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Antioxidant activity of kecombrang preserving powder using *foam mat drying* method

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Abstract. Food production is one of the easily damaged products, both in processing and during product storage. Therefore, to prevent damage made preservative powders from natural ingredients. One of them is obtained from the flowers of the kecombrang (*Etlingera elatior*). Kecombrang plant flowers contain bioactive components, namely, alkaloids, polyphenols, flavonoids, and essential oils. This study used a reasonably practical drying, namely foam-mat drying technique. This study aims to determine the best formulation in making kecombrang flower preservative powder using the foam mat drying method. This study used a randomized block design (RBD). The factors studied included maltodextrin concentration and drying temperature. The concentration of maltodextrin consists of three levels, namely 5%, 10%, and 15%, and the temperature consists of three levels, namely 50°C, 60°C, and 70°C. The variables observed were qualitative and quantitative analyses. The best treatment results qualitatively and quantitatively, namely at the drying temperature treatment of 50°C and 5% maltodextrin concentration with a water content value of 5.92%, antioxidant activity value 61.77%, total phenol levels 2.12 mg TAE / g, total flavonoid levels 0.13 mg TAE/g. The qualitative test shows that kecombrang flower preservative powder contains phenolic compounds, flavonoids, steroids, tannins, saponins, and alkaloids.

1. Introduction

The food product is one of the easily damaged products, both in processing and during product storage. Food that is damaged will experience changes caused by physical damage, chemical reactions, or the activity of organisms such as rats, parasites, insects, microbes, and others. Therefore, additional materials such as preservatives are needed to maintain food products' quality, so they are not easily damaged [1].

One of the natural ingredients used as a preservative, antioxidant, and antimicrobial is the kecombrang (*Etlingera elatior*). Kecombrang has been widely used in medicine and food additives, especially flowers, that effectively reduce microbiological damage. Kecombrang flower is an alternative natural preservative because it contains bioactive components: alkaloids, phenols, flavonoids, and essential oils [2]. The manufacture of natural preservatives from the kecombrang plant's flowers is quite useful because it contains suitable antimicrobials [3] and antioxidants [4] in preventing the damage of a food product.

The preservatives are made into powder form because the powder form has the advantages of being more durable, lighter, and has a smaller volume to make packaging more comfortable. Powdered preservatives are quite useful and can be stored for a long time compared to preservatives in

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extract or liquid form [5]. The manufacture of preservative powder uses the foam-mat drying method (foam drying technique). In this preservative powder formulation, a filler is needed, namely, maltodextrin. The use of this maltodextrin concentration is around 5-15%. This is consistent with the statement regarding the addition of maltodextrin in the range of 6-20% in the manufacture of apple powder products using the foam mat drying method, which is proven to reduce hygroscopic properties, stabilize empty cavities in the foam and increase granulation in powders [6]. The use of concentrations above 15% can reduce the quality of preservative powders. The addition of more than 20% maltodextrin can reduce polyphenol levels in kombucha tea instant drinks, where polyphenols act as antioxidants [7].

The foam drying technique's effectivity is determined by the drying speed, which can be done with the correct temperature setting and filler concentration. Too high a temperature will cause the loss of volatile compounds such as aroma and accelerate the material's browning reaction. Simultaneously, a too low temperature will cause the drying process to be less efficient and encourage damage during the process.

According to other studies, foam's presence will accelerate the evaporation process of water even without the temperature being too high; products that are dried using foam at a temperature of 50°C - 80°C can produce 2-3% water content. The powder from the foam mat drying method has a low density (light) and is crumbly [8]. Therefore, it is necessary to study the heating temperature and maltodextrin concentration as an effective filler in making preservative powder formulations in this study. Based on these descriptions, this study's objectives are to determine the best treatment on the kecombrang flower preservative powder formulation using the foam mat drying method.

2. Research Methodology

2.1. Experimental design

This research is an experimental study with the experimental design used was a Randomized Block Design. The factors observed were maltodextrin concentration, which consists of three levels, namely 5%, 10%, and 15%, and the temperature, which consists of three levels, namely 50, 60, and 70^{o}C. From these treatments obtained nine treatment combinations and carried out three times repetition to get 27 experimental units.

2.2. Preparation and Manufacture of Flower Simplicia Powder

The red kecombrang flowers are sorted into good ones, then washed first, then chopped into small pieces and evenly distributed with a knife. Then spread it out on a baking sheet and then dry it with a *cabinet dryer* at 50°C for 4 hours. The dried flowers are then ground with a disk mill grinder until a homogeneous powder is obtained.

2.3. Kecombrang Flower Extract

Kecombrang flower powder and water are put into the extractor with a ratio of 1:14. Kecombrang flower extraction process was carried out for 3 hours 42 minutes at a temperature of 60°C using an extractor.

2.4. Drying Preservative Powder with Foam-mat drying

The liquid extract of kecombrang flower was added with maltodextrin with each different concentration (5%, 10%, 15%) and 1% tween 80, then stirred using a mixer for 15 minutes foam was formed. Kecombrang flower liquid extract foam is poured into a glass plate and flattened; after that, it is put into a *cabinet dryer* for the drying process with a temperature of 50°C, 60°C, 70°C, then the dry product is mashed with mortar to obtain a homogeneous preservative powder.

2.5. Qualitative Analyses

After obtaining a homogeneous preservative powder, a qualitative analysis was carried out, namely the phytochemical test including the alkaloid, phenol hydroquinone, flavonoid, tannin, saponin, and steroid/triterpenoid test. Furthermore, the preservative powder will be analyzed

quantitatively, including moisture content, total phenol content, determination of flavonoid levels, antioxidant activity, and antibacterial activity.

2.6. Quantitative Analyses

2.6.1. Grading Total Phenol. A total of 0.4 ml of sample was added to 1.5 ml of Folin-Cicalteu 10% and stood for 5 minutes at 25°C. Then Sodium bicarbonate ($NaHCO_3$) 1.5 ml was shaken and left in a dark room for 90 minutes. Next step, the absorbance was measured using a spectrophotometer at a wavelength of 725 nm.

2.6.2. Grading Total Flavonoid. A total of 0.1 ml of sample supernatant was added to 1 ml $AlCl_3$ 2% (2 g $AlCl_3$ in 100 ml acetate acid glacial solid 5%) and 1 ml calium acetate solid 120 mm (1.176 g calium acetate in 100 ml distilled water). After that, it was incubated for 1 hour at 25°C and measured the absorbance using a spectrophotometer UV Vis of 435 nm.

2.6.3. Antioxidant Activity. 0.2 ml of sample solution for each dilution was taken and then mixed with 2.8 ml of 2,2-diphenil-1- pikrilhidrazil (DPPH) solution in a dark test tube. The solution was then vortexed and then incubated for 30 minutes; then, measured the absorbance using a spectrophotometer UV Vis of 517 nm.

Furthermore, the IC50 price is determined, namely the concentration of the sample, which has a DPPH absorbance inhibition of 50%, using regression soft wear with a value of y = 50% so that the IC50 inhibitory concentration value is obtained. The lower the IC50 value, the higher the free anti-radical activity.

2.7. Analysis Data.

The variable data obtained were analysed using ANOVA with a level of 95%, if the treatment had a significant effect, then continued with the Duncan Multiple Range Test with a level of 5%. The best formulation is done by testing the effectiveness index.

3. Result and Discussions

3.1 Qualitative Analyses

The bioactive content found in kecombrang is saponins, flavonoid, phenol, triterpenoid, tannin, and alkaloids which activities as antimicrobials and antioxidants for natural preservatives [9].

Table 1. Phytochemical analysis of kecombrang flower preservative powder

Treatment	Saponin	Flavonoid	Phenol	Triterpenoid	Tannin	Alkaloid
50°C, 5%	++	+++	++++	+++	++++	++++
50°C, 10%	++	+++	+++	++	+++	+++
50°C, 15%	++	++	+++	++	++	++
60°C, 5%	++	+++	+++	+++	+++	++++
60°C, 10%	++	++	++	++	++	+++
60°C, 15%	+++	+	++	++	-	+++
70°C, 5%	++	+++	+++	++++	++	+++
70°C, 10%	+++	++	+++	++	++	+++
70°C, 15%	+++	+	++	+	-	+++

Note: - (negative), + (weak), ++ (positive), +++ (strong), ++++ (very strong)

3.1.1. Alkaloid. In alkaloid testing, sample was added to HCl and then reagent [10]. A positive result in the Meyer test is indicated by the formation of a white precipitate. In contrast, a positive result for

alkaloids in the Wagner test is shown by forming a light brown to red precipitate. Positive alkaloid formation.

In Table 1, it can be seen that the formation of the most potent alkaloid compounds at treatment temperatures of 50°C and 60°C with a concentration of 5% maltodextrin. The appearance of an orange precipitate indicates a positive result for alkaloids in the Dragendorff analyses. These residues are metal complexes with alkaloids. In the Dragendorff reagent reaction, bismuth nitrate reacts with potassium iodide to form bismuth (III) iodide deposits, which dissolves in excess iodide potassium tetra iodo bismunate. In the alkaloid test with the Dragendorff reaction, the lone pair on nitrogen is used to form a covalent bond with bismuth to give an orange to red precipitate [11].

3.1.2. Phenol. Table 1 shows that the phenolic compounds produced from kecombrang flower preservative powder are the strongest at the lowest temperature (50°C) and a low concentration of 5% maltodextrin. The presence of phenolics is indicated by a blue to green color formed when the sample is exposed to $FeCl_3$. The more Phenol is contained, the darker the color will be. This is consistent with research that explains that phenol testing is carried out by reacting a sample of 1 mL with 5% $FeCl_3$. The presence of phenolic compounds is characterized by forming a bluish-green to blackish color [12].

3.1.3. Flavonoid. The table shows that the formed flavonoid compounds are more potent with temperature treatment and low maltodextrin concentration at 50°C. In contrast, the formed flavonoid compounds are weak if the temperature and the maltodextrin concentration are higher, 70°C. Flavonoids in kecombrang flowers contain phenolic compounds with carbonyl groups, flavones with 3-OH groups, and flavones with free ortho-dihydroxy and ortho-hydroxy carbonyls [13]. The flavonoid test with H2SO4 reagent shows a positive sample when the solution has a striking color change to yellow, red, or brown [10].

3.1.4. Saponin. In the table, it is known that the higher the temperature and the concentration of maltodextrin, the stronger the saponin content formed. Saponin testing is characterized by the formation of foam when the sample is shaken. Saponin test results yield a solution with the appearance of stable foam as high as ± 15 mm, which indicates a positive effect [14]. In general, if the result is positive, the addition of HCl 2 N is intended to increase polarity so that the hydrophilic groups will bind more stably and the formed foam becomes stable. When shaken, saponins form because of the hydrophilic groups that bind to water, while hydrophobes will attach to air. In the micellar structure, the polar groups face outwards while the non-polar groups face inwards; this forms the foam [15].

3.1.5. Steroid/Triterpenoid. In table 1, it is known that the formation of the most potent steroid content at 70oC treatment and 5% maltodextrin concentration. Test for terpenoids and steroids using Lieberman Burchard's reagent [16]. Steroid and triterpenoid tests using the Liebermann-Bouchard method, the extract was dissolved in chloroform and then added with Liebermann-Bouchard reagent (acetic acid anhydrous-H_2SO_4) showed positive results in the presence of a brownish-red color change for steroids and brown-purple for triterpenoids. The reaction of triterpenoids with Liebermann's reagent produces a red-purple color while steroids give a green-blue color. This is based on triterpenoid and steroid compounds' ability to form color by H_2SO_4 in an anhydrous acetic acid solvent. The difference in color produced by triterpenoids and steroids is due to differences in groups on the C-4 atom [17].

3.1.6. Tanin. In table 1, it can be seen that the tannin content is the strongest at a treatment temperature of 50°C and a concentration of 5% maltodextrin. The higher temperature and the maltodextrin concentration used, the lower the color density. In the treatment of maltodextrin concentration of 15%, temperature 60 and 70°C showed no tannin content in the preservative powder.

While kecombrang do not contain tannins, in Table 1, the tannin test results green to blackish blue colors were formed in several treatment samples. In testing, the $FeCl_3$ used can react with other compounds with the same color results. The main properties of tannins depend on the phenolic group contained in the tannins. Color reactions occur when combined with $FeCl_3$. $FeCl_3$ will give a green or

blue-black color when reacted with tannins. But this test is not good because, in addition to tannins which can give color reactions, other substances can also provide the same color reaction [18].

3.2. Analysis Result of Quantitative Data

3.2.1. Total Phenol. The results of the analysis of variance showed that the drying temperature had a very significant effect on the total phenol content and the maltodextrin concentration also had a very substantial impact on the total phenol content of kecombrang flower preservative powder. Meanwhile, the interaction of drying temperature and maltodextrin concentration did not significantly affect the total phenol content of kecombrang flower preservative powder. The principle of measuring total Phenol is by measuring the Folin-Ciocalteu reagent's ability to reduce phenol compounds that form blue complexes. Analysis of total phenol levels is necessary because there is a link between antioxidant and antimicrobial activity, which is influenced by phenolic compounds' composition [19].

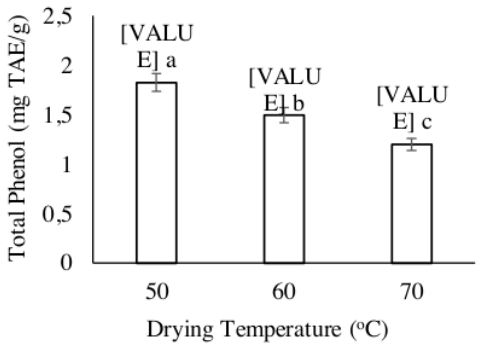

Figure 1. Effect of drying temperature

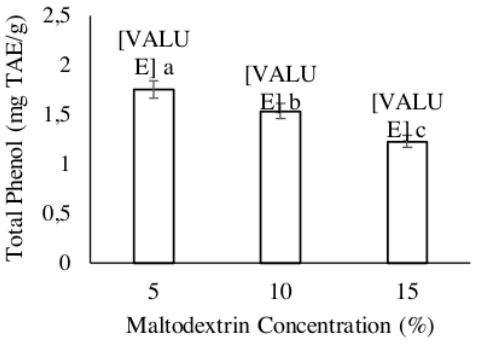

Figure 2. Effect of maltodextrin concentration

Total phenol levels against total phenol levels From Figure 1, it can be seen that the degree of drying temperature greatly affects the total phenol content, and the results are significantly different. The highest average total phenol value was obtained from the treatment at 50°C, namely 1.8248 mg TAE / g, at 60oC at 1.4948 mg TAE / g, and the lowest at 70°C at 1.1998 mg TAE / g. The decrease in the total amount of Phenol is due to the oxidation process due to heat treatment so that the drying process can reduce the content of phenol compounds [20]. The decrease may be due to chemical changes, decomposition of phenol compounds, or phenol-protein complexes' formation due to temperature and pressure [21]. The literature states that there is a relationship between temperature and phenolic content. Increasing the temperature causes an increase in phenolic content to a specific temperature, decreasing with higher temperature [22].

The phenol component in the extraction of kecombrang plant parts is thought to have a polarity close to ethanol's polarity. The use of ethanol solvents is more effective in dissolving phenol compounds [9][23]. The difference in total phenol content can be caused due to environmental factors for different kecombrang plants, such as differences in ultraviolet radiation, plant growth, soil composition, temperature, and rainfall [24].

Figure 2 also shows that maltodextrin concentration has a very significant effect on the total phenol value. The highest average total phenol value was obtained from the treatment of 5% maltodextrin concentration, namely 1.7544 mg TAE / g, at 10% maltodextrin concentration valued at 1.5358 mg TAE / g, and the lowest at 15% maltodextrin concentration worth 1.2291 mg. TAE / g. The higher the addition of maltodextrin causes a decrease in the total phenol content. This is due to the increasing number of total solids in the material, namely maltodextrin as a filler. The total Phenol measured is less, where the maltodextrin is white. Simultaneously, the complex color is blue, so when measured with a spectrophotometer, the blue color intensity decreases so that the total content phenol tends to decrease [25].

3.2.2. Total Flavonoid. The total flavonoid levels produced ranged from 0.06 to 0.13 mg TAE / g. From Figure 3, it can be seen that the degree of drying temperature has an effect on the total flavonoid levels with significantly different results. The highest mean value of total flavonoids was obtained from treatment at 50°C, namely 0.1254 mg TAE / g, at 60°C at 0.0978 mg TAE / g, and the lowest at 70°C at 0.0697 mg TAE / g. According to the literature, a temperature of 50°C is relatively safe and prevents damage to specific secondary metabolites, especially flavonoids. Flavonoids are phenolic compounds that have a conjugated aromatic system that is easily damaged on relatively long heating with high temperatures. Several groups of flavonoids have glycoside bonds with sugar molecules. Glycoside bonds will easily break or break on high-temperature heating [26].

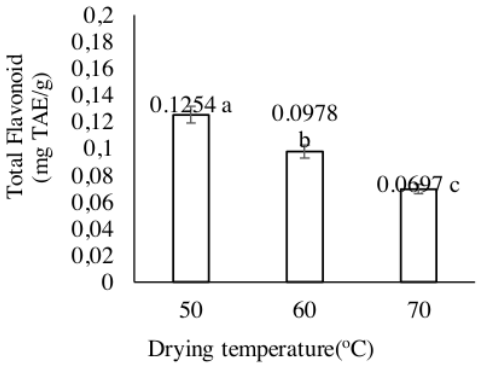

Figure 3. Effect of drying temperature on concentration total flavonoid levels

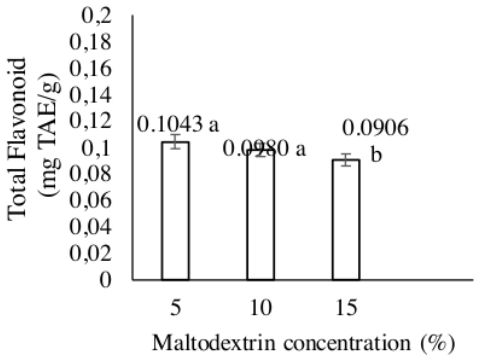

Figure 4. Effect of maltodextrin on total flavonoid levels

High temperature affects the increase in total flavonoids. This is because, at higher temperatures, contact between the materials and the solvent works well. As a result, the solvent's dissolve ability is higher when the solvent is hot and lower when it is cold. According to the literature, optimal resistance to flavonoid compounds has a temperature range of 0°C – 100°C [27].

Figure 4 also shows that the concentration of maltodextrin has a significant effect on the total flavonoid value. The highest mean value of total flavonoids was obtained from the treatment of 5% maltodextrin concentration, namely 0.1043 mg TAE / g, at 10% maltodextrin concentration worth 0.0980 mg TAE / g, and the lowest at 15% maltodextrin concentration worth 0.0906 mg TAE / g. This is directly proportional to the total phenol content and antioxidant activity, where flavonoids are phenolic compounds that act as anti-free radicals. It is related to the value of the sample's antioxidant activity. From the resulting data, it can be seen that the total flavonoid value is lower than the total phenol value. Increasing temperature and extraction time need to be considered; too high extraction temperature and long extraction time and exceeding the optimum time limit can cause the loss of compounds in the solution due to evaporation. Vice versa, if the extraction temperature is too low, it will cause not all active compounds to be extracted from ingredients, resulting in a low percentage of the active compounds obtained [28].

3.2.3. Antioxidant Activity. The analysis results showed that the treatment of drying temperature and maltodextrin concentration significantly affected kecombrang flower preservative powder's antioxidant activity. Simultaneously, the interaction of drying temperature and maltodextrin concentration has no significant effect on kecombrang flower preservative powder's antioxidant activity. The IC_{50} values obtained ranged from 49.49 - 204.44 ppm.

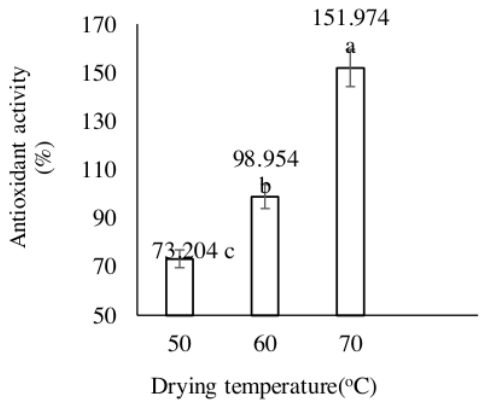

Figure 5. Effect of drying temperature on Antioxidant Activity

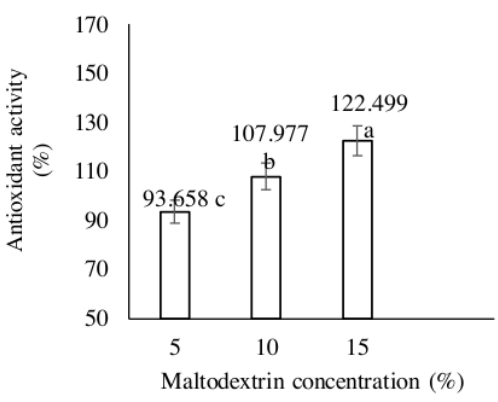

Figure 6. Effect of maltodextrin concentration on Antioxidant Activity

From Figure 5, it can be seen that the higher the temperature can cause the antioxidant activity to decrease. The most increased antioxidant activity was at 50°C with an IC_{50} value of 73.204%; at 60°C, it became 98.954%, and the lowest at 70°C was around 151.974%. The antioxidant activity obtained was calculated the IC_{50} value with a linear regression equation. The IC_{50} value is inversely proportional to the antioxidant ability of a compound contained in the test material. The smaller the IC_{50} value, the greater the antioxidant capacity. When the electrons become paired by a free radical scavenger's presence, the absorbance decreases stoichiometry according to the number of electrons taken. The presence of antioxidant compounds can change the color of the DPPH solution from purple to yellow. The absorbance changes resulting from this reaction have been used extensively to test the ability of some molecules to scavenge free radicals [29].

Other studies suggest that the antioxidant activity decreases when the drying temperature is high [30]. This is because the higher heating temperature causes the secondary metabolites that act as antioxidants (flavonoid compounds) to become damaged. The literature states that the higher the drying temperature, the lower the antioxidant activity and impair the sample's antioxidant activity [31].

Based on Figure 6, it can be seen that as well as temperature, the effect of maltodextrin concentration also has a very significant impact on antioxidant activity, so that the higher the maltodextrin concentration causes the IC_{50} value to increase, causing the ability to capture free radicals from bioactive compounds of kecombrang flowers to decrease. The moderate antioxidant activity against the highest concentration of maltodextrin was at a concentration of 5% worth 93.658%, while the lowest was at a concentration of 15%, namely 122.499%. A substance has antioxidant properties when the IC_{50} value is less than 200 ppm. If the IC_{50} value obtained is between 200-1000 ppm, the meaning is less active but still has potential as an antioxidant [32].

The addition of higher maltodextrin concentration causes a decrease in antioxidant activity levels. This is thought to be due to the increasing number of total solids contained in the material, namely maltodextrin as a filler so that the measured antioxidant activity is less so that with the increase in the total solids in a material, the measured levels of antioxidant activity will be smaller [33]. Also, it is thought that it is also caused by changes in antioxidant compounds due to the heating process, namely vitamin C and other phenol compounds that are oxidized. It is possible that heating causes phenol compounds to decompose so that their ability as an antioxidant decreases. The antioxidant activity in noni leaf instant drinks is closely related to total Phenol and vitamin C. So that by decreasing the concentration of phenol and vitamin C, the flavonoid content will decrease and cause the antioxidant activity to decrease [25].

4. Conclusion

Kecombrang flower preservative powder formulations positively contain bioactive compounds, including phenolic compounds, flavonoids, saponins, steroids, and alkaloids. Except for negative tannins at treatment temperatures of 60 and 70°C with a concentration of 15% maltodextrin. The best formulation was obtained at a temperature treatment of 50°C with a concentration of 5% maltodextrin. It produces the highest results at an antioxidant activity value of 61.77%, total phenol content of 2.12 mg TAE / g, and total levels of flavonoids 0.13 mg TAE / g.

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