

Research Article

Optimization of Polyvinyl Alcohol (PVA) and Xanthan Gum Edible Film Saffron (*Crocus sativus* L.) as Antibacterial against *Staphylococcus aureus*

Prihandi Surya Samudra*, Lilies Wahyu Ariani, Intan Martha Cahyani

College of Pharmaceutical Sciences, Yayasan Pharmasi Semarang, Jl. Letjend Sarwo Edie Wibowo KM 1 Plamongsari-Pucanggading, Semarang 50193, Jawa Tengah, Indonesia

*Corresponding author: Prihandi Surya Samudra | Email: handisurya67@gmail.com

Received: 15 September 2022; Revised: 20 November 2022; Accepted: 6 December 2022; Published: 31 December 2022

Abstract: *Staphylococcus aureus* is a normal bacteria flora in the oral cavity and often causes infections. Saffron (*Crocus sativus* L.) contains flavonoids that can inhibit the growth of *Staphylococcus aureus*. Saffron extract is formulated into edible film preparations because it controls drug release and maintains active substances, especially antibacterial ones. This study aims to determine the effect of comparing PVA and xanthan gum concentrations on edible film preparation physical characteristics and antibacterial activity. Also, to find out the optimal formula. This study uses the Simplex Lattice Design method and Design Expert to determine the concentration ratio. The optimization parameters of the preparation included pH, soluble time, water absorption, and antibacterial activity test using the well diffusion method. The ANOVA results showed that adding PVA concentration accelerated the dissolution time and increased the antibacterial inhibition. The addition of xanthan gum concentration increased the pH value and water absorption. The test results obtained a pH of 5.52, dissolution time of 22.25 seconds, water absorption capacity of 43.95%, and antibacterial inhibition of 0.53 cm. The optimal formula was received at the concentration of PVA at 5.59% and xanthan gum at 0.41%. The T-test shows that the equation of each optimal parameter is valid.

Keywords: Antibacterial against *Staphylococcus aureus*; Edible film; Optimization; PVA; Xanthan gum;

1. INTRODUCTION

Saffron (*Crocus sativus* L.) contains secondary flavonoids, terpenoids, anthocyanins, and carotenoid metabolites. Flavonoid compounds are compounds synthesized by plants as a defense system and respond to infection by microorganisms. Saffron (*Crocus sativus* L.) has an antibacterial activity of *Staphylococcus aureus* at a concentration of 1000 g/petri with an inhibitory diameter of 15 ± 0.14 mm [1].

Staphylococcus aureus is a normal flora bacteria in the oral cavity and can be pathogenic and cause infection. These bacteria enter through food and occupy different parts or surfaces in the oral cavity, which often causes disease in the mouth. The infection caused by this bacterium is the presence of signs such as inflammation, necrosis, and abscess formation [2].

The edible film is a thin layer of hydrophilic material made from proteins, carbohydrates, and fats or their mixtures [3]. The edible film is divided into three main components: hydrocolloids, polysaccharides, and composites [4]. One example of hydrocolloid is polyvinyl alcohol (PVA) which has good film-forming properties and chemical resistance. Still, PVA in edible films has high moisture sensitivity and water absorption, so it is necessary to have a crosslink, one of which is 0.5% citric acid which can support the formation of polysulfone membranes, increasing the mechanical properties and film stability [5]. Adding polysaccharides in edible films also increases the preparation's strength and antibacterial ability [6]. Xanthan gum is a polysaccharide material that can quickly return to its original shape and maintain the consistency of the preparation so that it is easy to pour, spread evenly, and form an even layer. In addition, it has a good appearance and spreadability and can be mixed with many pharmaceutical ingredients to improve the preparation's physical quality [7].

Based on the description above, in this study, researchers are interested in carrying out the physical characteristics of edible film preparations, including pH, soluble time, and water absorption. Testing of antibacterial activity on each run formula (I-VIII) and the optimal formula of the trial against *Staphylococcus aureus* to determine the ability of edible film preparations as antibacterial. Optimization of PVA and xanthan gum to determine the optimal procedure for manufacturing edible film preparations.

2. MATERIALS AND METHODS

2.1. Materials

The ingredients for the extract are saffron (*Crocus sativus* L.) and 96% ethanol. The ingredients for edible films are PVA, xanthan gum, citric acid, saccharin sodium, sorbitol, peppermint oil, menthol, and aquadest. For testing, the antibacterial inhibitory were *Staphylococcus aureus*, physiological NaCl 0.9%, Mueller Hinton agar media, McFarland reagent, artificial saliva, nutrient agar media, and Nutrient Broth media.

2.2. Methods

Remaceration is one of the extraction methods with characteristic of soaked the sample with suitable solvent [8], which can be applied with sample saffron (*Crocus sativus* L.). Dried pistils of saffron (*Crocus sativus* L.) flowers were weighed and soaked using ethanol 96% using ratio of 1:50. The soaking process was carried out during for five days. After the soaking process, filtrated the product which aims to separate the filtrate and liquid extract. The liquid extract was evaporated using a rotary evaporator machine of 70°C until viscous extract is obtained.

In making an edible film extract ethanol of saffron (*Crocus sativus* L.), the PVA component is dispersed in several parts of distilled water and then heated on the free fire and stirred until a clear liquid gel is formed. In the second step, the xanthan gum component was developed in distilled water at a temperature of $\pm 70^{\circ}\text{C}$ using a hotplate. The two gels were mixed in a mortar, added citric acid and other ingredients (saccharin sodium, sorbitol, peppermint oil, and menthol), then distilled water and crushed until homogeneous and poured into a beaker glass. The gel mixture was degassed for 10 minutes and then poured into a glass mold (20X20X1 cm). Drying is carried out in an oven at 40°C for 1-2X24 hours. After drying, the edible film is allowed to stand for 10-15 minutes, peeled off from the glass, and cut to a size of 2.2X3.2 cm [9].

The evaluation of edible film saffron (*Crocus sativus* L.) consists of pH, dissolution time, water absorption, and antibacterial inhibition of *Staphylococcus aureus*. The pH evaluation began with one stripe of edible film placed in a beaker glass and drabbled with aquadest until dissolved. Then, count the pH from the dissolved liquid with a pH meter [10]. The dissolution time evaluation started with weighing one stripe of edible film and then put into a beaker glass filled with factitious saliva as much as 10 milliliters. Observed and counted the time using a stopwatch until the edible film dissolved [11].

Table 1. Edible Film Formula Design Extract Ethanol of Saffron (*Crocus sativus* L.).

Ingredients	Run							
	I	II	III	IV	V	VI	VII	VIII
Extract Saffron (%)	8	8	8	8	8	8	8	8
PVA (%)	5	4	6	4.5	6	5	5.5	4
Xanthan Gum (%)	1	2	0	1.5	0	1	0.5	2
Citric Acid (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Saccharin Sodium (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sorbitol (%)	5	5	5	5	5	5	5	5
Peppermint Oil (%)	2	2	2	2	2	2	2	2
Menthol (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Aquadest Ad (%)	100	100	100	100	100	100	100	100

Water absorption evaluation used a stripe of the edible film, which was weighed (W_0) and then put into a beaker glass filled with aquadest for 10 seconds and weighed (W_1). Count the percentage of water absorption using the formula $(W_1 - W_0) / W_0 \times 100\%$ [12]. In antibacterial inhibition of *Staphylococcus aureus* evaluation, the first step is to inoculate the suspension of bacteria on Nutrient Broth media and hushed until dried. The well is made using cylinder cup metal. The dissolved edible film was then put into the well and incubated for 24 hours at 37°C. The inhibitory zone formed around the well counted using vernier calipers [13].

The test data for pH, dissolution time, water absorption, and antibacterial inhibition of *Staphylococcus aureus* for each run formula (I-VIII) were processed with Design Expert 10.0.1 software with Simplex Lattice Design method to obtain the optimal formula from the optimization of the two components, then made back with the same specs. The software optimization results were compared with the actual results using the T-test software SPSS 23 validity test. This test was used to determine the resulting formula and the formula in the software to obtain essential data (there or no significant difference) so that the formula used was valid.

3. RESULTS AND DISCUSSION

Extraction of saffron (*Crocus sativus* L.) by remaceration method obtained a result is 76.95%. The saffron extract obtained was subjected to a preliminary test of phytochemical screening and thin layer chromatography (TLC). The test results showed that the saffron extract contained secondary metabolites of flavonoids, tannins, alkaloids, terpenoids, and saponins.

3.1. Evaluation of Edible Film Saffron (*Crocus sativus* L.)

Table 2. Evaluation Results of Edible Film Saffron (*Crocus sativus* L.).

Test	Run							
	I	II	III	IV	V	VI	VII	VIII
pH	4.86	5.50	6.06	4.66	6.14	5.46	5.29	4.59
Dissolution Time (second)	30	33.7	12.7	37.3	12	28	26	30
Water Absorption (%)	77.21	147.03	19.71	92	36.46	88.71	41.94	154.74
Antibacterial Inhibitory (cm)	0.51	0.37	0.75	0.40	0.77	0.49	0.55	0.35

The test results on each optimization parameter were analyzed using Design Expert 10.0.1 software with the Simplex Lattice Design method. The effect of using PVA and xanthan gum on each response to saffron edible film preparations can be seen in Table 3 and Figure 1.

Table 3. Simplex Lattice Design Equation of Edible Film Saffron (*Crocus sativus* L.).

Response (Y)	Simplex Lattice Design Equation
pH	$Y = +1.01 (A) + 2.58 (B) - 0.53 (A) (B)$
Dissolution Time (second)	$Y = +2.09 (A) - 22.54 (B) + 8.62 (A) (B)$
Water Absorption (%)	$Y = +4.66 (A) + 118.54 (B) - 13.44 (A) (B)$
Antibacterial Inhibitory (cm)	$Y = +0.13 (A) + 0.25 (B) - 0.08 (A) (B)$

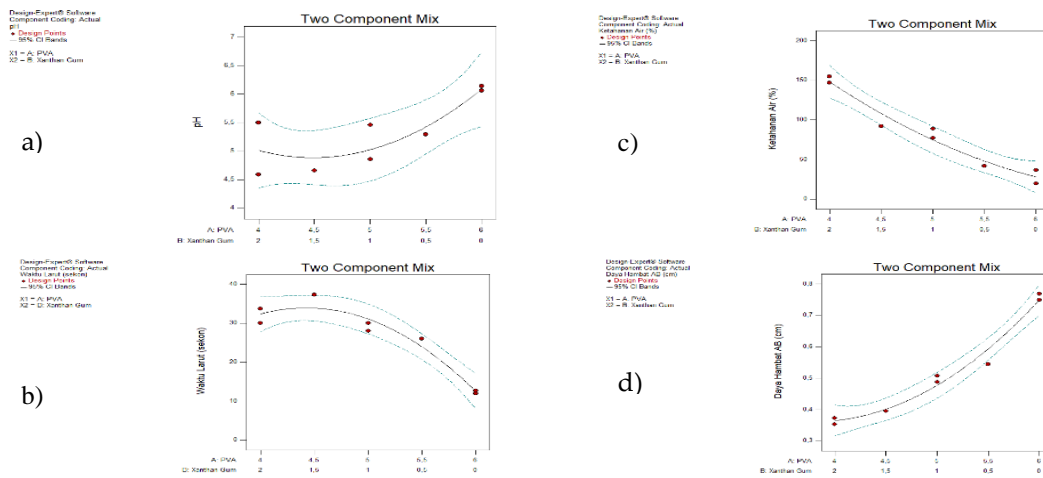


Figure 1. Profile of Physical Characteristics and Antibacterial Activity of Edible Film Saffron from Combination of PVA and Xanthan Gum Based on Simplex Lattice Design (a) pH; (b) Dissolution Time; (c) Water Absorption; (d) Antibacterial Inhibitory.

The pH response results on the xanthan gum showed an increase in the pH value compared to the PVA, which was due to the presence of D-glucuronic acid from xanthan gum so that the pH of the edible film increased or became more acidic [14].

The results of the dissolution time response on the xanthan gum showed an extension of time due to xanthan gum being a type of hydrocolloid with a rigid or tight molecular structure [15]. Increasing the concentration of xanthan gum in the preparation will increase the viscosity of the dispersion medium, thereby reducing the speed of dispersion of edible film particles [16]. In addition, the excessive use of xanthan gum will inhibit the interaction of xanthan gum with other polymers, which causes the hardening of the texture of the edible film layer [17].

The response to water absorption in the xanthan gum showed an influence in increasing water absorption compared to the PVA because xanthan gum was biopolymer or water soluble with an extreme and dense molecular chain conformation so that it absorbed more water [7]. The increased water absorption in edible film can be caused by adding xanthan gum concentration to bind water molecules through solid hydrogen bonds [18].

The results of the chart of the diameter response of the antibacterial inhibition of *Staphylococcus aureus* on the xanthan gum showed a decrease in antibacterial ability due to the mass of the preparation having a high viscosity resulting in the formation of a thick barrier layer [19]. After contact with the test media or MHA media, the edible film solution causes difficulties for the

active antibacterial substance to come out of the edible film, resulting in a small inhibitory zone diameter.

The optimal formula obtained was PVA at 5.59% and xanthan gum at 0.41%, with predicted results of pH 5.52, dissolution time of 22.25 seconds, water absorption capacity at 43.95%, and antibacterial inhibition is 0.53 cm. Equation validation with a One-Sample T-Test aims to validate the prediction equation on Design Expert obtained with experimental results. The optimal formula shows results that are not significantly different from theoretical results from the design expert so that the equations of each parameter are valid.

4. CONCLUSION

Comparison of the concentration of PVA and xanthan gum significantly affects the physical characteristics and antibacterial activity of *Staphylococcus aureus* edible film preparations of saffron (*Crocus sativus* L.) ethanol extract. PVA accelerates the dissolution time of edible film preparations and increases the antibacterial inhibition of *Staphylococcus aureus*. Xanthan gum has the effect of increasing pH and water absorption. The final result of this research concluded that a concentration of PVA 5.59% and xanthan gum 0.41% can produce edible film preparations of saffron ethanol extract (*Crocus sativus* L.) with optimal physical characteristics and antibacterial activity of *Staphylococcus aureus* including pH 5.52, dissolution time 22.25 seconds, water absorption is 43.95%, and antibacterial inhibition is 0.53 cm.

References

- [1] S. Muzaffar, T. A. Sofi, and K. Z. Khan, "Review Article Chemical Composition of Saffron : A Review," *Int. J. Biol. Med. Res.*, vol. 10, no. 4, pp. 6910–6919, 2019.
- [2] Y. T. Risky, A. Agrijanti, and N. Inayati, "Uji Screening Methicillin-resistant *Staphylococcus aureus* (MRSA) Menggunakan Antibiotik Cefoxitin (fox) 30 µg pada Pasien Penderita Abses Gigi di Klinik BPJS Mataram," *J. Anal. Med. Biosains*, vol. 6, no. 2, pp. 98–104, 2019, doi: 10.32807/jambs.v6i2.140.
- [3] F. Harmely, C. Deviarny, and W. S. Yenni, "Formulasi dan Evaluasi Sediaan Edible Film dari Ekstrak Daun Kemangi (*Ocimum americanum* L.) sebagai Penyegar Mulut," *J. Sains Farm. Klin.*, vol. 1, no. 1, p. 38, 2015, doi: 10.29208/jsfk.2014.1.1.10.
- [4] F. Syarifah, D. Mulyanti, and S. E. Priani, "Formula Edibe Film Ekstrak Biji Pepaya (*Carica Papaya* L.) dan Uji Aktivitasnya terhadap Bakteri *Klebsiella pneumoniae* dan *Staphylococcus aureus*," *Pros. Penelit. Spes. Unisba 2015*, vol. 2, no. Farmasi Gelombang, pp. 405–414, 2015.
- [5] A. K. Sonker, K. Rathore, R. K. Nagarale, and V. Verma, "Crosslinking of Polyvinyl Alcohol (PVA) and Effect of Crosslinker Shape (Aliphatic and Aromatic) Thereof," *J. Polym. Environ.*, vol. 26, no. 5, pp. 1782–1794, 2018, doi: 10.1007/s10924-017-1077-3.
- [6] F. Yulistiani, D. R. D. Kurnia, M. Agustina, and Y. Istiqlaliyah, "Pembuatan Edible Film Antibakteri Berbahan Dasar Pektin Albedo Semangka, Sagu, dan Ekstrak Bawang Putih," *J. Fluida*, vol. 12, no. 1, pp. 29–34, 2019, doi: 10.35313/fluida.v12i1.1621.
- [7] K. W. Song, H. Y. Kuk, and G. S. Chang, "Rheology of Concentrated Xanthan Gum Solutions: Oscillatory Shear Flow Behavior," *Korea Aust. Rheol. J.*, vol. 18, no. 2, pp. 67–81, 2006.
- [8] A. Guntur *et al.*, "Kemangi (*Ocimum basilicum* L.): Kandungan Kimia, Teknik Ekstraksi, dan Uji Aktivitas Antibakteri," *J. Food Pharm. Sci.*, vol. 9, no. 3, pp. 513–528, 2021.
- [9] A. D. Putra, V. S. Johan, and R. Efendi, "Penambahan Sorbitol Sebagai Plasticizer dalam Pembuatan Edible Film Pati Sukun," *Jom Fak. Pertan.*, vol. 4, no. 2, pp. 1–15, 2017.

- [10] D. E. Ermawati and C. I. Ramadhani, "Formulation of Anti-Acne Gel of Moringa oleifera L Ethanolic Extract and Antibacterial Test on Staphylococcus epidermidis," *J. Food Pharm. Sci.*, vol. 7, no. 1, pp. 34–44, 2019.
- [11] N. Gonthard, C. Duchez, J. -L Cuq, and S. Guilbert, "Edible Composite Films of Wheat Gluten and Lipids: Water Vapour Permeability and Other Physical Properties," *Int. J. Food Sci. Technol.*, vol. 29, no. 1, pp. 39–50, 1994, doi: 10.1111/j.1365-2621.1994.tb02045.x.
- [12] A. Qurrata and Tusniyawati, "Karakterisasi Fisik dan Mekanik Edible Film dengan Penambahan Pektin Kulit Pisang Kepok (Musa Paradisiaca Linn)," *Pros. Konf. Nas. Mat. dan IPA Univ. PGRI Banyuwangi*, vol. 1, no. 1, pp. 283–296, 2019.
- [13] A. Hamad, M. G. P. Mahardika, and D. Hartanti, "Antimicrobial and Volatile Compounds Study of Four Spices Commonly Used in Indonesian Culinary," *J. Food Pharm. Sci.*, vol. 4, no. 1, pp. 1–5, 2016.
- [14] C. E. Brunchi, M. Bercea, S. Morariu, and M. Dascalu, "Some Properties of Xanthan Gum in Aqueous Solutions: Effect of Temperature and pH," *J. Polym. Res.*, vol. 23, no. 123, pp. 1–8, 2016, doi: 10.1007/s10965-016-1015-4.
- [15] H. N. Imtihani, R. A. Wahyuono, and S. N. Permatasari, *Biopolimer Kitosan dan Penggunaannya dalam Formulasi Obat*. Gresik: Graniti, 2020.
- [16] B. Pudyastuti, Marchaban, and R. Kuswahyuning, "Pengaruh Konsentrasi Xanthan Gum Terhadap Fisik Krim Virgin Coconut Oil (VCO)," *J. Farm. Sains dan Komunitas*, vol. 12, no. 2, pp. 6–14, 2015.
- [17] Sukamto, "Perbaikan Tekstur dan Sifat Organoleptik Roti yang Dibuat dari Bahan Baku Tepung Jagung Dimodifikasi oleh Gum Xanthan," *Agrika*, vol. 4, no. 1, pp. 54–59, 2010.
- [18] Febriyenti, N. Fitria, N. Mohtar, S. Umar, D. Noviza, and S. Rineldi, "Honey Gel and Film for Burn Wound," *Int. J. Drug Deliv.*, vol. 6, no. 1, pp. 1–7, 2014, [Online]. Available: <http://www.arjournals.org/index.php/ijdd/index>
- [19] N. Zhang *et al.*, "Effect of Gellan Gum and Xanthan Gum Synergistic Interactions and Plasticizers on Physical Properties of Plant-Based Enteric Polymer Films," *Polymers (Basel)*, vol. 12, no. 1, pp. 1–13, 2020, doi: 10.3390/polym12010121.

