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RESEARCH ARTICLE

Formulation and Optimization Peel-Off Gel Mask with Polyvinyl Alcohol and Whey Protein-Based using Factorial Design from Ethanolic Extract of Mangosteen Peel (*Garcinia Mangostana*) as Antioxidant

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ABSTRACT:

Mangosteen peel (*Garcinia mangostana*) contains xanthone compounds that have high antioxidant activity so it can be used as active ingredients in cosmetic preparations. This study aims to make a peel off-gel mask preparation of mangosteen peel ethanol extract using a factorial design and to test the antioxidant activity of the optimum formula. Mangosteen peel was extracted with 96% ethanol solvent and then formulated into a peel-off gel mask. The peel-off gel mask formulation was designed using a factorial design of 2³ where the factors and levels used were mangosteen peel ethanolic extract (1 and 2%), whey protein (2.5 and 5%), and PVA (12 and 14%), and whey protein (2.5 and 5%). The physical properties of the mask were analyzed using an expert design in order to obtain the optimum formula for continued antioxidant testing. Based on the results of statistical analysis, there was a significant influence between the factors and the response to adhesion (p<0.05) but not significant on the dispersion and drying time (p>0.05). Formula 6 was chosen as the optimum formula and had an IC50 value of 3.82ppm while vitamin C as a positive control was 13.98ppm and has the potential to be developed into cosmetic preparations.

KEYWORDS: *Garcinia mangostana*, Ethanolic Extract, Peel-Off Gel Mask, Factorial Design, Antioxidant.

INTRODUCTION:

Mangosteen peel (*Garcinia mangostana*) is a part of the mangosteen fruit plant which is often considered as waste, whereas mangosteen peel has great health benefits. Mangosteen peel has been shown to have activity as antidiabetic¹, antibacterial^{2,3}, antidyslipidemic⁴, antiproliferative⁵, anti-inflammatory⁶, and antioxidants^{7,8}.

Mangosteen peel contains flavonoid compounds, anthocyanins, saponins, tannins, monoterpenes, and xanthone derivatives such as α -mangostin, β -mangostin, and γ -mangostin⁹⁻¹¹. The antioxidant activity of mangosteen peel is higher than in other parts.

Mangosteen peel has an antioxidant effect that was approximately 27-fold and 8.6-fold higher than pulp and seed extracts, respectively³. The antioxidant activity of mangosteen peel is also strongly influenced by the extraction solvent used. Based on the research of Tjahjani et al.⁷, the antioxidant activity of mangosteen peel with 96% ethanol solvent produced the smallest IC50 compared to other solvents. The content of xanthenes causes the mangosteen peel to have a high antioxidant effect. Xanthenes are suitable as antioxidants for cosmetic preparations. Xanthenes contain the hydroxyl groups, attached to the unsaturated heterocyclic xanthone core⁴ which can scavenge free radicals¹². Then there are the prenyl substituents that might enhance skin penetration by increasing their lipophilicity, and, thus, the affinity to cell membranes¹³.

A peel-off gel mask is one of the widely used cosmetic preparations and can increase the delivery of active substances into the skin. Peel-off gel masks have

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occlusive properties that can maintain skin moisture so that the delivery of active compounds is easier to enter into the skin¹⁴. In addition, the peel-off gel mask is easily removed like an elastic membrane, so it can be applied easily and is painless when removed¹⁵. The occlusive ability and elasticity of the peel-off gel mask are related to the presence of film-forming in the peel-off gel mask formula. In this study, a combination of PVA and Whey Protein was used as film-forming. PVA can produce a gel that dries quickly and forms a transparent, strong, plastic film, and adheres well to the skin¹⁶. According to research by Beringsh et al.¹⁶, PVA at a concentration of 13% is the optimum concentration in the manufacture of peel-off gel masks. In this study, PVA concentrations of 12% and 14% were used. Whey protein is an edible film, extensively interacting polymer network that possesses a three-dimensional gel-type structure. Whey protein produces a film that is transparent, flexible, odorless, colorless and has aroma-retaining properties¹⁷. Films based on whey protein are stabilized by disulfide bonds and are not easily soluble in water so the film is less able to maintain water evaporation¹⁸. Therefore, it is necessary to combine it with PVA. The concentration of Whey Protein used in this study was 2.5% and 5%.

Based on the description above, the formulation and optimization of peel-off gel masks from mangosteen peel ethanolic extract was carried out using a 2³ factorial design which used 3 factors with 2 levels. The factors and levels used in the factorial design were as follows: Mangosteen peel ethanolic extract (1 and 2%), PVA (12 and 14%), and Whey Protein (2.5 and 5%). Data analysis was carried out on the evaluation of the peel-off gel mask preparation, namely spreadability, adhesion, and drying time to determine the optimum formula. The

optimum formula will be tested for antioxidant activity using the DPPH method.

MATERIALS AND METHODS:

Materials:

The materials used in this research include mangosteen peel ethanolic extract, Whey Protein, polyvinyl alcohol (PVA), HPMC, Propylene glycol, methylparaben, propylparaben, aquadest, ascorbic acid, DPPH, 96% ethanol, n-hexane, ethyl acetate, Dragendroff's reagent, Mayer's reagent, Liebermann-Burchard reagent, 2 N HCl, acetic anhydrous acid P, sulfuric acid P, chloroform, 10% solution of iron (III) Chloride, acetone P, boric acid P, oxalic acid P, ether P, and methanol pa.

Preparation of Mangosteen Peel Ethanolic Extract:

Mangosteen peel (*Garcinia mangostana*) was collected in South Sumatra, Indonesia. Fresh peels are washed with running water and then dried. The simplicia is mashed using a blender. The simplicia is macerated with 96% ethanol for 48 hours and re-macerated for 24 hours. The obtained macerate was concentrated with a rotary evaporator at a temperature of 50°C to obtain a thick extract¹⁹.

Phytochemical Screening:

Phytochemical screening carried out included examination of flavonoids, alkaloids, steroids and triterpenoids, glycosides, saponins and tannins, and flavonoids. The test was carried out qualitatively using color reagents²⁰.

Design Formula of Peel-Off Gel Mask:

The formula is designed with formula 2³ factorial design which consisting of three factors with two levels. The design of the formula can be seen in Table 1.

Table 1: Factorial Design 2³ Formula

Factor	Level Low (%)	Level High (%)
Extract (A)	1	2
Whey Protein (B)	12	14
PVA (C)	2.5	5

Based on the 2³ factorial design, 8 formulas were obtained for the manufacture of peel-off gel masks which can be seen in Table 2.

Table 2: Formulation of Peel-Off Gel Mask

Ingredients	Formula (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
Mangosteen peel ethanolic extract	1	1	1	1	2	2	2	2
Whey Protein	2.5	5	2.5	5	2.5	5	2.5	5
PVA	12	12	14	14	12	12	14	14
HPMC	1	1	1	1	1	1	1	1
Propilenglycol	10	10	10	10	10	10	10	10
Propylparaben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Methylparaben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Aquadest ad	100	100	100	100	100	100	100	100

Preparation of Peel-Off Gel Mask:

The concentration of ingredients in the manufacture of peel-off gel masks can be seen in Table 2. PVA was

dispersed into water and stirred using a magnetic stirrer for 24 hours. In a separate container, HPMC was developed in hot water and allowed to stand for 30

minutes. Mix PVA and HPMC homogeneously (Mixture A). In a separate bowl, dissolve the whey protein with warm water and mix it into mixture A (Mixture B). Methylparaben and propylparaben were dissolved in propylene glycol and put into mixture B. Put the Mangosteen peel ethanolic extract and the remaining water little by a little while stirring homogeneously.

Evaluation of Peel-Off Gel Mask:

Organoleptic Test:

The organoleptic test was carried out by observing directly at the color, shape, and smell of the mask of each formula.

Homogeneity Test:

The homogeneity test was carried out by applying the peel-off gel mask to a transparent glass preparation. It was observed visually. If there were no particles so the peel-off gel mask was homogenous

pH test:

The preparation was weighed 1gram dissolved in a beaker glass with 10ml of distilled water and measured with a pH meter.

Spreadability Test: 5

Spreadability was determined by measuring the spreading diameter of 1g of the sample between two horizontal glass plates (10cm x 20cm) after one minute. The standard weight applied to the upper plate was 25g. Each formulation was tested three times²¹.

Adhesion Test:

A 0.25grams sample is placed between 2 object glasses. Then pressed with a load of 1kg for 5 minutes. Then the additional load of 80grams attached to the device was removed and then the release time between 2 glasses of peel-off gel mask object was recorded. Each formulation was tested three times.

Drying Time:

A total of 0.2g peel off mask gel was weighted and spread using a glass slide with an area of 5x2.5cm. The formulations were monitored for 30min until the drying process was completed. Each formulation was tested three times.

Data Analysis of Peel-Off Gel Mask Evaluation:

The spreadability, adhesion, and drying time data were analyzed according to the factorial design method using software *Design Expert 11*® to determine the influence of factors and factor interactions on the response.

Formula Optimization:

Formula optimization is carried out using software *Design Expert 11*®. The formula is expected to provide

maximum spreadability and adhesion response, and minimal drying time.

Antioxidant Activity Test:

The testing procedure is carried out based on the DPPH method. A 6 ml test solution containing a peel-off gel mask with concentrations 25, 50, 100, 125,150 dan 200 µg/mL mixed with 3.8 ml of DPPH 1 mM. The mixture was incubated in a dark room for 35 minutes, then its absorbance was measured at a wavelength of 515 nm with a UV-Visible spectrophotometer. Ascorbic acid was used as positive control. Measurements were made three times. Percent value of radical scavenging calculated by following the formula 1:

$$\% \text{ Radical Scavenging} = \frac{A \text{ blank} - A \text{ test}}{A \text{ test}} \times 100\% \dots\dots\dots 1$$

Where: A Blank = Absorbance of DPPH Solution

A Test = Absorbance of Sample

IC₅₀ values are calculated when the % radical scavenging value is 50%²².

RESULT:

Preparation of Mangosteen Peel Ethanolic Extract:

The mangosteen peel ethanolic extract produced is greenish-brown, thick, and has a distinctive smell. The percent yield value obtained is 31.15%. The yield percentage describes the effectiveness of extraction. The high yield percentage was obtained because many reasons but the main reason was related to the solvent used in this study. Xanthone was soluble in organic solvent with a moderate polarity such as ethanol, acetone, ethyl acetate, and methanol. Based on the research of Kusmayadi et al.²³, more xanthenes were extracted in ethanol as compared to other solvents. In this study, the yield obtained is quite high which proves that the extracted content is quite large.

Phytochemical Screening:

Phytochemical screening of the mangosteen peel ethanolic extract was carried out to determine the compounds contained in the extract. The compounds identified were flavonoids, alkaloids, steroids/triterpenoids, saponins, glycosides, and tannins. The results of phytochemical screening proved that the extract contains flavonoids, alkaloids, steroids, triterpenoids, saponins, glycosides, and tannins as shown in Table 3.

Table 3: Phytochemical Screening Result

Test	Result
Flavonoid	+
Alkaloid	+
Steroid	+
Triterpenoid	+
Saponin	+
Glycosides	+
Tannin	+

13 Evaluation of Peel-Off Gel Mask:

Evaluation of the peel-off gel mask of the mangosteen peel ethanolic extract has been carried out on 8 formulas to determine the physical properties of the preparation. The evaluation of the peel-off gel mask of the

11 mangosteen peel ethanolic extract that was carried out included organoleptic tests, homogeneity tests, pH tests, spreadability tests, 2 adhesion tests, and drying time tests. The results of the evaluation of the peel-off gel mask can be seen in Table 4.

13 Table 4: Evaluation of Peel-Off Gel Mask Result

Test	Result			
	F1	F2	F3	F4
Organoleptic	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	5.60±0.01	5.40±0.02	5.60±0.02	5.00±0.03
Spreadability (cm)	4.80±0.01	5.20±0.01	5.80±0.01	6.10±0.07
Adhesion (sec)	4.00±0.01	3.90±0.01	4.10±0.01	3.80±0.07
Drying Time (min)	16.32±0.06	16.54±0.03	17.55±0.04	17.48±0.03
Test	F5	F6	F7	F8
Organoleptic	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor	Brown, transparent, semisolid, distinctive odor
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	5.20±0.01	5.80±0.01	5.00±0.02	5.40±0.02
Spreadability (cm)	6.50±0.06	6.80±0.06	4.60±0.01	4.80±0.04
Adhesion (sec)	3.60±0.01	4.00±0.01	4.20±0.01	4.30±0.07
Drying Time (min)	18.12±0.02	18.31±0.03	18.47±0.03	19.08±0.03

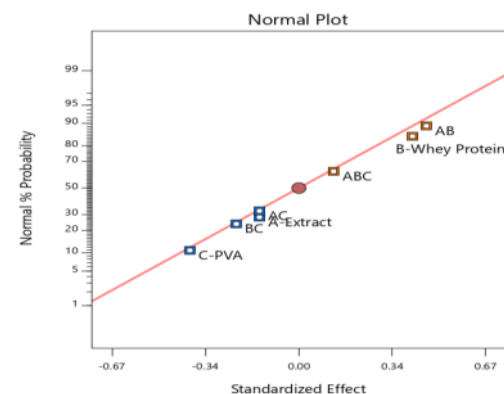
Data Analysis of Peel-Off Gel Mask Evaluation:

The spreadability, adhesion, and drying time data were analyzed using software *Design Expert 11®*. The results of the ANOVA test for the spreadability, adhesion, and drying time can be seen in Table 5. The graph of normal plot, normal plot of residual, and prediction vs actual for spreadability can be seen in Figure 1 while the adhesion can be seen in Figure 2 and drying time can be seen in Figure 3.

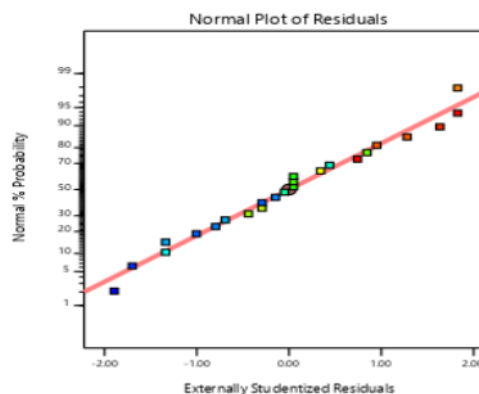
Table 5: The Results of ANOVA Analysis

Source	p value		
	Spreadability	Adhesion	Drying Time
A- Mangostin Peel Ethanolic Extract	0.6753	0.0848	0.5556
B- Whey Protein	0.2365	0.5489	0.5402
C- PVA	0.2553	< 0.0001*	0.2329
AB	0.1864	< 0.0001*	0.0187*
AC	0.6753	< 0.0001*	0.4616
BC	0.5076	0.0075*	0.7628
ABC	0.7115	0.5489	0.8864

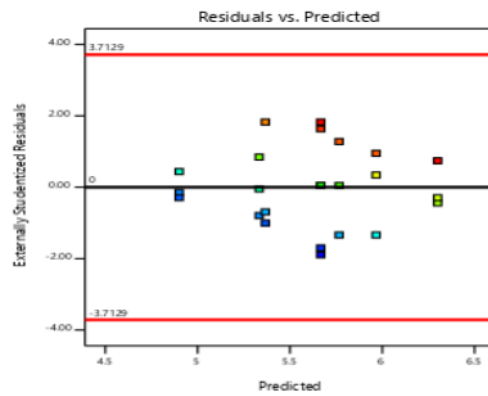
*p value <0.05 indicates that the factor has a significant effect on the response



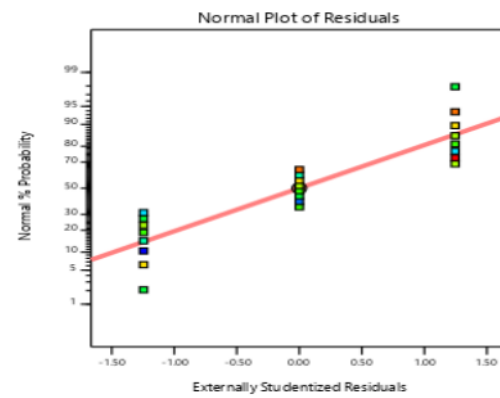
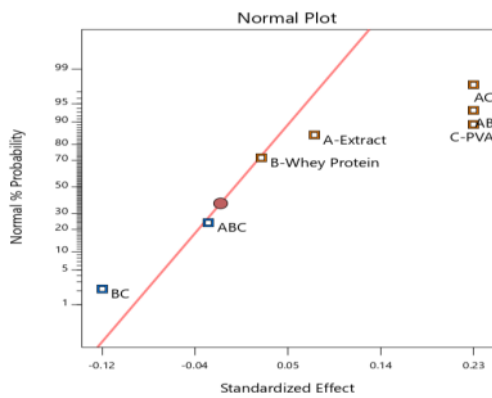
A



B

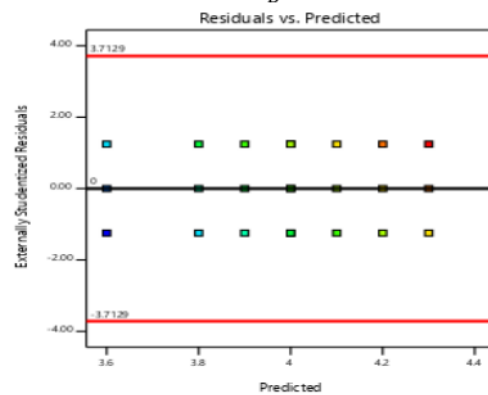


6
C
Figure 1: The Graph of Normal Plot (A), Normal Plot of Residual (B), and Predicted vs Actual (C) from Spreadability Data



A

B



6
C
Figure 2: The Graph of Normal Plot (A), Normal Plot of Residual (B), and Predicted vs Actual (C) from Adhesion Data

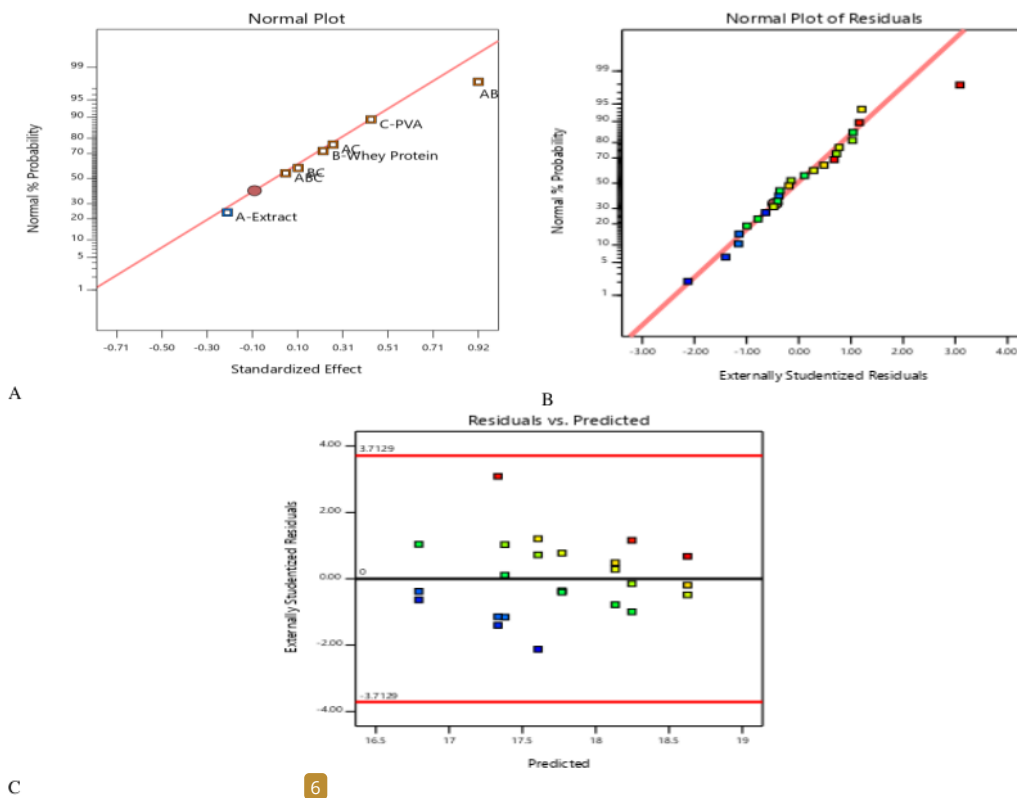


Figure 3: The Graph of Normal Plot (A), Normal Plot of Residual (B), and Predicted vs Actual (C) from Drying Time Data

In the analysis of the factorial design data, coefficient values are also generated to make the adhesion and drying time response equations which can be seen in equations 2 and 3.

Adhesion Response Equation:
 $Y = 3.9875 + 0.1125 C + 0.1125 AB + 0.1125 AC - 0.0625 BC \dots\dots 2$

Drying Time Response Equation:
 $Y = 17.7363 + 0.4579 AB \dots\dots\dots 3$

Formula Optimization:

The optimization of the formula was carried out using software *Design Expert 11*® by entering several criteria such as the maximum spreadability and adhesion response value and the minimum drying time response value. Based on this analysis, the optimum formula for making peel-off gel masks was obtained, namely the concentration of the mangosteen peel ethanolic extract was 2%, PVA was 12%, and Whey Protein was 5%. The desirability value was 0.808. Based on this result, F6 was chosen as the optimum formula by system.

Antioxidant Activity Test:

In this test, F6 was used with a concentration of the mangosteen peel ethanolic extract was 2%, Whey

Protein was 5%, and PVA was 12% as the optimum formula and ascorbic acid as a comparison or positive control. The results of the antioxidant activity test can be seen in Table 6.

Table 6: Antioxidant Activity Result

Formula	IC ₅₀ (ppm)
F6	3.82
Ascorbic Acid	13.98

DISCUSSION:

The mangosteen peel ethanolic extract produced is greenish-brown, thick, and has a distinctive smell. The percent yield value obtained is 31.15%. The mangosteen peel ethanolic extract in this study was proven to contain flavonoids, alkaloids, saponins, tannins, glycosides, steroids, and terpenoids. The presence of flavonoids in the mangosteen peel ethanolic extract has the potential to be an antioxidant. The mangosteen peel was contained xanthone derivatives such as α-mangostin, β-mangostin, and γ-mangostin. Xanthenes contain the hydroxyl groups, attached to the unsaturated heterocyclic xanthone core, which can scavenge free radicals¹². Then there are the prenyl substituents that might enhance skin penetration by increasing their lipophilicity, and, thus, the affinity to cell membranes¹³.

The mangosteen peel ethanolic extract was formulated into a peel-off gel mask preparation using a 2³ factorial design. The factors and levels used in the factorial design were the concentrations of the mangosteen peel ethanolic extract (1% and 2%), whey protein (2.5% and 5%), and PVA (12% and 14%). The selection of these factors is based on the function of the material in the manufacture of peel-off gel masks. The peel-off gel mask produced has the characteristics, namely yellowish-brown to brown color, homogeneous, pH around 5, spreadability in the range of 4.6 to 6.8 cm, adhesion in the range of 3.6 to 4.2 sec, and drying time the range of 16 to 20 minutes. The good characteristic of peel-off gel mask is based on the value of pH, spreadability, adhesion, and drying time. The pH requirements range from 4.5 to 6.5, spreadability range from 5 to 7 cm, adhesion more than 4 sec, and a drying time of 15 to 30 minutes²⁴.

The analysis data was conducted to spreadability, adhesion and drying time using software *Design Expert 11*® to see the influence of factors and interaction factors on the response and to determine the optimum formula for the peel-off gel mask. The influence of the factor and interaction factor on the response can be seen in Table 5. In the ANOVA result, the p-value < 0.05 was indicated that the factor has a significant effect on the response. All factors and interaction factors were not significant on the spreadability and drying time test but significant on the adhesion test. The spreadability response was not influenced by factors and interaction factors indicated by the p-value > 0.05. The adhesion response was influenced by PVA(C), the interaction between extract and whey protein (AB), the interaction between extract and PVA (AC), and the interaction between whey protein and PVA (BC). The drying time was influenced by the interaction between extract and whey protein (AB). The data generated on the response of spreadability, adhesion, and drying time are normally distributed which can be seen in the Normal Plot of Residual Graphs in Figures 1, 2, and 3. Predicted and actual interaction can be seen in the Predicted vs Actual graphs in Figures 1, 2, and 3. The spreadability and drying time response had predicted results that are not close to the actual. This may indicate a large block effect or a possible problem with the model and/or data. The adhesion response has the close Predicted results to the actual so that the resulting data is quite good.

The factors and interaction of factors can have a positive effect or negative effect which can be seen in the Normal Plot Graph in Figures 1, 2, and 3. In the normal plot graph on the spreadability response, there is no effect so the response equation was not obtained. In the normal plot graph on the adhesion response, it can be seen that the PVA (C), the interaction between extract

and whey protein (AB), and interaction between extract and PVA (AC) have a positive effect while the interaction between whey protein and PVA (BC) has a negative effect on the response with the equation $Y = 3.9875 + 0.1125 C + 0.1125 AB + 0.1125 AC - 0.0625 BC$. The positive effect illustrates that the greater concentration of the factors used, the adhesion value will be increased. PVA act as film-forming in this study but PVA can be used as a viscosity-increasing agent. PVA has a hydrocarbon backbone structure with -OH Hydroxyl groups. The hydroxyl group makes the PVA soluble in water and swell in water because the intermolecular hydrogen bonds occur between hydroxyl groups on PVA chains with water^{25,26}. A high concentration of PVA will make the viscosity, tensile strength, stability, and adhesivity increase²⁷. Whey protein is also a polymer that can increase viscosity because whey protein can form a three-dimensional gel structure. However, if whey protein is used at sufficient concentrations, the resulting viscosity will be good. Whey protein at a concentration of 5% still showed good viscosity properties^{28,29}. However, when whey protein is combined with the mangosteen peel ethanolic extract which is in semisolid form, the viscosity of the preparation increases so that the adhesivity of the preparation will be higher. The interaction between extract and PVA also gives the positive effect same with the interaction between extract and whey protein. But, when PVA interact with Whey Protein, the effect was negative. The combination of whey protein and PVA at high concentrations will cause more hydrogen bonds and hydrophobic interactions to occur so the aggregation will be formed^{30,31}. The aggregation causes the formation of an insoluble precipitate so that the viscosity of the preparation will be decreased³². In accordance with Stokes' law, the more precipitate formed, the lower the viscosity of the preparation. When the preparation has a low viscosity, the adhesivity of the preparation will be decreased.

In the normal plot graph on the drying time response, there was one interaction influenced the response, that is interaction between extract and whey protein (AB). The effect was positive to the response so the higher concentration of extract and whey protein used in this study will make the drying time is longer. The equation for this response was $Y = 17.7363 + 0.4579 AB$. Whey protein is less able to maintain evaporation of water so that the preparation obtained has a long drying time. Long drying time is also associated with the formation of insoluble precipitates when using high concentrations of whey protein³³.

Based on the results of spreadability, adhesivity, and drying time, the formula optimization is carried out with several criteria such as high spreadability and

adhesivity, and low drying time. Formula optimization was also carried out using software *Design Expert 11*®. The program gives the recommendation for the optimum formula which had a concentration of the mangosteen peel ethanolic extract was 2%, Whey Protein was 5%, and PVA was 12 with desirability value of 0.808. A desirability value close to 1 indicates that the formula is close to the criteria desired by the researcher³⁴. So, the F6 was chosen to be the optimum formula by the system.

The peel-off gel mask antioxidant activity test was carried out on the optimum formula, namely F6 and ascorbic acid as the comparison or positive control. Based on the result in Table 6, both F6 and ascorbic acid had the IC₅₀ less than 50 ppm so it is included in the category of antioxidants with very strong activity³⁵. From the result, F6 has a smaller IC₅₀ value than ascorbic acid, respectively 3.82 ppm and 13.98 ppm. This proves that the antioxidant activity of peel-off gel mask from ethanol extract of mangosteen peel with a concentration of 2% is better than ascorbic acid. Based on the research of Tjahjani et al.⁷, the antioxidant activity of the ethanol extract of mangosteen rind resulted in an IC₅₀ value of 7.48 ppm. The release of the active substance from the preparation is strongly influenced by the characteristics of the preparation produced, such as in the peel-off gel mask preparation, the properties of spreadability, adhesion, and drying time are important things that can affect the release of the active substance. When the viscosity of the semisolid preparation is too high, the penetration of the active substance into the skin will be smaller^{36,37}. The IC₅₀ value of the mangosteen peel ethanolic extract and peel-off gel mask of the mangosteen peel ethanolic extract was similar, it can be concluded that the peel-off gel mask preparation used in this study was able to increase the delivery of active substances so that the antioxidant activity produced was also good.

CONCLUSION:

The peel-off gel mask produced in this study has good physical properties, namely brown, pH is around 5-6, homogeneous and has good spreadability, adhesion, and drying time. Based on data analysis, there is an effect of the use of the concentration of the mangosteen peel ethanolic extract, whey protein, PVA, and the interaction between factors on the response of spreadability, adhesion, and drying time. The optimum formula produced based on the software *Design Expert 11*® is F6. The results of the antioxidant activity test showed that there was an effect of using the optimum base to the active substances release. F6 with a 2% of the mangosteen peel ethanolic extract had a better IC₅₀ than ascorbic acid as positive control.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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