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# CHEMICAL PROPERTIES AND TOTAL PLATE COUNT OF DODOL PACKAGED IN ACTIVE PACKAGING WITH THE ADDITION OF KLUTUK BANANA LEAF (*MUSA BALBISIANA COLLA*) EXTRACT

Safinta Nurindra Rahmadhia*
Naufal Haudry Sigit
Titisari Juwitaningtyas

Food Technology Study Program, Faculty of Industrial Technology, Universitas Ahmad Dahlan

## ABSTRACT

Food packaging is a material or tool used to contain or wrap food, whether in direct or indirect contact. Active packaging is packaging designed to extend shelf-life or to maintain or improve the quality of packaged food. The concept of active packaging is to add certain components to the packaged food system or the surrounding environment. Dodol is a perishable food because it has a high-water content. The deterioration typically manifests as the presence of microbial growth and rancidity on the dodol. To increase the shelf life and quality of dodol is by wrapping it with active packaging. This study aimed to determine the effect of using active packaging on the chemical properties (water content, peroxide number, and antioxidant activity) and total plate count (TPC) of dodol. Active packaging made from cassava starch with the addition of klutuk banana leaf extract (KBLE) of the wulung variety as a source of antioxidant compounds is made by casting method. This research was conducted in two variables, namely the Wulung variation of KBLE concentration (0% and 20% w/v) and storage time (0; 5; 10; 15; 20; 25; and 30 days). The results of this study indicate that the active packaging (F2) applied to dodol has a significant effect ($p<0.05$) on the water content, peroxide value, antioxidant activity, and TPC values of dodol. Dodol packaged using active packaging after 30 days had lower water content, peroxide value, and TPC values than dodol packaged without KBLE. In addition, the value of antioxidant activity in dodol and active KBLE packaging obtained higher values (37.5% on days 30th) than packaging that was not given KBLE.

**Keywords:** active packaging; antioxidant; banana leaf; cassava starch; shelf life

## ABSTRAK

Pengemas pangan adalah bahan atau alat yang digunakan untuk mewadahi atau membungkus pangan, baik yang bersentuhan langsung maupun tidak langsung. Pengemasan aktif adalah pengemasan yang dirancang untuk memperpanjang umur simpan atau untuk mempertahankan atau meningkatkan kualitas makanan yang dikemas. Konsep pengemasan aktif adalah menambahkan komponen tertentu ke dalam sistem makanan yang dikemas atau lingkungan sekitarnya. Dodol merupakan makanan yang mudah rusak karena memiliki kandungan air yang tinggi. Kerusakan yang terjadi biasanya ditandai dengan munculnya jamur dan ketengikan pada dodol, Untuk meningkatkan daya simpan dan kualitas dodol adalah dengan membungkusnya dengan kemasan aktif. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh penggunaan kemasan aktif terhadap sifat kimia (kadar air, angka peroksida, dan aktivitas antioksidan) dan angka lempeng total (TPC) dodol. Kemasan aktif berbahan dasar pati singkong dengan penambahan ekstrak daun pisang klutuk (KBLE) varietas Wulung sebagai sumber senyawa antioksidan dibuat dengan metode pengecoran. Penelitian ini dilakukan pada dua variabel yaitu variasi konsentrasi KBLE Wulung (0% dan 20% b/v) dan lama penyimpanan (0; 5; 10; 15; 20; 25; dan 30 hari). Hasil penelitian ini menunjukkan bahwa kemasan aktif yang diaplikasikan (F2) pada dodol berpengaruh nyata ($p<0.05$) terhadap kadar air, bilangan peroksida, aktivitas antioksidan, dan nilai TPC dodol. Dodol yang dikemas menggunakan kemasan aktif setelah 30 hari memiliki kadar air, bilangan peroksida, dan nilai TPC yang lebih rendah dibandingkan dodol yang dikemas tanpa KBLE. Selain itu, nilai aktivitas antioksidan pada kemasan dodol dan KBLE aktif diperoleh nilai yang lebih tinggi (37.5% pada hari ke 30) dibandingkan kemasan yang tidak diberi KBLE.

**Kata kunci**: antioksidan; daun pisang; kemasan aktif; tepung singkong; umur simpan

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**Corresponding author:**
**Safinta Nurindra Rahmadhia**
Tangerang 15138, Indonesia
Email:
safinta.rahmadhia@tp.uad.ac.id

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## INTRODUCTION

Food packaging is an important matter in the food industry which functions to wrap, protect, and serve food ingredients or products [1]. In addition, packaging also plays an important role in presenting information and during the process of food transportation. Packaging is also a major part of food which plays a role in increasing consumer interest in buying the product [2], [3]. Consumer awareness regarding food safety and the use of biodegradable food packaging to enhance the shelf life of packaged items is growing in this decade. Therefore, several researchers have conducted research on packaging made from natural ingredients that can protect and increase the shelf life of food products [4]–[7].

Active packaging is a system designed to maintain or improve the quality of packaged food products in terms of sensory, safety and quality. The use of active packaging as a wrapper is usually used as a primary packaging in direct contact with the packaged product [8]. Antioxidant and/or antimicrobial compounds are compounds that are commonly used as additives in packaging. Several types of active packaging can prevent the growth of microorganisms, respiration, rancidity, improve freshness, safety, quality, and reduce the water content of food products. Hence, it is very possible that active packaging can increase the shelf life of the packaged food products [7], [9]. The ingredients for active packaging are very diverse, ranging from fat, starch and protein [10].

Starch is one of the active packaging materials that is widely used because of its abundant availability and low price [11]. Starch is a semi-crystalline polymer that is produced naturally in granular form by certain plants, such as roots, tubers, and cereals, and acts as an energy reserve [12], [13]. Starch-based biofilms have the properties of being renewable, degradable, good oxygen barrier ability, transparent, odorless, and flexible [14]. The use of cassava starch as an active packaging material has been carried out in various studies. So, it has been proven that cassava starch has an important role in the biodegradable and active packaging industry [15], [16].

Dodol is a food made from fruit and perishable. The critical point of dodol product that often occurs is rancidity and spoilage [17]. Prevention of rancidity in dodol can be done by using ingredients containing antioxidant compounds. Then, rotting can also be prevented by using materials that contain antimicrobial compounds. Based on the compounds inserted in active packaging, one of the principles is to be able to release these compounds into food, absorb, and block so that product quality and safety increase [7], [18]. This project is designed to develop novel dodol packaging that incorporates active chemicals to enhance its shelf life. To enhance the durability of dodol, one can incorporate antioxidant and antibacterial substances into the film matrix. This will effectively hinder the development of rancidity and the proliferation of harmful microorganisms in dodol.

In this study, active packaging was made to pack dodol using klutuk banana leaf extract (KBLE) of the wulung variety. The active packaging produced is based on cassava starch. Dodol was stored at room temperature for 30 days and analyzed for water content, peroxide value, antioxidant activity, and total plate count (TPC) every 5 days. Antioxidant activity was also analyzed on the film used to pack dodol on the same day.

## MATERIALS AND METHOD

The materials used in this study were klutuk banana leaves of the wulung variety obtained from the Dinas Pertanian dan Pangan Kota Yogyakarta and cassava starch (Pak Tani Gunung, Indonesia). Salak’s dodol obtained from one of the SMEs in Yogyakarta. The chemicals reagent used for analysis were aquadest, gliserol (Gamma Scientific Biolab, Indonesia), petroleum eter (Merck, Germany), N-heksane (Merck, Germany), and plate count agar (Himedia, USA).

The tools used in this study were oven (Memmert, Germany), blender (Philips 2115, Indonesia), 60 mesh sieve, waterbath shaker (Memmert, Germany), hotplate (Thermo Scientific Cimarec, USA), desiccator, centrifuge (Oregon, China), vortex (Gemmy WM-300, Taiwan), and spectrophotometer UV-Vis (B-One, China).

### 1. Extraction of Klutuk Banana Leaf

The production of KBLE includes two stages, namely the manufacture of klutuk banana leaf powder and extraction. The manufacture of klutuk banana leaf powder refers to research [9] with modifications. Banana leaves that have been cleaned using tap water are then cut and dried in a cabinet dryer. Drying was carried out for 24 hours at 50°C then the banana leaves were mashed using a blender and sieved with a 60-mesh sieve.

The extraction process was carried out using the maceration method with distilled water for 1 hour at room temperature. The 10 g of klutuk banana leaf powder of the wulung variety is mixed with 100 ml of distilled water and then shaken using a shaker-waterbath at 50°C at 150 rpm for 1 hour. Furthermore, it was filtered using wathmann filter paper no. 41 to obtain a yellowish klutuk banana leaf extract [19].

### 2. Active Packaging Production

Active packaging is made based on research [20] which has been modified. Tapioca flour was weighed as much as 7.5 g and then put into a 250 ml glass beaker. A 91 ml of distilled water was added, then mixed and heated at 130°C at 200 rpm for 15 minutes. Next, 20% w/v KBLE and 1.5 g glycerol were added and stirred for 1 hour. The film solution was casted on a glass plate (15 × 21 cm) and dried in an oven at 50°C for 14 hours. Control films are made without adding KBLE.

### 3. Application of Active Packaging on Dodol

The salak fruit dodol is coated with KBLE film on its entire surface. Plastic wrap was chosen as secondary packaging to prevent release of active compounds into the environment. Dodol packed with KBLE film was stored for 30 days at 25ºC.

### 4. Water Content Analysis

Analysis of water content using the gravimetric method [21]. Moisture analysis was only carried out on dodol packed with KBLE film on days 0, 5, 10, 15, 20, 15 and 30. The water content value is calculated using Equation 1.

$$Water\ content\ (\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \qquad (1)$$

Description:
$W_0$ = weight of empty cup (g)
$W_1$ = weight of cup and sample before heating (g)
$W_2$ = weight of cup and sample after heating (g)

### 5. Peroxide Number Analysis

Analysis of peroxide number using method [22]. Peroxide number was only carried out on dodol packed with KBLE film on days 0, 5, 10, 15, 20, 15 and 30. The peroxide number is calculated using Equation 2.

$$Peroxide\ number\ (meq/kg) = \frac{1000 \times N \times (V_0 - V_1)}{W} \qquad (2)$$

Description:
N = normality of $Na_2S_2O_3$ solution
$V_0$ = initial volume of $Na_2S_2O_3$ solution (ml)
$V_1$ = final volume of $Na_2S_2O_3$ solution (ml)
W = weight of sample (g)

### 6. Antioxidant Activity Analysis

Analysis for antioxidant activity in film and dodol used a modified method [23]. The film that had been applied as dodol packaging was weighed as much as 0.04 g and then crushed using a mortar for 15 minutes. Then 10 ml of 96% methanol was added, then baked at 60°C for 10 hours. After that, the DPPH test was carried out to determine the levels of antioxidants in the applied KBLE film.

Analysis of the antioxidant activity of dodol was carried out by taking 1 g and then crushing it using a mortar for 15 minutes. Added 10 ml of 96% methanol, then vortexed at 300 rpm for 5 minutes. Then put it in a centrifuge at 4000 rpm for 1 hour and then do the DPPH test. Antioxidant activity on film and dodol were carried out on days 0, 5, 10, 15, 20, 15 and 30. The antioxidant activity is calculated using Equation 3.

$$\%RSA = \frac{(Absorbansi\ blanko - Absorbansi\ sampel)}{Absorbansi\ blanko} \times 100\% \quad (3)$$

### 7. Total Plate Count Analysis

Bacterial analysis testing used the TPC method which refers to research [24]. The TPC test uses the pour/pour plate method. Samples were taken as much as 1 ml (all dilution series) and poured into a sterile petri dish. Then 10 ml of plate count agar (PCA) media was poured into the petri dish. Then flatten by shaking the petri dish and incubated at 20°C for 24 to 48 hours.

## RESULTS AND DISCUSSION

### 1. Water Content

The results of the analysis of water content in *dodol* packaged with KBLE film can be seen in Table 1. The water content values ranged from 13.85 ± 0.12% - 22.80 ± 0.33%. From these data (Table 1) it can be seen that the water content of *dodol* has increased significantly with storage time. On the 30th day, the water content of *dodol* with F1 packaging had a higher value than that of *dodol* with F2. KBLE active packaging is proven to reduce the rate of water absorption in *dodol*. This is in accordance with research [25] which added citronella essential oil to starch films which also showed better moisture barrier properties compared to films without essential oils.

The film's ability to block water vapor from entering food is known as water vapor permeability (WVP). The water content in *dodol* is related to the ability of WVP on the KBLE film. Film based on cassava starch with the addition of anthocyanin extract *Lycium ruthenicum Murr* was proven to be able to reduce WVP values. This happens because there are intermolecular bonds such as hydrogen bonds between starch and extracts. Newly formed hydrogen bonds can reduce the availability of hydrophilic hydroxyl groups in starch and extracts, thereby reducing the affinity of the starch-extract film for water vapor [26], [27]. Hydrogen/covalent interactions between biopolymer networks and polyphenolic compounds (flavonoids, tannin, and other phenolic compounds) limit the availability of hydroxyl groups to form hydrophilic bonds with water [28], [29].

**Table 1.** Water content of *dodol* with KBLE film

| Time (day) | F1 (%) | F2 (%) |
|---|---|---|
| 0 | 13.85 ± 0.12$^{a}$ | 13.90 ± 0.07$^{a}$ |
| 5 | 14.78 ± 0.21$^{b}$ | 14.83 ± 0.32$^{b}$ |
| 10 | 15.02 ± 0.22$^{c}$ | 16.34 ± 0.24$^{d}$ |
| 15 | 20.90 ± 0.36$^{f}$ | 20.51 ± 0.17$^{e}$ |
| 20 | 21.83 ± 0.11$^{g}$ | 21.60 ± 0.19$^{g}$ |
| 25 | 22.74 ± 0.24$^{h}$ | 21.96 ± 0.04$^{g}$ |
| 30 | 22.80 ± 0.33$^{h}$ | 21.89 ± 0.21$^{g}$ |

Note: Letter symbols indicatesignificant differences for each treatment (p<0.05) in the same column and row. F1: without KBLE; F2: KBLE 20 g.

### 2. Peroxide Number

The peroxide value is generally related to the rancidity of a food ingredient. Rancidity can appear due to the presence of oxidized fat. Oxidation can be prevented by the packaging materials used, avoiding exposure to direct sunlight, and adjusting the humidity accordingly during the storage process [30], [31]. In addition, the peroxide value can also increase due to enzymatic reactions. The mold that grows in *dodol* secretes an enzyme which is included in the lipase enzyme which can convert fat into free fatty acids and glycerol [32].

**Table 2.** Peroxide number of *dodol* with KBLE film

| Time (day) | F1 (meq/kg) | F2 (meq/kg) |
|---|---|---|
| 0 | 3.30 ± 0,53$^{ab}$ | 3.20 ± 0,48$^{ab}$ |
| 5 | 3.86 ± 0,61$^{bc}$ | 3.86 ± 0,80$^{bc}$ |
| 10 | 4.53 ± 0,50$^{cd}$ | 4.07 ± 0,50$^{bcd}$ |
| 15 | 5.06 ± 0,70$^{de}$ | 4.53 ± 0,50$^{cd}$ |
| 20 | 5.66 ± 0,80$^{e}$ | 4.73 ± 0,58$^{cde}$ |
| 25 | 3.73 ± 0,64$^{bc}$ | 2.47 ± 0,46$^{a}$ |
| 30 | 3.33 ± 0,99$^{ab}$ | 2.40 ± 0,53$^{a}$ |

Note: Letter symbols indicate significant differences for each treatment (p<0.05) in the same column and row. F1: without KBLE; F2: KBLE 20 g.

The results of the analysis of the peroxide value of *dodol* packed with KBLE film can be seen in Table 2. The peroxide value ranged from 2.40 ± 0.53 meq/g - 5.66 ± 0.80 meq/g. From these data (Table 2) it can be seen that the *dodol* peroxide value increased significantly until the 20th day, but then decreased until the 30th day. The presence of KBLE containing antioxidants is thought to prevent oxidation from occurring in the sample, so that *dodol* with F2 has a lower peroxide value. The inclusion of natural extracts with antioxidant activity in packaging materials can protect foods from lipid oxidation, thereby increasing their shelf life [31].

This research is in line with research [33] which states that active packaging made from chitosan with essential oils containing antioxidants can slow down oxidation when applied as a packer (sachet) to butter. This phenomenon can occur due to the free radical barrier properties of antioxidants [34]. Antioxidants act as donors of hydrogen atoms which can retard or prevent oxidative reactions from occurring [35], [36].

## 3. Antioxidant Activity

The results of the analysis of the antioxidant activity of *dodol* and film KBLE can be seen in Table 3 and Table 4 respectively. The antioxidant activity of *dodol* ranged from 34.42 ± 0.13% - 70.99 ± 1.06%, whereas antioxidant activity of film KBLE ranged from 3.08 ± 0.13% - 9.93 ± 0,07%. The antioxidant activity of both decreased with storage time. The value of antioxidant activity in *dodol* packaged using F2 was higher than F1. This can happen because the antioxidants in the KBLE film migrate into the *dodol*. This phenomenon is in accordance with research [9] which simulated the release of antioxidants in watery and fatty foods. The antioxidant activity of the KBLE films had the same migration profile, which increased in the first 2 hours and remained constant or decreased after 72 hours.

The decreased antioxidant activity in the KBLE film was the result of the release of antioxidants in the film. By migrating antioxidant compounds from the KBLE film, *dodol* has a lower peroxide value than *dodol* packed with F1. In the study [37] it was also explained that a film made from potato starch with the addition of thymol, which was tested for the release of antioxidant compounds, showed that the three food simulants that were given the film had increased antioxidant activity. This shows that there is a process of migrating compounds from the film to the food.

The incorporation of an active compound of technological interest (e.g., antimicrobial, antioxidant, etc.) into a packaging material constitutes active packaging [38]. The active compound is then gradually released at an appropriate rate from the active packaging into the atmosphere surrounding the food product. As a result, active packaging is an effective technique for extending product shelf life [39].

**Table 3.** Antioxidant activity of *dodol* with KBLE film

| Time (day) | F1 (%RSA) | F2 (%RSA) |
|---|---|---|
| 0 | 65.73 ± 0.77$^{h}$ | 68.02 ± 0.74$^{i}$ |
| 5 | 67.93 ± 0.50$^{i}$ | 70.99 ± 1.06$^{k}$ |
| 10 | 65.86 ± 0.26$^{h}$ | 69.87 ± 0.13$^{j}$ |
| 15 | 63.71 ± 0.92$^{g}$ | 64.46 ± 0.13$^{g}$ |
| 20 | 53.08 ± 0.13$^{e}$ | 56.16 ± 0.26$^{f}$ |
| 25 | 39.83 ± 0.13$^{c}$ | 45.24 ± 0.13$^{d}$ |
| 30 | 34.42 ± 0.13$^{a}$ | 37.50 ± 0.26$^{b}$ |

Note: Letter symbols indicate significant differences for each treatment ($p<0.05$) in the same column and row. F1: withoutKBLE; F2: KBLE 20 g.

**Table 4.** Antioxidant activity of used KBLE film

| Time (day) | F1 (%RSA) | F2 (%RSA) |
|---|---|---|
| 0 | 9.50 ± 0.12$^{gf}$ | 9.62 ± 0.01$^{fh}$ |
| 5 | 9.82 ± 0.05$^{fh}$ | 9.93 ± 0.07$^{h}$ |
| 10 | 9.24 ± 0.13$^{g}$ | 9.79 ± 0.13$^{h}$ |
| 15 | 8.40 ± 0.26$^{f}$ | 8.21 ± 0.26$^{f}$ |
| 20 | 4.66 ± 0.26$^{c}$ | 7.74 ± 0.13$^{e}$ |
| 25 | 5.88 ± 0.13$^{d}$ | 4.66 ± 0.26$^{c}$ |
| 30 | 3.08 ± 0.13$^{a}$ | 4.20 ± 0.13$^{b}$ |

Note: Letter symbols indicate significant differences for each treatment ($p<0.05$) in the same column and row. F1: without KBLE; F2: KBLE 20 g.

## 4. Total Plate Count

The results of the TPC of *dodol* packed with KBLE film can be seen in Table 5. The TPC value ranged from 2.50 ± 0.70 CFU/g – 31.00 ± 2.80 CFU/g. *Dodol* packed with F2 has a significantly lower TPC value than *dodol* packed with F1. KBLE which also has antimicrobial properties plays a role in reducing the microbes in the *dodol*. By controlling the release rate, active substances on the food surface can maintain the optimal concentration to inhibit microorganism growth and ensure maximum food quality and safety [40]. In fatty foods such as peanut butter and chocolate, it has been observed that these foods often contain microorganisms such as *Salmonella, Escherichia coli and Enterobacteriaceae* [41].

The results of TPC observations show that with increasing storage time, the number of microorganisms that grow on these foodstuffs will also increase. As with time, temperature also affects the growth of microorganisms. Temperature is an important factor for the growth of microorganisms because each microorganism has an optimum temperature for its growth [42].

Table 5. TPC of *dodol* with KBLE film

| Time (day) | F1 (CFU/g) | F2 (CFU/g) |
|---|---|---|
| 0 | 2.50 ± 0.70$^{a}$ | 2.50 ± 0.00$^{a}$ |
| 5 | 3.50 ± 0.70$^{a}$ | 3.00 ± 1.41$^{a}$ |
| 10 | 11.00 ± 1.41$^{cd}$ | 5.50 ± 0.71$^{ab}$ |
| 15 | 14.00 ± 1.41$^{de}$ | 8.50 ± 0.71$^{bc}$ |
| 20 | 17.50 ± 0.71$^{f}$ | 12.50 ± 0.71$^{d}$ |
| 25 | 25.50 ± 2.1$^{h}$ | 17.00 ±1.41$^{ef}$ |
| 30 | 31.00 ± 2.8$^{i}$ | 21.50 ± 2.12$^{g}$ |

Note: Letter symbols indicate significant differences for each treatment (p<0.05) in thesame column and row. F1: without KBLE; F2: KBLE 20 g.

The release packaging system is a type of active packaging in which active substances diffuse to the food surface or top wrapper to inhibit microbial growth, lipid oxidation, and rancidity of foods. Some technologies allow the rate of release of some active substances over time to be regulated so that their concentrations on the food surface can be maintained to prevent food oxidation and spoilage, thereby extending shelf life [43]. In this KBLE film, it can be proven that the packaged *dodol* has decreased the amount of TPC. So that the KBLE film can be called a release packaging system.

## CONCLUSION

Based on the research that has been done, it can be concluded that cassava starch-based active packaging with KBLE can significantly (p<0.05) reduce the water content, peroxide value, and TPC in dodol. In addition, the antioxidant compounds from KBLE present in the film can be released into food by decreasing the value of antioxidant activity in the KBLE film.

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