



Application of a Two-Level Full Factorial Design for the Synthesis of Composite Bioplastics from Durian Seed Flour and Yellow Konjac Flour Incorporating Ethanolic Extract of *Syzygium myrtifolium* Leaves and its Characterization

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ABSTRACT

Increasing environmental problems related to synthetic plastics for food packaging encourage the creation of more environmentally friendly plastics from Indonesia's local natural resources, such as durian seed, yellow konjac, and *Syzygium myrtifolium* leaves, which are abundant in nature. The purpose of the study was to evaluate the effect of the durian seed flour (DSF) mass, yellow konjac flour (YKF) mass, and the concentration of ethanolic extract of *S. myrtifolium* leaves (5-25%) on the tensile strength, elongation, and inhibition zone area of composite bioplastics. The two-level full factorial design was conducted for this experiment with 3 independent factors: DSF mass (0.5-1 g), YKF mass (0.5-1 g), and the concentration of ethanolic extract of *S. myrtifolium* leaves (5-25% b/v), and 3 responses were observed: tensile strength, elongation, and inhibition zone area. The physicochemical characteristics were then used to further describe the best combination. The results showed that the DSF mass had only affected tensile strength, whereas the YKF mass had affected tensile strength and elongation of composite bioplastics. Meanwhile, the concentration of ethanolic extract of *S. myrtifolium* leaves only affects the inhibition zone area. The best combination was found in the DSF mass of 0.5 g, YKF mass of 1 g, and the concentration of ethanolic extract of *S. myrtifolium* leaves of 25%, with the tensile strength of 3.30 MPa, elongation of 50.00%, and inhibition zone area of 15.33 mm. Moreover, these combinations also had a thickness of 0.115 mm, modulus young of 0.066 MPa, density of 1.37 g.cm⁻³, moisture content of 17.14%, and water solubility of 76.91%.

INTRODUCTION

Synthetic plastics have posed significant problems and challenges to the world today. It produces over 400 Mt per year globally and takes a long time to decompose. Synthetic plastics have unfriendly environmental properties that pollute humans indirectly (Fahim et al. 2021). Thus, environmentally friendly plastics are needed to reduce the impact of synthetic plastics. Generally, eco-friendly plastics are made from renewable materials such as starch (Thakur et al. 2019) or whole flour (Retnowati et al. 2015). Durian seed is an exotic natural resource that is relatively abundant and underutilization in Indonesia as the by-product of durian

fruit. The production of durian seeds as a bioproduct is approximately 233960.4 tons, according to data published by the BPS-Statistics Indonesia 2020 (BPS-Statistics Indonesia 2020). For further applications, durian seeds can be transformed into durian seed flour (DSF).

DSF contains high starch, gum, trace elements, and minerals (Leemud et al. 2020). Starch, gums, proteins, and lipids are constituent components in manufacturing eco-friendly plastics (Thakur et al. 2019, Permatasari et al. 2021). Our previous study reported the DSF had a starch content of 41.42%, a protein content of 6.26%, a fat content of 0.57%, a carbohydrate content of 80.61%, and a low fiber of 0.85%

(Permatasari et al. 2022), so it is promising as a source of bioplastics materials. Previously, the bioplastic production from DSF has been carried out and reported by Retnowati et al. (2015). However, Retnowati and her team found that bioplastics of DSF in individual composition had undesirable characteristics such as low modulus young, tensile strength, and elongation. Furthermore, in our preliminary study, the utilization of DSF alone as raw material could not form bioplastics properly. Thus, to improve its characteristics, it is necessary to substitute it with other ingredients, such as yellow konjac flour (YKF).

YKF is a product of processing yellow konjac (*Amorphophallus muelleri*) tubers through a dry method (Witoyo et al. 2020, 2021). It contains glucomannan, a type of polysaccharide with diverse food and non-food application (Zhang et al. 2014). Glucomannan is one of the biopolymer-based packaging film materials which attracts attention because it is classified as a safe, edible, and renewable polysaccharide (Zhang & Rhim 2022). Kurt and Kahyaoglu (2014) reported that it succeeded in making bioplastics from sahlepl glucomannan. Another study reported that the substitution of konjac glucomannan in biofilms from keratin could produce biofilms with better tensile strength and modulus young than those made from pure keratin (Strnad et al. 2019). To improve the anti-bacterial properties of the bioplastics produced, ethanol extract from *S. myrtifolium* leaves is added. Ahmad et al. (2021) reported that the ethanolic extract of *S. myrtifolium* leaves could inhibit bacterial growth of *A. baumannii*, *B. cereus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* in vitro study. However, no one has applied ethanol extract from *S. myrtifolium* leaves to produce bioplastics as natural anti-bacterial agents.

Two-level full factorial is a recently introduced method used to study the interaction of various parameters during the process. Two-level factorial design helps determine significant independent variables that affect the dependent variable with few data compared with the conventional one factor (Lim et al. 2018). However, this approach is still rarely used to synthesize composite bioplastics and evaluate the effect of the independent factors on the specific responses of composite bioplastics. Thus, this study uses a two-level full factorial design to synthesize bioplastics from DSF and YKF incorporated with ethanolic extract of *Syzygium myrtifolium* and evaluated their effect on the mechanical properties and inhibition zone area of composite bioplastics.

MATERIAL AND METHODS

Materials

Durian seed flour (DSF) used in this study was obtained from our previous study (Permatasari et al. 2022), with the

characteristics as follows: starch content of 41.42%, amylopectin content of 21.79%, the protein content of 6.26%, the fat content of 0.57%, the carbohydrate content of 80.61%, and fiber of 0.85%. *S. myrtifolium* was obtained from the center of flowers and ornamental plants, Sidomulyo, Batu, and identified in Materia Medica, Batu, East Java, Indonesia. Yellow konjac flour (YKF) was obtained from the previous study (Witoyo et al. 2021) and used further purified using ethanol 3 times, then powdered using the roller mill (locally made), which contained glucomannan content of 81% d.b and a viscosity of 11000 mPa.s. All chemical reagents were purchased in analytical grade from Merck in Germany through the Chemical and Food Biochemistry Laboratory in Universitas Brawijaya.

Extraction of *Syzygium myrtifolium*

The extraction of *S. myrtifolium* is followed by methods described by Ahmad et al. (2022) with minor modifications. Briefly, the fresh red leaves of *S. myrtifolium* were selected, cleaned, and blended into small pieces. Then, mixed and agitated (120 rpm) with 96% of ethanol for 48 h at room temperature, evaporated, and dried using a rotary evaporator at 60°C until the paste of the extracts was obtained. For further applications, the paste extracts were stored in a dark bottle (5°C in refrigeration condition).

Synthesis of Composite Bioplastics

The production of composite bioplastics adopts Kurt and Kahyaoglu (2014) and Nasution and Wulandari (2021) methods, with the total of solid on bioplastics blends being 1.5% b/v. Briefly, DSF (0.5 or 1 g) was mixed with distilled water (100 mL) and stirred until the temperature reached 75 °C. Then, the YKF (0.5 or 1 ram) and 5 mL of ethanol extract of *S. myrtifolium* leaves (5% or 25% b/v) were mixed in the DSF solution until homogenized, respectively. After that, 5 mL of glycerol was added to the mixture solution, stirred, heated until fully gelatinized, and stood for 10 min. Then, the composite bioplastic solution was poured into a petri dish with a 14 cm diameter, followed by drying at 50°C for 48 h. After drying, the opaque composite bioplastic was input into a desiccator for stabilization and stored for further analysis.

Determined Bioplastic Characteristics

The elongation and tensile strength of composite bioplastics were measured using the ASTM- Methods D-638 (Umiyati et al. 2020). Before the measurement, the composite bioplastics were incubated at 28°C for 2 days in the humidity-controlled chamber. The anti-bacterial of bioplastics was evaluated using methods adopted by Wang et al. (2019). *E. coli* bacteria suspensions (10^5 CFU.mL⁻¹) were subsequently inoculated and cultured on agar media in Petri dishes. Then,

the bioplastic was cut in diameter of 10 mm, placed on a petri dish, and incubated for 12 h at 37°C. The diameter of the inhibition zone area was measured and recorded. The best composite bioplastic was further characterization, like thickness (Oluwasina et al. 2017), density (Salgado et al. 2010), moisture content (Oluwasina et al. 2017), water solubility (Rhim et al. 2007), and microstructure (Retnowati et al. 2015). The characterization was conducted in duplicate.

Experimental Design and Statistical Analysis

Eight experiments ($2^3 = 8$) were conducted to evaluate three independent factors at two levels (Lim et al. 2018). The independent factors used in the study were durian seed flour mass (Latter known as DSF mass), yellow konjac flour (Latter known as YKF mass), and the concentration of ethanolic extract of *S. myrtifolium* leaves. The response data, namely tensile strength, elongation, and inhibition zone area, were analyzed using Design Expert Ver. 13 (trial version, Stat-Ease, Inc., Minneapolis, MN, USA). The design experiment of bioplastics synthesis is listed in Table 1.

Table 1: The design experiment of composite bioplastics synthesis.

No.	DSF mass [g]	YKF mass [g]	The concentration of ethanolic extract of <i>S. myrtifolium</i> leaves [%]
1	1.0	1.0	5.0
2	0.5	1.0	5.0
3	1.0	0.5	5.0
4	0.5	0.5	5.0
5	0.5	1.0	25.0
6	1.0	0.5	25.0
7	1.0	1.0	25.0
8	0.5	0.5	25.0

Table 2: The characteristics of composite bioplastics.

No.	Independent Factor			Response		
	DSF mass [g]	YKF mass [g]	The concentration of ethanolic extract of <i>S. myrtifolium</i> leaves [%]	Tensile Strength [MPa]	Elongation [%]	Inhibition Zone Area [mm]
1	1.0	1.0	5.0	3.40	46.67	12.85
2	0.5	1.0	5.0	3.60	50.00	7.92
3	1.0	0.5	5.0	2.20	40.00	11.92
4	0.5	0.5	5.0	2.80	43.33	7.93
5	0.5	1.0	25.0	3.30	50.00	15.33
6	1.0	0.5	25.0	2.00	30.00	14.88
7	1.0	1.0	25.0	3.00	46.67	15.08
8	0.5	0.5	25.0	3.00	43.33	13.57

RESULTS AND DISCUSSION

The Tensile Strength

The tensile strength of composite bioplastic was 2.0 to 3.6 MPa, as listed in Table 2. This result is in the range of tensile strength data reported by Retnowati et al. (2015), which is 1.07 to 5.31 MPa in durian seed flour (DSF)–jack seed fruit (JSF) bioplastics. Based on the statistical analysis in Table 3, the tensile strength of composite bioplastics was affected by the DSF mass and YKF mass. The Pareto chart for tensile strength of composite bioplastics, as presented in Fig. 1A, showed that the YKF mass had a higher t-value, followed by the DSF mass and the concentration of ethanolic extract of *S. myrtifolium* leaves. It indicates that the YKF mass had a dominant effect on the tensile strength of composite bioplastics. Kalaydzhiev et al. (2019) stated that the higher t-value observed on the Pareto graph indicates the dominant factor's effect on a specific response. Fig. 1B shows the individual percentage contribution of each independent factor on the tensile strength of composite bioplastic. For tensile

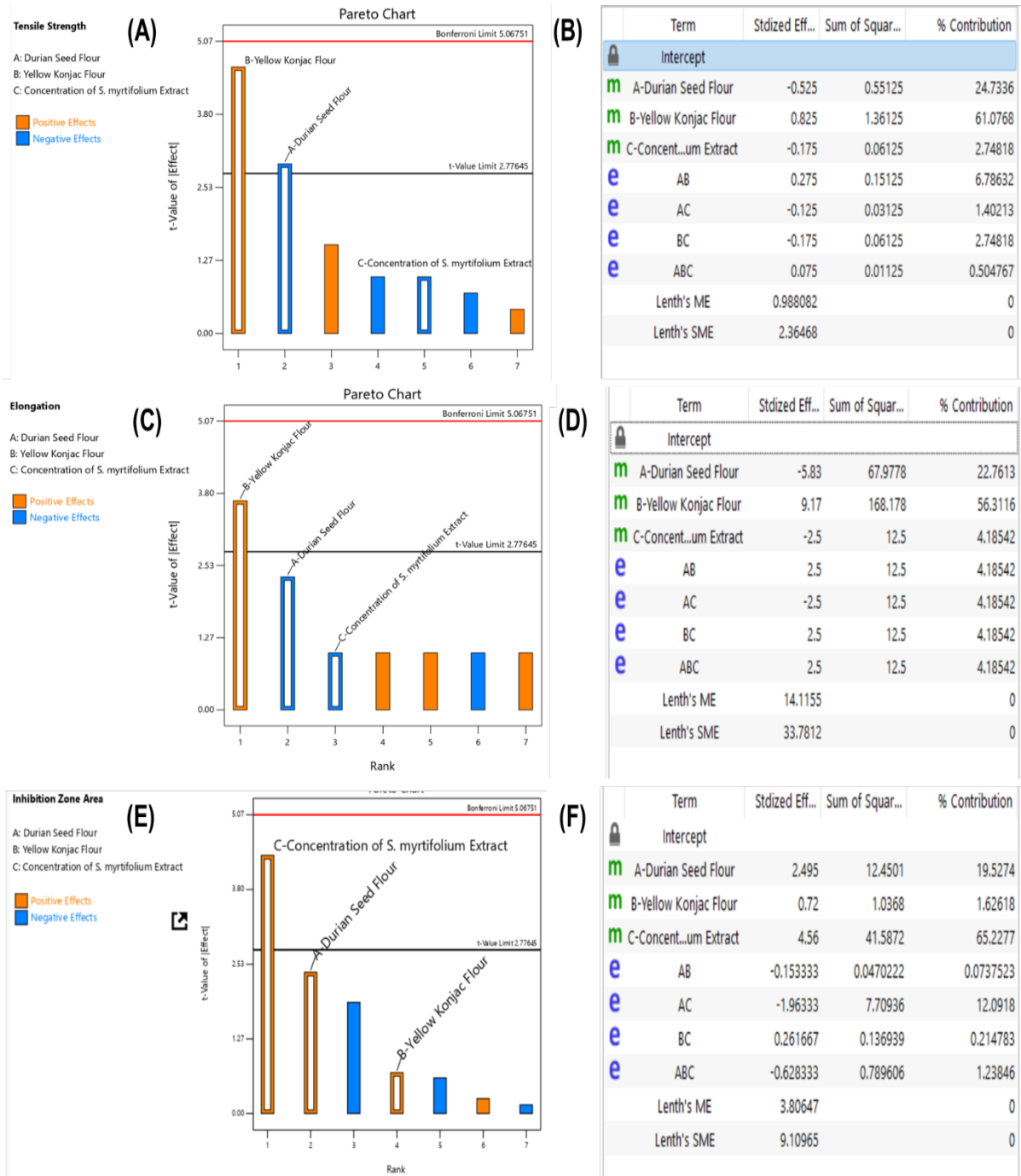


Fig. 1: Pareto chart for tensile strength (A), elongation (C), and inhibition zone area (E) of composite bioplastics, and percentage contribution chart for tensile strength (B), elongation (D), and inhibition zone area (F) on composite bioplastics.

strength response, the YKF mass had the highest percentage of contribution of 61.08%, followed by DSF mass and concentration of ethanolic extract of *S. myrtifolium* leaves with a contribution of 24.73% and 2.75%, respectively. The interaction also contributed between factors of 0.5 to 6.98%.

As a result, YKF mass is more critical than DSF mass, and the concentration of ethanolic extract of *S. myrtifolium* leaves, has a more significant impact on tensile strength, as evidenced by a higher percentage contribution to change measured variable.

Fig. 1A and Table 3 showed that the DSF mass had a significantly negative effect on the tensile strength. The increasing DSF mass from 0.5 to 1.0 g significantly decreased the tensile strength of composite bioplastics, as depicted in Fig. 2A. The decrease in tensile strength was due to the high amylopectin content in our DSF samples, which is 21.79% compared to amylose (19.64%), which contributed in composite bioplastics. Woggum et al. (2014) reported that the amylose content of starch contributes to film strength and the branched structure of amylopectin, leading to lower mechanical properties in films. However, this result was in line with Retnowati et al. (2015), who reported that the increase in the DSF ratio significantly affected the decrease in tensile strength. In addition, the Pareto chart (Fig. 1A) and Table 3 showed that the YKF mass had a significantly positive effect on the tensile strength. The tensile strength of composite bioplastic was significantly improved with increasing YKF mass, as presented in Fig. 2B. It is most likely due to the intermolecular interactions of glucomannan macromolecules in YKF, which can maintain bioplastics' compactness and stability and prevent brittleness. Moreover, the interaction of glucomannan in YKF with polymers in DSF (such as starch, gum, protein, and fat) and the plasticizer through hydrogen

bonding might contribute to the increased tensile strength of composite bioplastics. Fahrullah et al. (2020) reported that adding polysaccharides, namely konjac flour, at high concentrations increased tensile strength and prevented the brittleness of whey-based edible films. Ma et al. (2021) stated that the increased tensile strength in film-based chitosan with the addition of konjac glucomannan (KGM) is caused by their hydrogen bonding interaction. Furthermore, the concentration of ethanolic extract from *S. myrtifolium* leaves had an insignificantly negative effect on tensile strength, as presented in Fig. 1A, Fig. 2C, and Table 3.

The Elongation

The elongation of composite bioplastic was 30.00 to 50.00%, as listed in Table 2. This result had higher elongation than data reported by Retnowati et al. (2015), which is 29.26 to 44.11 % in durian seed flour (DSF)– jack seed fruit (JSF) bioplastics. Based on the statistical analysis in Table 3, the elongation of composite bioplastics was only affected by YKF mass. Pareto chart for elongation of composite bioplastics, as presented in Fig. 1C, showed that the YKF mass had a higher t- value, followed by DSF mass and the concentration of ethanolic extract of *S. myrtifolium* leaves.

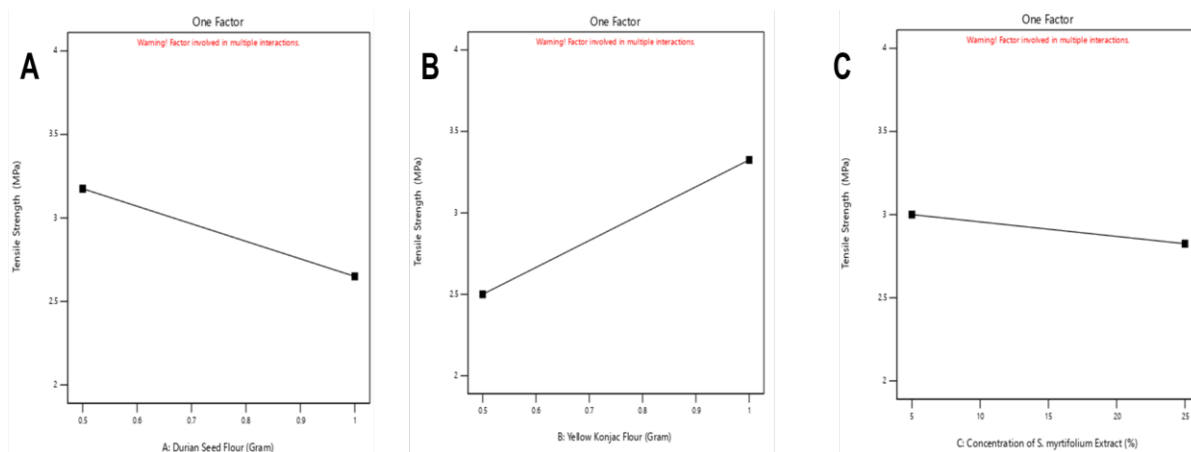


Fig 2: Effect of DSF mass (A), YKF mass (B), and the concentration of ethanolic extract of *S. myrtifolium* leaves (C) on the tensile strength of composite bioplastics.

Table 3: Coefficient estimates and significance analysis of composite bioplastics responses.

Factor	Coefficient estimates		
	Tensile Strength	Elongation	Inhibition Zone Area
Intercept	2.9125	43.75	12.43
DSF mass	-0.2625 ^s	-2.915 ^{ns}	1.25 ^{ns}
YKF mass	0.4125 ^s	4.585 ^s	0.3600 ^{ns}
The concentration of ethanolic extract of <i>S. myrtifolium</i> leaves	-0.0875 ^{ns}	-1.25 ^{ns}	2.28 ^s

Remarks: s: significant, and ns: not significant

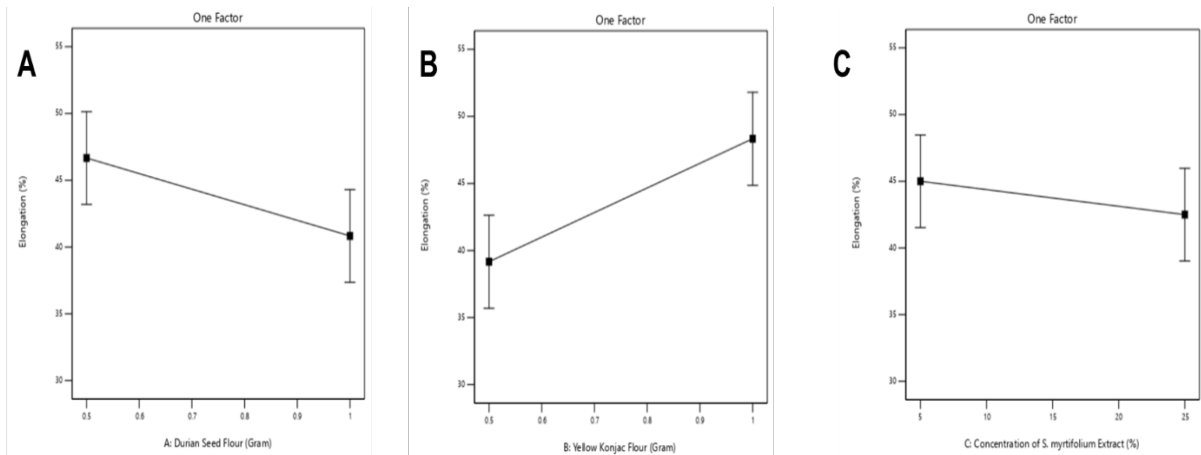


Fig 3: Effect of DSF mass (A), YKF mass (B), and the concentration of ethanolic extract of *S. myrtifolium* leaves (C) on the elongation of composite bioplastics.

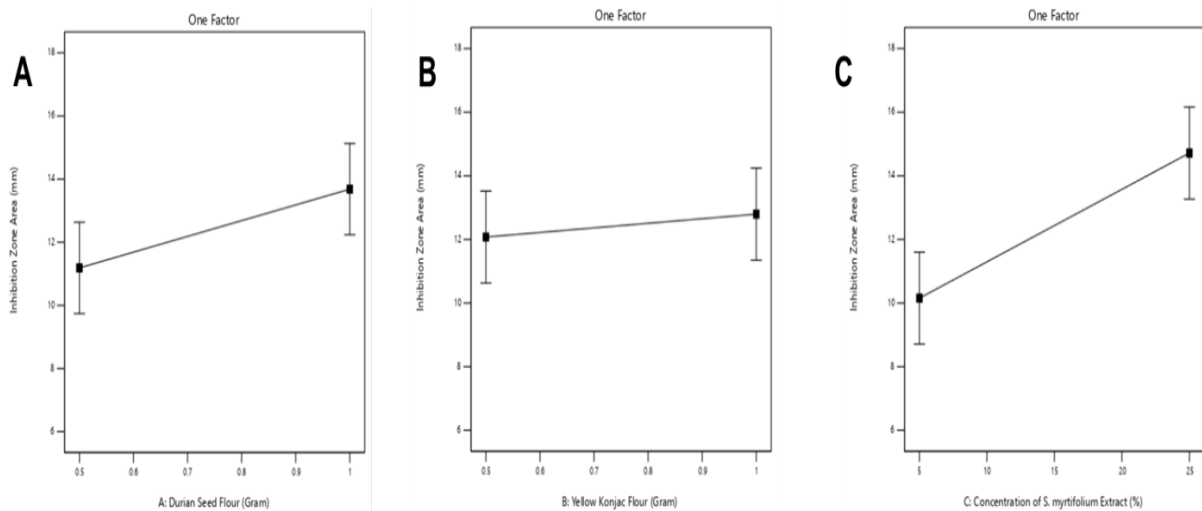


Fig. 4: Effect of DSF mass (A), YKF mass (B), and the concentration of ethanolic extract of *S. myrtifolium* leaves (C) on the inhibition zone area of composite bioplastics.

It indicates that the YKF mass had a dominant effect on the elongation of composite bioplastics. Kalaydzhev et al. (2019) that the higher t-value observed on the Pareto graph indicates the dominant factor effect on a specific response. Fig. 1D shows the individual percentage contribution of each independent factor to the elongation of composite bioplastic. The YKF mass had the highest effect on the elongation with a contribution of 51.31%, followed by DSF mass and concentration of ethanolic extract of *S. myrtifolium* leaves with a contribution of 22.76% and 4.18%, respectively. The interaction also had a contribution between factors was 4.19% in all factor interactions. As a result, YKF mass is more critical than DSF mass, and the concentration of ethanolic extract of *S. myrtifolium* leaves, has a more significant impact on

elongation, as evidenced by a higher percentage contribution to change measured variable.

Fig. 1C and Table 3 showed that the DSF mass had an insignificantly negative effect on elongation. The increase of DSF mass from 0.5 to 1.0 g tends to decrease the elongation of composite bioplastics, as depicted in Fig. 3A. This result was in line with the Retnowati et al. (2015), who reported that the increase in the DSF ratio significantly affected the decrease in elongation. In addition, the Pareto chart (Fig. 1C) and Table 3 showed that the YKF mass had a significantly positive effect on elongation. The elongation of composite bioplastic was significantly improved with increasing YKF mass, as presented in Fig. 3B. It might be due to the glucomannan intermolecular interaction in YKF, which affects

the elasticity and matrix strength of composite bioplastics. Additionally, the interaction of glucomannan in YKF with polymers in DSF (such as starch, gum, protein, and fat) and the plasticizer through hydrogen bonding might contribute to the increasing elongation of composite bioplastics. Fahrul-lah et al. (2020) stated that the increase in the elongation of whey-based edible films with the addition of konjac flour was caused by the polysaccharide content in konjac flour. Ma et al. (2021) stated that adding konjac glucomannan (KGM) improved film-based chitosan's elongation, probably the interaction hydrogen bonding of chitosan – KGM in molecular stages. The concentration of ethanolic extract from *S. myrtifolium* leaves had an insignificantly negative effect on elongation, as presented in Fig. 1C, Fig. 3C, and Table 3.

The Inhibition Zone Area

The inhibition zone area of composite bioplastic was 7.92 to 15.33 mm, as listed in Table 2. This result was reported in the range of previous work by Yuniarni et al. (2020) (9.00 to 10.37 mm). Based on the statistical analysis in Table 3, the inhibition zone area of composite bioplastics was only affected by the concentration of ethanolic extract of *S. myrtifolium* leaves. The Pareto chart for the inhibition zone area of composite bioplastics, as presented in Fig. 1E, showed that the concentration of ethanolic extract of *S. myrtifolium* leaves had a higher t- value, followed by DSF mass and YKF mass. It indicates that the concentration of ethanolic extract of *S. myrtifolium* leaves had a dominant effect on the inhibition zone area of composite bioplastics. Kalaydzhiev et al. (2019) that the higher t-value observed on the Pareto graph indicates the dominant factor effect on a specific response. Fig. 1F shows the individual percentage contribution of each independent factor to the inhibition zone area of composite bioplastic. The concentration of ethanolic

extract of *S. myrtifolium* leaves had the highest effect on the inhibition zone area with a contribution of 65.23%, followed by DSF mass and YKF mass with a contribution of 19.53% and 1.63%, respectively. The interaction also contributed between factors of 0.07 to 1.23%. It indicates that the highest contribution of independent factors reflects a dominant effect on the specific response in bioplastic composites. As a result, the concentration of ethanolic extract of *S. myrtifolium* leaves is more critical than YKF mass and DSF mass, it has a more significant impact on the inhibition zone area, as evidenced by a higher percentage contribution to change measured variable.

Fig. 1E and Table 3 showed that the DSF mass had an insignificantly positive effect on the inhibition zone area. The increase of DSF mass from 0.5 to 1.0 g tends to increase the inhibition zone area of composite bioplastics, as depicted in Fig. 4A. In addition, the Pareto chart (Fig. 1F) and Table 3 showed that the YKF mass had an insignificantly positive effect on the inhibition zone area, as presented in Fig. 4B. Moreover, the concentration of ethanolic extract of *S. myrtifolium* leaves had a significantly positive effect on the inhibition zone area, as illustrated in Fig. 1E and Table 3. The increase in the concentration of ethanolic extract of *S. myrtifolium* leaves from 5.0 to 25%, increase the inhibition zone area of composite bioplastics, as depicted in Fig. 4C. Yuniarni et al. (2020) reported that the ethanol extract of *S. myrtifolium* could inhibit the growth of *E. coli* with inhibition diameter zones of 9.00, 9.30, and 10.37 mm at concentrations of ethanol extract of *S. myrtifolium* of 6, 12.5, and 12.5% in the *in-vitro* study, respectively. Ahmad et al. (2022) also reported that the ethanol extract of *S. myrtifolium* showed potent inhibitory activity against *E. coli* with a minimum inhibitory concentration of 0.63 mg mL⁻¹ in the *in-vitro* study. Chemical compounds in ethanolic

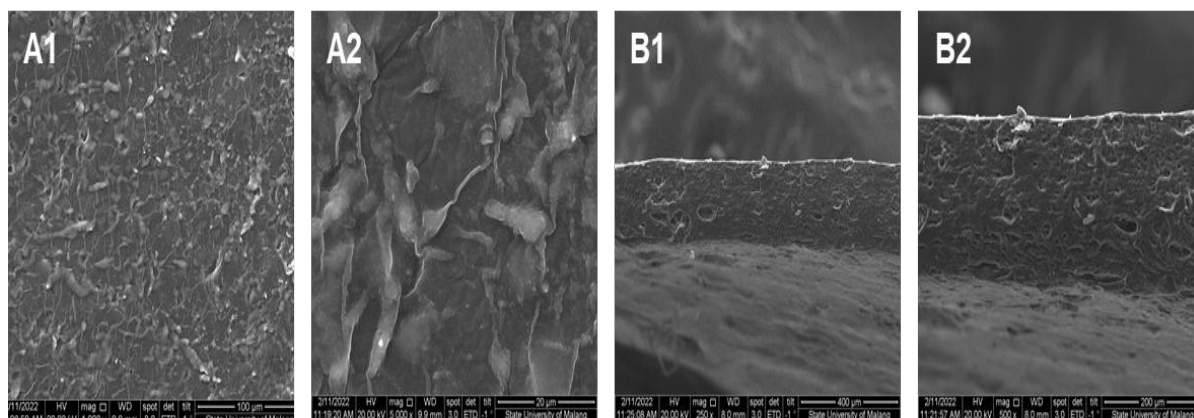


Fig. 5: The microstructure of the surface composite bioplastics in 1000x (A1), 5000x (A2) magnifications, and cross-section in 250x (B1), and 500x (B2) magnifications.

extract of *S. myrtifolium* leaves that act as an anti-bacterial are auraptinol, calopiptin, quercetin-3-o--L-arabinopyranoside, and quercetin-3-o--D-glucuronide (based on LC-MS screening analysis and data not shown). Tan et al. (2017) reported that crude extracts of the roots of *Prangos hulussii* that contain auraptinol compounds had anti-bacterial activity to inhibit the growth of g-positive and g-negative bacteria. Jaisinghani (2017) reported that the quercetin compound had anti-bacterial properties in *E. coli* NCIM2065 with a minimum inhibitory concentration (MIC) of 400 mcg.mL⁻¹ and minimum bactericidal concentration (MBC) of >500 mcg.mL⁻¹. Elansary et al. (2020) reported that the quercetin and quercetin 3-glucuronide had moderate anti-bacterial activities on *B. cereus*, *P. aeruginosa*, *L. monocytogenes*, *E. coli*, *M. flavus* and *S. aureus* in the *in-vitro* study.

Determination of the Best Treatment of Composite Bioplastics

The criteria were used to determine the best treatment of composite bioplastics: the DSF mass, YKF mass, and the concentration of ethanolic extract of *S. myrtifolium* leaves were set in the range, the tensile strength, elongation, and the inhibition zone area were set in maximize. Based on the criteria, the best treatment was found at the 0.5 g of DSF mass, 1 g of YKF mass, and 25% of the concentration of ethanolic extract of *S. myrtifolium* leaves, with the tensile strength of 3.30 MPa, elongation of 50.00%, and inhibition zone area of 15.33 mm. Additionally, further characterization showed that the best composite bioplastics had a thickness of 0.115 mm, modulus young of 0.066 MPa, density of 1.37 g.cm⁻³, moisture content of 17.14%, and water solubility of 76.91%.

Microstructure

The surface and cross-section microstructure of the best composite bioplastics were presented in Fig. 5. Fig. 5 A1 and A2 revealed that the composite bioplastics had some starch granules, which means that the starch was not fully gelatinized during the film formation process. Moreover, the irregularities can be seen on the surface and cross-section of the composite bioplastics (Fig. 5 A-B). It could be due to the presence of many polymer molecules in the matrix of the composite bioplastics, such as starch, protein, lipid, and cellulose (Retnowati et al. 2015). In addition, the cross-section (Fig. 5 B1-B2) of the composite bioplastics also shows the presence of ungelatinized starch granules, as reported by Wu et al. (2009). Durian flour contains many amylopectins, which can cause flour not to dissolve in the film (Garcia-Hernandez et al. 2017). In our DSF used in this study, the amylopectin was 21.79%. Furthermore, the uniformity of the film's surface could reveal its mechanical properties.

The film matrix's uniformity will improve the polymer's integrity and improve the film's mechanical properties (Dias et al. 2010). The uneven and irregular surface of the bioplastic composites confirms the low tensile strength (Table 2) and low modulus young (0.066 MPa).

CONCLUSION

The effect of the DSF mass, YKF mass, and concentration of ethanolic extract of *S. myrtifolium* leaves on composite bioplastics were successfully investigated using a two-level full factorial design. The YKF and DSF mass significantly affected the tensile strength of bioplastic composites. In addition, the YKF mass also had a significant effect on the elongation, and the concentration of the ethanolic extract of *S. myrtifolium* leaves only had a significant effect on the inhibition zone area. The best treatment was found at the 0.5 g of DSF mass, 1 g of YKF mass, and 25% of the concentration of ethanolic extract of *S. myrtifolium* leaves, with the tensile strength of 3.30 MPa, elongation of 50.00%, and inhibition zone area of 15.33 mm. Moreover, these conditions also had 0.115 mm of thickness, 0.066 MPa of modulus young, 1.37 g.cm⁻³ of density, 17.14% of moisture content, and 76.91% of water solubility.

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