscuits, and tea. Fast food is also classified as a food that has the potential to be cariogenic because it is accompanied by seasonings containing sugar [4].

Implementation of innovation needs to be done to prevent tooth decay in children due to consuming cariogenic foods. Healthy gummy xylitol based on edible film is an action that can be taken to prevent tooth decay because of the use of xylitol as a natural sugar substitute that has been approved by the FDA in 1963 that xylitol can be used as a nutritional additive. Studies have reported that consumption of xylitol (5-10 g/day) reduces caries by between 30% and 80% [5]. The formulation of making healthy gummy requires other ingredients as antioxidant compound and natural dyes, one of the ingredients that can be used as a dye is red dragon fruit peel. The peel of red dragon fruit can be used as a natural dye because it contains color pigments, namely betalain. In addition, the skin of the red dragon fruit contains many antioxidants. These pigments dissolve in water so that they can be extracted with polar solvents [6]. The development of betalain and xylitol as antimicrobials, especially *Streptococcus mutans*, requires a delivery system in the form of edible film.

Edible film is a thin layer made of hydrocolloid, fat, and composite materials. Edible film contains xylitol as a delivery system that is able to release xylitol gradually which functions as an anti-*Streptococcus mutans* which acts as protection against oxygen, reduces the rate of water evaporation, improves the appearance of the product, and can be used as carrier of antioxidant or antibacterial compounds that can protect the product, and inhibit microbial growth [7]. Gelatin is a substance sourced from protein with levels of 85-92%. Gelatin has water-soluble properties and is able to form a gel, able to form an elastic thin layer, forming a transparent, and strong packaging [8]. Xylitol combined with betalain and gelatin is expected to produce healthy gummy xylitol based edible film that has anti-*Streptococcus mutans* activity with chewy, elastic, not easily torn characteristics, and high resistance to oxygen and carbon dioxide so as to maintain the quality and shelf life of packaged products and have attractive colors. Based on the description above, further research is needed on the application of healthy gummy based on edible film with xylitol, gelatin and betalain in terms of inhibitory zone, water content, water activity and optical microscopy.

2 Materials and Methods

2.1 Material and Equipment

The ingredient used in this research were xylitol (Soho) from the Online Market (Shopee), gelatin (Hakiki) obtained from a food store in Malang, dragon fruit peel extract (*Hylocereus polyrhizus*), chemicals obtained from the Chemical Shop in Malang such as aquades (Hydrobatt), nutrient broth (Himedia), nutrient agar (Himedia), spiritus and *Streptococcus mutans* isolate (Agavilab) obtained from the Online Market (Tokopedia).

The equipment used in this research includes digital scale (Taffware Digipounds I-2000), optical microscope (Olympus CX 21 FS 1), hot plate (SH-2), object glass, cover glass, measuring cup (100 mL) (Iwaki), measuring cup (10 mL) (Pyrex), beaker

glass (100 mL) (Duran), Erlenmeyer (250 mL) (Iwaki), filter paper, blue tip, test tube (Iwaki), bunsen, thermometer (30 cm), filter cloth, petri dish, analytical scale (BC Series OHAUS Centrogram Balance), spatula spoon, aluminum foil, magnetic stirrer (3 cm), oven (Memmert), refrigerator (Polytron SCN-140L), silicone mat (3x3.9x1cm), measuring pipette (Iwaki), filler, cotton, rubber bands, test tube rack, petromax plastic, autoclave (Hirayama HL 36 AE), desiccator, and Aw meter (Landtek WA-60A).

2.2 Research Method

This study employed a Completely Randomized Design (CRD) to evaluate the effect of xylitol concentration on edible film properties. Four xylitol concentrations were tested T1 (3.6% w/v), T2 (3.9% w/v), T3 (4.2% w/v), and T4 (4.5% w/v), with each treatment replicated three times. All data underwent analysis using one-way analysis of variance (ANOVA). Significant differences detected ($P < 0.05$) prompted further examination through Duncan's Multiple Range Test (DMRT) to identify specific differences between treatment means. This statistical approach provided a robust framework for determining the optimal xylitol concentration for antimicrobial edible film formulation.

2.3 Extraction Process of Dragon Fruit Peel

This method employed Microwave Assisted Extraction (MAE) to extract bioactive compounds from dragon fruit peel (*Hylocereus polyrhizus*) using distilled water as the solvent, with modifications to the protocol described by [9]. The extraction procedure began with sample preparation of red dragon fruit was peeled, and 50 g of the peel was cut into small uniform cubes. The prepared peel samples were transferred to a 250 mL Erlenmeyer flask, followed by the addition of 100 mL distilled water. The mixture was then subjected to microwave irradiation at 100°C for 1 h. To prevent overheating and maintain temperature below 100°C, the microwave operation followed an intermittent pattern with a 2-min cooling interval at the 30th min of extraction. After the extraction period, the mixture was filtered through a filter cloth. The resulting filtrate was collected as the final dragon fruit peel extract for subsequent analysis.

2.4 Preparation of Healthy Xylitol Gummy

The preparation of healthy xylitol gummy followed a modified protocol based on [10]. Initially, 8 g of gelatin was dissolved in 30 mL of distilled water. To this solution, 10 mL of dragon fruit peel extract was added. The mixture was then heated and homogenized using a hot plate-magnetic stirrer. Xylitol was incorporated at various concentrations (3.6, 3.9, 4.2, and 4.5% w/v) according to the experimental design. The xylitol was added gradually with continuous stirring to ensure uniform distribution and prevent lump formation in the edible film solution. Subsequently, the solution was filtered through a filter cloth to remove any remaining particulates. For

gummy formation, 10 mL of the prepared edible film solution was measured using a measuring cup and poured into silicone mat. The gummies were allowed to dry at room temperature for 24 hours. Once set, the gummies were removed from the silicone mat and stored in sealed plastic bags for further analysis.

2.5 Inhibitory Zone Test

The inhibitory zone analysis procedure with some modifications on the number of inhibitory holes and healthy gummy solution [11]. Inhibitory zone analysis begins by take 15 mL of nutrient agar solution at a temperature of 50°C. Pour it into a petri dish aseptically. Take 1 mL of bacterial isolate using a measuring pipette. Allow it to solidify as a base layer. Make 4 holes using a blue tip. Pour 0.5 mL of health gummy solution into the whole hole. Leave it to solidify as a flat layer. Incubate at 37°C for 24 h. Measure the diameter of the inhibition zone using the formula:

$$\text{Inhibitory zone (\%)} = \frac{(dv-dh)-(do-dh)}{2}$$

Remarks:

dv = Diameter vertical

dh = Diameter hole

do = Diameter horizontal

2.6 Water Content Test

The water content analysis procedure with some modifications on the size of the samples and the length of oven time for the samples [12]. Analysis of the water content begins by take one healthy gummy. The healthy gummy was weighed using an analytical scale. The petri dish was dried in an oven for 30 min at a temperature of 100-105°C. The dish was cooled in a desiccator for 30 min. Weighed when the dish was cold. The healthy gummy sample and filter paper were put into the dish. Dried in an oven at a temperature of 100-105°C for 30 min until it reached a constant weight. The dish was cooled in a desiccator for 30 min. The healthy gummy was weighed after drying. The water content was calculated using the formula:

$$\text{Water content (\%)} = \frac{\text{Initial sample weight(g)} - \text{Final sample weight (g)}}{\text{Initial sample weight(g)}} \times 100\%$$

2.7 Water Activity Test

Water activity (A_w) was determined using a water activity meter following a modified method described by [13]. The sample was placed in a plastic container, ensuring complete coverage of the container surface. The container with the sample was then positioned in the sample test chamber and sealed with a sample reader sensor. Prior to measurement, the instrument display was verified to show "reset" status, indicating readiness for operation. The analysis was initiated by pressing the enter button, which

changed the status from "reset" to "dwell," signifying the beginning of the acclimatization process. Subsequently, the instrument status changed to "running" during measurement, accompanied by an audible signal. Completion of the analysis was indicated by another audible signal and a status change to "stop," with a corresponding checkmark on the display. The measured sample was then removed from the test chamber, and the water activity value was recorded.

2.8 Optical Microscopy Test

Analysis of the optical microscopy begins by take one healthy gummy One healthy gummy sample was placed on an object glass. Covered with a cover glass. Observed using an optical microscope with a magnification of 4x. Photos were taken as documentation of the observation results using a camera. Observed using an optical microscope with a magnification of 10x. Photos were taken as documentation of the observation results using a camera [14].

3 Results and Discussion

The result of the research of healthy gummy xylitol based on edible film gelatin can be seen in Table 1.

Table 1. The average value of healthy gummy xylitol based on edible film gelatin..

Treatment	Inhibitory Zone	Water Content	Water activity (Aw)
T0	12.25 ± 2.69	2.60 ± 0.21	0.90±0.01 ^a
T1	8.08 ± 1.13	2.65 ± 0.03	0.87±0.01 ^{ab}
T2	12.87 ± 3.52	2.31 ± 0.16	0.87±0.01 ^b
T3	12.79 ± 1.01	2.69 ± 0.14	0.88±0.01 ^c

Remarks: ^{a, b, c} Different superscripts in the same column indicate significant differences ($P < 0.05$).

3.1 Antibacterial Activity

Based on the analysis results in Table 1 shows that the inhibitory zone of healthy gummy xylitol based on edible film gelatin from T1 to T4 decreased at the different percentages did not show any significant differences ($P > 0.05$). The data of the inhibitory zone of *Streptococcus mutans* T1, T2, T3 and T4 produced an average value of 12.25 ± 2.69, 8.08 ± 1.13, 12.87 ± 3.50 and 12.79 ± 1.01. The highest average value of the inhibitory zone of *Streptococcus mutans* was found in the xylitol treatment T3 (4.2%) which was 12.87. The lowest average value of the inhibitory zone of *Streptococcus mutans* was found in the T2 treatment (3.9%) which was 8.08. The resulting difference in inhibitory zones occurs due to the number of *Streptococcus mutans* growing in each petri dish is uneven. The difference between the diameter of the in-

hibitory zone that appears proves that the effect of antibacterial activity is still in the moderate and strong range. The difference in inhibition zones is caused by the amount of extract absorbed on filter paper and the elimination of *Streptococcus mutans* due to uneven agar media in each part [15].

The lowest inhibition zone value was found in the use of xylitol treatment T2 of 3.9%, which was 8.08 mm in diameter. This can be interpreted as a treatment with a moderate inhibition value. The low inhibition zone value occurs due to the thickening or viscosity process. The higher the viscosity value, the lower the diffusion process. This results in a low diffusion process into the agar medium so that it can reduce the inhibition zone value. This also occurs because antibacterial substances do not kill all bacteria, they only stop their growth [16]. The highest inhibition zone value occurred in the use of xylitol treatment P3 of 4.2%, namely a diameter of 12.87 mm. This indicates that the betalain compounds contained in the red dragon fruit skin that have been combined with xylitol and betalain are still good so that the active antioxidant substances can help xylitol in inhibiting *Streptococcus mutans* bacteria and produce an inhibition zone diameter that is in the strong range. The growth of *Streptococcus mutans* is disrupted due to the combination of xylitol and good betalain compounds. Xylitol is known to inhibit the growth of *Streptococcus mutans* because it has toxic xylitol properties. Xylitol has toxic properties because it cannot be fermented by *Streptococcus mutans* [17].

3.2 Water Content

Based on the analysis results in Table 1 shows that water content of healthy gummy xylitol based on edible film gelatin from T1 to T4 decreased at the different percentages did not show any significant differences ($P>0.05$). The water content data in T1, T2, T3 and produced average values of 2.60 ± 0.21 , 2.65 ± 0.03 , 2.31 ± 0.16 and 2.69 ± 0.14 . The highest average water content value was found in the xylitol T4 treatment (4.5%) which was 2.69. Meanwhile, the lowest average water content value was found in the T3 treatment (4.2%) which was 2.31. At T1 and T2, as xylitol was added, there was an increase in the water content. However, there was a significant decrease in T3 and an increase in the water content value in T4, so that the results for the most optimal use were 4.2%. This is because xylitol is in the form of a white crystalline powder that is soluble in water, so it can increase the volume of the water itself. Xylitol is also a compound that has the ability to bind to water (hydrophilic) and can bind water molecules (hygroscopic). The addition of the concentration of xylitol can increase the water content in it because xylitol has the ability to absorb water [18].

In the results of T3 treatment results, the water content decreased. This is due to the material used in the manufacture of the product in the form of xylitol. Xylitol is a solid material that can be dissolved in water which is used as a component in the manufacture of healthy gummy solutions. This causes the formation of hydrogen bonds between molecules of the healthy gummy components. These hydrogen bonds cause the free water contained in the healthy gummy xylitol product to decrease [19].

3.3 Water Activity

Based on the results of the variety above, it is known that the administration of xylitol with different percentages shows a real difference in each treatment of treatment ($P < 0.05$). Data on water activity results at T1, T2, T3 and T4 produced average A_w values of 0.90 ± 0.01 , 0.87 ± 0.01 , 0.87 ± 0.01 and 0.88 ± 0.01 . The highest average value of water activity was found in the treatment of xylitol T1 (3.6%), which was 0.90. Meanwhile, the lowest average value of water activity was found in the T3 treatment (4.2%), which was 0.87. The results of this water activity test are classified as high because of the different additions of xylitol. At T1 to T3, there was a significant decrease. However, at T4, there was an increase in the water activity value again. The optimum use of xylitol was 4.2% (T3). The high water activity value is because xylitol is a humectant. Humectant means a material that can bind water or is hygroscopic so that it can bind the water contained in a product. Humectants can also reduce the water activity value and can be used as a plasticizer [20]. High water activity values in a material can result in rapid damage to the material due to microorganisms such as fungi, yeast and bacteria.

The value of this water activity indicates the results that can cause various microorganisms to grow in the product because the value of the water activity results is in the range of 0.87-0.90. This can be interpreted that the edible film-based healthy gummy xylitol product is included in high-risk products because it has a water activity value of > 0.85 and is wet. Products that have a high water activity value of > 0.9 are products that are easily damaged because they can produce various microorganisms. The amount of water activity value contained in a material can affect the characteristics of the resulting product, such as edible film-based healthy gummy xylitol. The minimum water activity value that can produce microorganisms is 0.90 in bacteria, 0.80 to 0.90 in yeast and 0.60 to 0.70 in mold [21].

The lowest water activity value was found in the use of xylitol in the T3 treatment of 4.2%, which was 0.87. This is because the use of xylitol can increasingly suppress water activity in healthy xylitol gummy based on edible film. The decrease in water activity in T3 occurs because xylitol is hygroscopic, which means that the high amount of bound water will reduce the free water content. Xylitol has many hydroxyl groups that bind to water so that the more water is bound, the free water content will decrease and affect the water activity value [22]. compounds that have many hydroxyl groups result in the OH group that binds to water will increase the water binding capacity. The freer water content that is bound, the lower the water activity value of the edible film. The use of xylitol can suppress water activity but remains wet, this is because the process of making healthy gummy xylitol products based on edible film requires a lot of water because it is used to dissolve the ingredients to create a homogeneous healthy gummy xylitol solution based on edible film without leaving any sedimentation residue. A small water activity value has an effect on the length of shelf life because the growth of microorganisms such as bacteria, molds and yeasts appears at high water activity values starting from 0.8 to 0.9 [23].

3.4 Optical Microscopy

Microscopy testing performed on edible film-based healthy gummy xylitol samples using optical microscopy with 4x and 10x magnification. The use of this preparation uses one gummy sample which is then placed on a glass object. This microscopic test aims to determine the presence of bubbles whether or not they are created in each sample and can be a reference for the resulting healthy gummy solution is homogeneous or not. The results of optical microscopy testing of healthy gummy xylitol-based edible film looked slightly different from each treatment.

In the optical microscopy test results of the T4 treatment (4.5%), it can be seen that only in this treatment there are many bubbles and are spaced apart. This is because the addition of xylitol can cause the formation of hydrogen bonds. Its increasingly dense structure can cause the distance in the membrane to become denser so that the hydrogen bonds between the chains become denser and have an impact on the tensile strength value of the healthy gummy. However, in this treatment it is known that the presence of bubbles is created because the solution used in the manufacture of healthy xylitol gummy based on edible film has not been mixed perfectly, resulting in non-uniform bubbles and spaces. This is different from the results of the optical microscopy test of the T2 treatment (3.9%) which obtained results with a smooth surface and a clear color. This is because the healthy gummy xylitol solution based on edible film has been perfectly mixed so that it does not cause bubbles. The addition of xylitol to each treatment gives clear surface results. The perfect mixing of the solution gives clear and smooth results for the product to be tested [24]. The optical microscopy results can be seen in Fig 1.

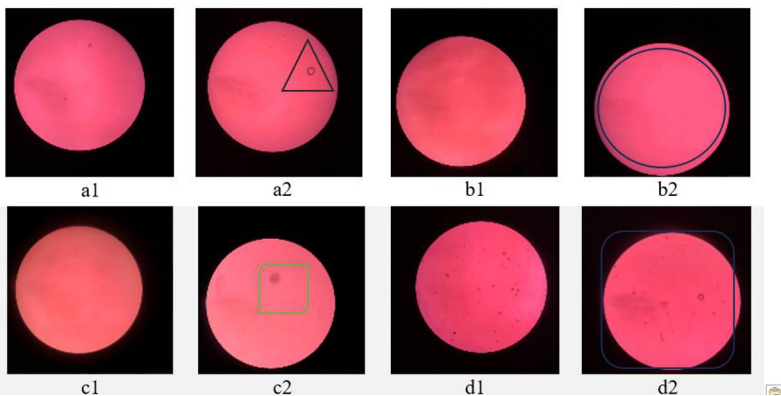


Fig. 1. Optical microscopy display of all samples each treatment. The indicator 1 for magnification 4 \times and 2 for magnification 10 \times .

In the optical microscopy test results of healthy gummy xylitol based on edible gelatin film T3 treatment (4.2%) it was found that the addition of xylitol as much as 4.2% can produce a smooth surface with a slightly faded color. This happens because there is a bond with the amount of water content contained in the product. The more water that is bound, the free water content will decrease. This happens because xylitol

has hydrophobic properties so that this polymer causes a bond between xylitol and gelatin to have a stronger strength in binding water [25]. The water content value can change the reflection of light on the surface of the film so that this causes a decrease in color, resulting in the color of the product not being too red [26]. This occurs due to the light being reflected by the water so that the color that appears is not too thick. The moisture content within an edible film significantly influences its visual appearance, particularly its color intensity. Elevated or reduced water content can lead to diminished color vibrancy due to light scattering and reflection caused by water molecules within the film matrix. As a result, the perceived color becomes less saturated and appears faded [27].

4 Conclusion

This study established that xylitol concentration significantly influences the properties of gelatin-based edible films in healthy gummy formulations. The optimal formulation was achieved with 4.2% (w/v) xylitol incorporation, exhibiting superior characteristics compared to other concentrations tested. This optimal formulation revealed enhanced antimicrobial efficacy against *Streptococcus mutans* with an inhibition zone of 12.875 mm, favorable moisture characteristics (water content of 2.31%, water activity of 0.873), and excellent structural properties as evidenced by optical microscopy, which revealed minimal bubble formation, smooth surface texture, and clear appearance. These findings provide valuable insights for the development of functional, antimicrobial edible films with potential applications in the confectionery industry, particularly for products targeting oral health benefits.

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