



## ANTIOXIDANT ACTIVITY AND COLOR OF BEEF JERKY WITH KLUWEK

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### Abstract

Spoilage that often occurs in jerky is generally caused by the fat oxidation process, either during the manufacturing process, heating, or storage, and can lead to health hazards. This study was conducted to examine the potential of kluwak as a natural antioxidant that can reduce the oxidation process, by exploring its effects on antioxidant activity and physical properties, namely  $L^*a^*b^*$  color values and cooking loss of jerky with the addition of kluwak. Kluwak, originating from community gardens in Soppeng Regency, Makassar, Indonesia, was used as an additional ingredient in making ground beef jerky. The part of meat used was the thigh, obtained from a slaughterhouse, and different levels of kluwak — namely 0%, 2%, 4%, and 6%—were added. The research results show that the addition of kluwak to jerky increases antioxidant activity, as indicated by higher values in the antioxidant activity test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies increased antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters  $a^*$  and  $b^*$ , indicating positive changes in the visual appearance of the product. However, no significant effect was found for the  $L^*$  color parameter, which measures brightness, and no difference was observed in the cooking loss of the jerky. In conclusion, kluwak can function as an effective natural antioxidant in reducing fat oxidation in jerky, while providing positive changes in product color without affecting brightness or cooking loss.

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### Introduction

Beef is a highly nutritious food commodity with an average content of 77.65% water, 14.7% fat, and 18.26% protein. It is commonly used in ground form or various processed forms in food menus [1]. The role of meat is very important, especially in meeting the community's need for animal protein. Meat is a food product derived from livestock that is rich in nutrients but highly perishable. Meat spoilage can be caused by physical, chemical, or biological contamination [2]. The high water and protein content in meat makes it prone to spoilage, which reduces its usability and shelf life. To overcome this, preservation or processing methods are applied [3].

Preserving and processing meat into various products aims to minimize quality degradation and extend shelf life, while also adding value to the final products [4]. *Dendeng*, or Indonesian beef jerky, is a well-known processed beef product, both domestically and internationally. It is generally prepared with different spice blends, resulting in varying aromas and flavors [5]. *Dendeng* is produced using drying technology to reduce moisture content, thus making the product safe and inhibiting bacterial growth and reproduction [6]. *Dendeng* is classified as an Intermediate Moisture Food (IMF), typically with an  $a_w$  range of 0.60–0.90 and a moisture content of 10–50% [7]. The maximum allowable water content for

beef jerky is 12%. The use of sugar can help reduce the water content in beef jerky, inhibit microbial growth, and extend its shelf life [3].

Through the drying process, jerky can be stored longer due to its lower water content compared to fresh meat. Jerky is a sheet-shaped product made from fresh or frozen meat that is cut or ground, then seasoned and dried [8]. In addition, the process of making *dendeng* also considers the homogeneous distribution of spices, which is a key factor in enhancing shelf life [9]. Ground beef jerky is a meat product made from ground beef that is seasoned, shaped into thin sheets, and dried [4]. In the production of ground beef jerky, spice absorption is better compared to sliced jerky. The grinding process improves the taste and texture of the jerky as the added seasonings are more evenly distributed throughout the product [10]. However, ground jerky is more susceptible to oxidation during processing.

Processing meat into jerky increases the formation of malondialdehyde (MDA), a secondary product of lipid oxidation [11]. Spoilage caused by fat oxidation leads to a reduction in nutritional value and deterioration of flavor in the final product [8]. Lipid oxidation negatively affects meat quality by altering its sensory attributes and nutritional composition [12]. The oxidation process during processing and storage is influenced by fat content, myoglo-

bin, oxidative enzymes, heat, light, and water activity [13]. Fat oxidation in meat produces off-flavor compounds, especially aldehydes [14]. The oxidation of myoglobin to metmyoglobin also causes discoloration, turning the meat brown [15].

Free radicals formed during oxidation can damage meat molecules and accelerate spoilage. An imbalance between free radicals and antioxidants can cause oxidative stress, which may negatively impact health [15]. Free radicals generated during meat processing oxidize fatty acids, especially polyunsaturated fatty acids, through radical chain reactions [16]. The level of lipid oxidation in jerky can be reduced by adding ingredients or spices that contain antioxidants [8]. Antioxidants play a crucial role in preventing and treating various chronic diseases associated with oxidative stress [17]. Antioxidant compounds found in fruits and vegetables include vitamin C, vitamin E,  $\beta$ -carotene, and polyphenols [18]. Natural antioxidants in plants are generally phenolic or polyphenolic compounds, including flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids [19].

Antioxidants can be derived from both natural and synthetic sources. Several studies have reported plant-derived bioactive compounds as natural antioxidants. Some natural sources of antioxidants include cloves, kluwak, cinnamon, cumin, and fennel seeds [20]. In addition to preventing rancidity, natural antioxidants in food also offer potential health benefits for consumers.

One of the traditional spices with antioxidant potential is kluwak (*Pangium edule* Reinw.). Kluwak contains flavonoids, vitamin C, iron ions, and  $\beta$ -carotene, all of which act as antioxidants [21]. Kluwak can serve not only as a flavor enhancer but also as a natural colorant. Color influences the quality and appeal of meat products, reflecting their biochemical, physiological, and technological status [22]. Food with visually appealing colors is more likely to attract consumers, making color an important indicator of food quality [23,24].

Kluwak fruit and seeds are commonly used in Indonesia as ingredients in *ise' pangi* and *lope' pangi* dishes, as well as in traditional cooking [25]. Kluwak is valued for imparting a blackish-brown color in cooking. The best time to use kluwak is when it is ripe, which can be identified by the sound produced when the seed is shaken [26]. The addition of kluwak to meat products such as beef sausages has been shown to increase antioxidant activity during storage [17]. However, there have been no previous reports on the use of kluwak seeds in jerky production. This gap forms the basis for the present study, which explores the use of kluwak (*Pangium edule* Reinw.) as an antioxidant and enhancer of the physical properties of jerky. Therefore, this study aims to determine the antioxidant activity and physical characteristics, including color ( $L^*a^*b^*$ ) and cooking loss, of jerky with the addition of kluwak at concentrations of 0%, 2%, 4%, and 6%.

## Objects and methods

The objects of the study were beef samples obtained from the Manggala Slaughterhouse, Makassar City, South Sulawesi Province, Indonesia. Thigh cuts of Balinese cattle were immediately transported to the laboratory using a Modena MD20 A freezer (Modena, Italy) at a low temperature of  $4 \pm 1^\circ\text{C}$  under strict aseptic conditions. Prior to processing, the meat was thawed in a refrigerator for 24 hours.

Kluwak was obtained from community gardens (Figure 1), and brown sugar was purchased from traditional markets in Soppeng Regency, Makassar City, South Sulawesi Province. The research samples were analyzed at the Meat and Egg Processing Technology Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar.



**Figure 1.** Kluwak (primary data, taken from the kluwak tree garden in Soppeng district)

Other ingredients in this study, such as coriander, salt, pepper, garlic, and galangal, were obtained from the Tello Market in Makassar City, South Sulawesi Province, Indonesia. The materials tested included 0.01% butylated hydroxytoluene (BHT) as a synthetic antioxidant for comparison, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and methanol.

This research was conducted experimentally using a Completely Randomized Design (CRD), with four levels of kluwak treatment (P0: 0%, P1: 2%, P2: 4%, and P3: 6%) and an additional reference solution treatment (BHT) (P4), each with three replications. P0 was used as a control and P4 as a comparison for synthetic antioxidants.

To make fermented kluwak, kluwak seeds were washed first to remove dirt, then boiled for 1 hour, and then dried. The kluwak seeds were buried in the soil for 40 days [24]. After that, they were cleaned, and brownish kluwak seeds were obtained. Kluwak seeds were cracked, then the non-bitter kluwak flesh was taken by prying it from the shell, and the kluwak was ready to use. It was then mixed with other spices and applied to the ground beef.

The ground meat, processed using a meat grinder (Type TC-12C, Gea Getra, China) with a 6 mm plate hole size, was then weighed and grouped based on its treatment. After that, fine spices were added to 250 g of meat according to each treatment: 3% salt (7.5 g), 34% brown sugar (85 g), 2.5% coriander (6.25 g), 1.5% garlic (3.75 g), 0.3% galangal (0.75 g), 0.3% pepper (0.75 g), tamarind (0.25 g), and kluwak at 0%, 2%, 4%, 6% (0 g, 5 g, 10 g, 15 g) [15]. Beef jerky without the addition of kluwak (0%) was added with BHT

at 0.01% of the meat weight (250 g). The mixture was then evenly blended using a food processor (Braun, Germany) and stored for 24 hours in a refrigerator Model MD10 W (Modena, Italy) [27].

After that, the dough was molded using a 3 mm thick mold, and the jerky was dried using a mold made from acrylic to maintain the desired thickness as preferred by consumers in Makassar City, Indonesia. Then, it was dried using a food dehydrator (Getra, China) by air-drying (at a temperature of 70°C for 4 hours) so that the outer layer of the meat dried first. Heating was continued (at a temperature of 70°C for 2 hours) by rotating the tray so that the heat could be evenly distributed [8,28,29]. The dried jerky was cooled at room temperature in the oven and then analyzed.

**Antioxidant activity analysis**

Testing was carried out using the method used by [29]. The sample extraction ratio to methanol was 1:5 for homogenized and modified foods [30]. A total of 0.4 ml of beef jerky extract was reacted with 3.6 ml of DPPH (with a concentration of 0.1 mM). The mixture was then incubated at 37°C for 30 minutes. Pure methanol was used as a reference material in the calibration of the SHIMADZU UV-1800 UV-VIS spectrophotometer (Shimadzu Corporation, Japan). The absorbance value of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [29]. The amount of the antioxidant activity was calculated using the formula:

$$\text{DPPH inhibition (\%)} = \frac{\text{Absorbance of DPPH} - \text{absorbance of sample}}{\text{Absorbance of DPPH}} \times 100\% \quad (1)$$

*L\*a\*b\* color of jerky*

The color testing of beef jerky was carried out using the CIE Lab method using a color reader or TES-135A Color Meter Color Analyzer Portable (TES Electrical Electronic Corp, Taiwan) and included L (brightness), a\* (redness) and b\* (yellowness) colors [31]. The “L” value indicates the brightness level from 0 to 100, with 0 indicating black and 100 indicating white. The “a” value reflects the red and green colors from -80 to 100. A negative “a” indicates green, a positive “a” indicates red. The “b” value indicates yellow and blue colors from -70 to +70. A negative “b” indicates yellow, a positive “a” indicates blue [31].

*Cooking loss*

Cooking loss is a major indicator of the nutritional value of meat and is related to the amount of water bound in the cells between muscle fibers [32]. To determine cooking loss of ground beef jerky cooked in the oven, meat samples were weighed before and after cooking. Cooking loss (CL) was calculated using the formula:

$$\text{CL (\%)} = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

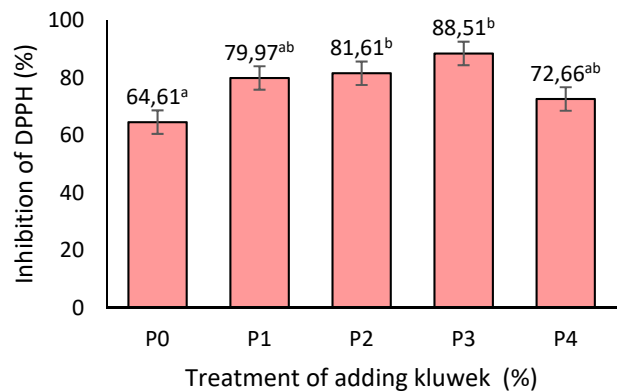
where:  $W_1$  = Weight of sample before cooking;  $W_2$  = Weight of sample after cooking.

*Statistical analysis*

The data obtained from the research were analyzed by statistical data processing using the MS Excel and IBM SPSS Statistics 24 computer programs, the analysis of variance or ANOVA method. Analysis was continued with Tukey’s advanced test with a 5% confidence interval or ( $P < 0.05$ ) [32].

**Results and discussion**

**Antioxidant activity**



**Figure 2.** Bar chart of the average antioxidant activity of ground beef jerky with the addition of kluwak (P0: 0%, P1: 2%, P2: 4%, and P3: 6%) and addition of BHT reference solution (P4) *Note:* Different superscripts in the figures indicate significant differences ( $P < 0.05$ ).

As can be seen from the bar diagram above, the average antioxidant activity of beef jerky with the addition of kluwak (P1: 2%, P2: 4%, and P3: 6%) ranged from 79.97% to 88.51%, while it was 64.61% in beef jerky without the addition of kluwak (P0: 0%) and 72.66% in the samples with 0.01% BHT. These results show that there was an increase in antioxidant activity of 15.36% to 23.9%. This indicates that kluwak can increase the antioxidant activity of beef jerky. The data above demonstrate that the antioxidant levels followed this descending order: P3 (6% kluwak) > P2 (4% kluwak) > P1 (2% kluwak) > P4 (0.01% BHT) > P0 (control, without kluwak). Several previous studies also confirmed that the addition of kluwak can increase the antioxidant activity in meat products [17].

The results of the analysis of variance show that the addition of kluwak had a significant effect ( $P < 0.05$ ) on the antioxidant activity of jerky. Further results from the Tukey test indicated significant differences in antioxidant activity. This is thought to be due to the beta-carotene, flavonoid, and vitamin C content in kluwak, which function as antioxidants [21]. Antioxidant compounds present in spices inhibit lipid oxidation reactions, thereby reducing the formation of malondialdehyde [11]. The antioxidant compounds in kluwak have the ability to capture free radicals, making them a potential alternative to synthetic antioxidants [33]. Therefore, treatment P3 (6% kluwak) produced the best effect compared to P2 (4% kluwak), P1 (2% kluwak), P4 (addition of BHT), and P0 (control, without kluwak). This occurs because kluwak contains antioxidants that can reduce the negative impact of oxidants in the body



by donating an electron to the oxidant compound, thereby reducing its activity [34]. Kluwak contains beta-carotene, which functions as an antioxidant by protecting and maintaining the integrity of cell membranes against free radicals, thus indirectly preventing lipid peroxidation in cell membranes [35].

Research on the antioxidant compounds in kluwak and processed meat products containing kluwak has been reported by several researchers. Kluwak contains alkaloids (2.69 ppm), tannins (16.0 ppm), flavonoids (1.23 ppm), and cyanide (122.7569 ppm) [36]. In addition, the antioxidant activity of beef sausage with kluwak fermented for 0 and 40 days was  $32.43 \pm 8.14\%$  and  $34.39 \pm 6.94\%$ , respectively, and the addition of kluwak at levels of 1%, 2%, and 3% was shown to increase antioxidant activity [17]. Other studies also suggest that kluwak can function as a natural antioxidant. For example, the addition of 4% kluwak seeds was found to help maintain the quality of patin fish after storage at room temperature for 4 days [20].

The results of this study, along with findings from several other studies mentioned above, show that processed meat products without the addition of kluwak still exhibit antioxidant activity derived from the ingredients and spices used in their preparation. In addition, kluwak, which is traditionally used as a spice in various dishes and herbal remedies, is also beneficial for boosting immunity, maintaining overall health, preventing cardiovascular disease, and functioning as a natural antioxidant [37].

## Color

### *L\** Value (brightness)

The results of the color measurement of jerky (Table 1) showed that the addition of kluwak did not have a significant effect ( $P > 0.05$ ) on the *L\** value (brightness) of the jerky. The brightness values of jerky with the addition of kluwak ranged from 21.58 to 22.14, while the control sample without kluwak (0%) had a brightness value of 21.37. This indicates that the addition of kluwak slightly reduces the brightness of jerky, making the color appear darker or more blackish. The low brightness in jerky with kluwak is caused by the presence of tannin compounds in kluwak, which result in a darker product. This is supported by phytochemical test results, which showed that water and ethanol extracts of kluwak reacted with  $FeCl_3$  to produce a blackish green color, due to the formation of complex compounds between tannins and  $FeCl_3$  [22].

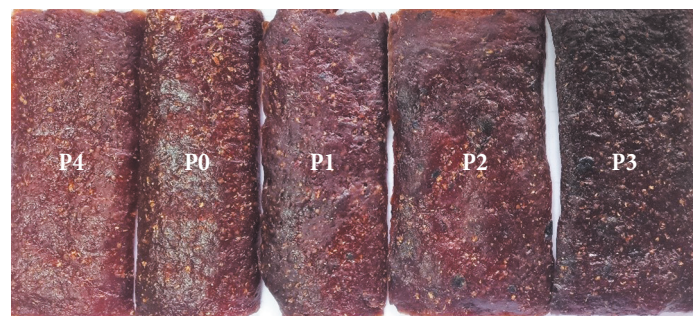
Additional ingredients in jerky also affect its color, such as brown sugar. The higher the sugar content, the

lower the protein level, which influences the occurrence of the Maillard reaction (a reaction between amino acids and the carbonyl groups in sugar) and leads to the formation of a brown color [38]. Jerky is generally brown or blackish due to the Maillard reaction that occurs during the drying process [30]. The brown color may also be influenced by tannins, which are yellowish to light brown in color and, when added to processed meat products, can intensify the brown color, making it darker and more intense [39].

### *Color a\** (redness)

The results of the measurement of the *a\** color value of dendeng (Table 1) showed that the *a\** value of dendeng with the addition of kluwak ranged from 4.89 to 7.33, while without the addition of kluwak (0%) it was 8.28. These results indicate that the addition of kluwak had a significant effect ( $P < 0.05$ ) on the *a\** value of dendeng. As the concentration of kluwak increased, the *a\** value tended to decrease, although it remained within the reddish color range. This is due to the presence of flavonoid and tannin compounds in kluwak, where flavonoids contribute to reddish coloration and tannins produce a blackish green tone. In dye extraction from kluwak, a red, yellow, or orange color appearing in the amyl alcohol layer indicates the presence of flavonoid compounds, while the formation of a dark blue or blackish green color indicates the presence of tannins [26,40].

Kluwak seeds contain tannins and flavonoids, which can serve as natural alternatives to synthetic dyes such as chocolate brown FH (used in fashion products) and chocolate brown HT food (used in food products) [25]. Dendeng is generally light brown to dark brown in color due to the Maillard reaction, which involves a reaction between the carbonyl groups of reducing sugars and the amino groups of proteins and amino acids [39].



**Figure 3.** Ground beef jerky with added kluwak.

(Source: primary data (personal documentation, 2024))

Note: The color of jerky with the addition of kluwak (P0: 0%, P1: 2%, P2: 4%, P3: 6%, and P4: addition of BHT comparison solution).

**Table 1.** Color of ground beef jerky with the addition of kluwak

Parameter	P0	P1	P2	P3	P4
<i>L*</i>	$21.37 \pm 0.43$	$22.14 \pm 1.12$	$21.79 \pm 1.93$	$21.58 \pm 0.74$	$22.10 \pm 0.73$
<i>a*</i>	$8.28 \pm 1.72^{ab}$	$7.33 \pm 0.77^{ab}$	$5.86 \pm 0.89^{ab}$	$4.89 \pm 0.57^a$	$9.15 \pm 2.18^b$
<i>b*</i>	$6.21 \pm 1.64^b$	$5.348 \pm 0.26^{ab}$	$3.98 \pm 0.93^{ab}$	$3.29 \pm 0.48^a$	$5.27 \pm 0.39^{ab}$

Note: Different superscripts in the same column indicate significant differences ( $P < 0.05$ ). *L\** (brightness) = 0 (black) – 100 (white); *a\** (redness) (a = 0 – 80 for red, a = 0 – (–80) for green); *b\** (yellowness) (b = 0 – 70 for yellow, b = 0 – (–70) for blue).

### Color $b^*$ (yellowness)

The average  $b^*$  value of jerky color with and without the addition of kluwak (0%) ranged from 3.29 to 6.21. The addition of kluwak had a significant effect ( $P < 0.05$ ) on the  $b^*$  color value of jerky (Table 1). The  $b^*$  value decreased as the concentration of added kluwak increased. This is because kluwak contains more tannin compounds than flavonoids, where tannins play a role in producing a dark blue color, while flavonoids contribute to a yellow hue.

In general, jerky has a dark or dark brown color [41]. The yellow color in meat is caused by low levels of pigments such as myoglobin and hemoglobin. The amount of marbling fat in the meat also influences the yellowish color of stored meat due to the presence of beta-carotene [4].

### Cooking loss

**Table 2. Cooking loss of jerky with the addition of kluwak**

Treatment	Cooking loss (%)
P0	55.38 ± 2.92
P1	56.06 ± 0.96
P2	55.44 ± 1.31
P3	54.62 ± 0.53
P4	55.38 ± 1.37

The addition of kluwak to jerky in amounts of 0–6% did not have a significant effect ( $P > 0.05$ ) on cooking loss, which ranged between 54.62% and 56.06% (Table 2). The cooking loss of jerky with the addition of 6% kluwak tended to be lower. Low cooking loss in meat products can positively affect their quality. Meat with lower cooking loss is considered to have relatively better quality because fewer nutrients are lost during cooking [40,42].

The non-significant effect is likely due to the meat storage method during transportation, which used ice packs to maintain the meat's temperature [43].

Based on the research results, the relationship between cooking loss and beef pH shows that the more acidic the meat, the lower its cooking loss. Cooking loss refers to the loss of water and soluble nutrients, which are components that influence meat texture and tenderness. This loss typically ranges from 15% to 40% during cooking and significantly affects the eating quality of the meat [44]. Meat shrinkage can be influenced by several factors, including muscle fiber structure, cut length, meat weight, and cooking time [45]. Meat with a cooking loss percentage of less than 40% is considered to have better quality, as it retains more nutrients during cooking compared to meat with higher cooking loss percentages [46].

### Conclusion

This study generally found that kluwak has oxidation resistance, functions as a natural antioxidant, and is effective in inhibiting oxidation in ground beef jerky products, which have a high risk of oxidation that can lead to product spoilage and pose potential health risks if consumed. The addition of kluwak to jerky has been shown to increase antioxidant activity, as indicated by higher antioxidant values in the antioxidant activity test using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which signifies increased antioxidant capacity. In addition, the use of kluwak also showed a significant effect on the color parameters  $a^*$  and  $b^*$ , indicating positive changes in the visual appearance of the product. However, no significant effect was found for the color parameter  $L^*$ , which measures brightness, and no differences were observed in the cooking loss during the jerky preparation process. Based on these findings, there is a need to promote the use of natural antioxidants such as kluwak in jerky products to maintain product quality and protect consumer health.

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