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Antioxidant Activities of Beef Sausage with Addition of Kluwak (*Pangium edule* Reinw)

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Abstract

Oxidation is one of the important factors that affect the quality of meat products including sausages. To inhibit oxidation, several ingredients need to be added in the process of making sausages which act as antioxidants. One of the plants that functions as a natural antioxidant is kluwak. Kluwak can be used to extend the shelf life, kluwak 0 days and 40 days fermentation. Aside from being a preservative, kluwak seeds also contain antioxidant compounds and flavonoid groups. Antioxidant compounds that function as anti-cancer in kluwak seeds include vitamin C, iron ions, and Beta carotene which are the work of fermented kluwak seeds for 40 days. This study aims to determine the effect of different types and levels of kluwak and their interactions on the physical characteristics and antioxidant activity of beef sausages during storage. This study uses two types of kluwak (0 days and fermentation 40 days), different levels and length of storage. The results of this study can increase antioxidant activity and reduce oxidation of lipid.

Keywords : Sausages, Kluwak, *Flavonoids*, Antioxidants, Oxidation of Lipid

INTRODUCTION

Sausages are meat products that are preferred by consumers. Because of the taste, shape and sausage served. Consumer preference is influenced by the quality of the material, the way of making, storing, and the product prsesntation (Davidek. 2004). Sausages can last for a long time with some trait changes. All changes are frozen storage, except changes in oxidation (Mapiye C, 2012). Therefore, oxidation must be controlled and even prevented by the use of antioxidants. Antioxidants are from plants and include vitamins, phenolic compounds and flavonoids (Hudson, 1990; El Ghorab et al., 2007).

Antioxidants play an important role in the prevention and treatment of various chronic diseases, such as premature aging, age-related impairment of the immune system, cardiovascular disease and cancer, which are largely mediated by oxidative stress (Sun, 1990). One plant that has antioxidants is kluwak. Pangium edule Reinw (kluwak) is a tropical tree that grows in the Southeast Asian Islands and South Pacific which was previously included in the family of the Flacourtiaceae (Burkill, 1935) and is currently part of the Gnetaceae family (Kato et al., 1995).

Kluwak is divided into two types, namely raw kluwak and cooked kluwak. Aside from being a preservative, kluwak seeds also contain antioxidant compounds and groups *flavonoid*. Antioxidant compounds that function as anti-cancer in kluwak seeds include vitamin C, iron ions, and Beta carotene which are the work of fermented kluwak seeds for 40 days. While the group *flavonoid* civetwhich has anti-bacterial activity namely cyanide acid, hydnocarpic acid, khaulmograt acid, gorlat acid and tannin. Especially cyanide and tannin compounds, these two compounds are able to provide a preservative effect on fish (Widyasari, 2005). Antioxidants also have the potential to improve product quality (Singh, 2010; Kochhar, 1997). Therefore, researchers want to see the antioxidant activity in beef sausages with the addition of kluwak during the storage period.

MATERIALS AND METHODS

This study uses fresh beef and kluwak obtained from traditional markets. Other ingredients for making sausages are fermented kluwak fat, nutmeg, flour, protein isolates, salt, sugar, ice cubes, and flavoring ingredients (pepper and garlic). The ingredients for testing the antioxidant activity of sausages are DPPH 20 ppm and methanol.

The testing instruments used in this study were glassware, plastic clips, micropipets, petri dishes, measuring cups, UV-VIS *Spectrophotometer SHIMADZU* UV-1800.

This research was carried out experimentally with a completely randomized design. Factorial pattern with treatment of 2 kluwak fermentation (0 days and 40 days), 4 levels of kluwak (0, 1%, 2%, and 3%), and length of storage (0, 5, and 10). Each treatment is 3 replications.

The 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH, Sigma, Germany) was analyzed using method of Alam et al., (2013). The DPPH solution was prepared by mixing 0.2 mM DPPH in ethanol. The control was determined using 2 ml of distilled water with 2 ml of DPPH solution. The mixture of 2 ml of distilled water with 2 ml of ethanol was used as a blank of control. The samples used 2 ml of meat supernatant with 2 ml of DPPH solution and the blank of sample used 2 ml of meat supernatant with 2 ml of ethanol. The decrease in absorbance (Abs) was measured at 517 nm after 30 min. incubation at room temperature in the dark. The percentage of inhibition was calculated as:

$$DPPH\ radical\ cleaning\ activity\ (\%) = \frac{(AB) - (CD)}{(AB)} x100$$

Where A is the control absorbance, B is the absorbance of the control sheet. C is the absorbance of the sample and D is the absorbance of the sample blank. All experiments were carried out in triplicate.

Lipid oxidation was performed using the distillation method to analyse thiobarbituric acid reactive substances (TBARS) as adapted from Cooper et al., (2017). A 10 g sample of meat product with 48 ml of distilled water and 1 ml of 0.2 % of BHT were homogenized. Homogenate was then poured into a digestion tube and tubes were vortexed and rinsed with 30 ml of distilled water. The homogenise was transferred into another tube4 with 1 ml of 5N HCl immediately before distillation. After distillation, Adding 5 ml of sample with 5 ml of thiobarbituric acid reagent (TBA, Sigma-Aldrich, Germany) into a glass tube and vortexed individually. Tubes were boiled in water bath for 35 mins at 95˚C immediately and then removed from the water bath. Tubes were

submerged into an ice bath for 35 mins. The absorbance was measured at 538 nm using a spectrophotometer.

RESULTS AND DISCUSSION

The addition of kluwak has an effect on antioxidant activity and fat oxidation. There are interactions between fermented kluwak, level and storage.

Table 1. Antioxidant activity (%) and fat oxidation (%) sausages with fermented kluwak administration, level and duration of storage.

	Fermentation (day)				rvalue	SEM
	0			40		
Antioxidant activity	32,42			34,39	0,008	0,89
TBA	0,086			0,112	0,000	0,006
	Level (%)					
	0	1	2	3		
Antioxidant activity	25,81	40,07	31.93	35.8	0,000	0,89
TBA	0,165	0,086	0,070	0,076	0,000	0,006
	Storage (day)					
	0	5	10			
Antioxidant activity	29,82	35,42	34,98		0,000	0,89
TBA	0,098	0,081	0,112		0,000	0,006

In Tabel 1, kluwak 0 days and 40 days fermentation are very real different. The value of the antioxidant activity of sausages with kluwak 0 days and 40 days fermentation was 32.43 ± 8.14 and 34.39 ± 6.94. Phenol compounds are components in kluwak which have an antioxidant role. Antioxidants are needed by the body, in addition to maintaining health can also prevent and treat various diseases. Antioxidants have free radical capture properties, reduce tumor formation, DNA damage and cell damage (Idris, 2005). This is in accordance with the opinion of Estiasih et al (2009) saying that the antioxidant activity in kluwak ranges from 50.96 - 76.67%. From this study the highest antioxidant value of kluwak fermented in 40 days. This is consistent with the opinion of Meiriyanto (1988) stating that antioxidant activity in fermented picung seeds increases from day 0 to day 40 (already in the form of kluwak).

The increase and decrease in the level of kluwak administration may be due to the influence of the compounds contained in the kluwak. Increased antioxidant activity in sausages by giving kluwak is due to the high content of phenols and flavonoids contained in kluwak. According to Fardiaz and Romlah (1992), methanol extract of fermented picung seeds has higher antioxidant activity than fresh methanol extract from fresh picung seeds. From this study the administration of kluwak level was 1% higher than other levels.

The value of antioxidant activity at storage of 0 days, 5 days and 10 days was 29.82 ± 5.51; 34.42 ± 8.18 and 34.98 ± 7.72%. A relatively long storage period and storage conditions at cold temperatures cause antioxidant activity in the sausage to change. This is in line with the statement of Rachmawati *et al.* (2009) that temperature has a significant effect on the content of the material. The higher the storage temperature, the active content of food decreases.

The results of the variance analysis showed that the interaction of kluwak 0 days and 40 days fermentation, level and storage period had a very significant effect on antioxidant activity. These results mean that fermentation, kluwak level and different storage periods influence the antioxidant activity. Antioxidant activity increases with the administration of different types of kluwak, kluwak level and storage period. Can be seen and pictures 1, 2 and 3.

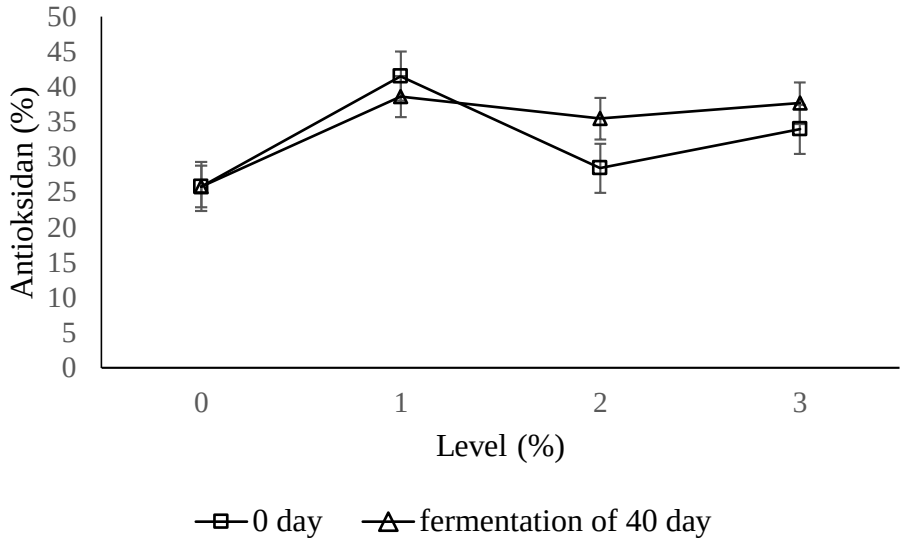

Figure 1. Antioxidant value (%) of raw sausage using kluwak and fermentation of 40 days at each kluwak level

In figure 1, level 0 (without kluwak) has an interaction between 0 days (raw) and 40 days fermentation, at 1% level there is an increase in kluwak 0 days and 40 days fermentation caused by the presence of phenol compounds that react to kluwak and interactions occur. At level 2% there is a decrease in 0 days kluwak and 40 days fermentation, because the phenol compound is not working optimally. At level 3% there is an increase in each kluwak fermentation, this is because oxidation begins to decrease due to storage time. This is in accordance with Sánchez A (2001) 's opinion to regenerate the compounds responsible for this reaction through synergistic effects among antioxidant compounds in sausage.

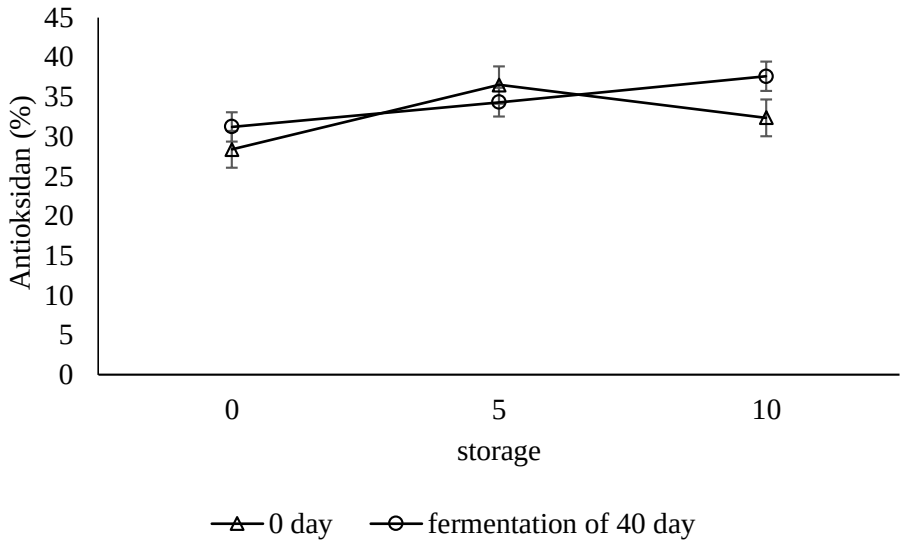

Figure 2. Antioxidant value (%) of raw sausage using kluwak and fermentation 40 days at each storage time

In Figure 2, 40 days fermentation storage has a value higher than 0 days (raw) on day 0, day 2 storage increases Antioxidant value between 0 days and 40 days fermentation and interaction occurred on day 3 but on the 10th day there is a decrease in fermentation of 40 days and increase in kluwak 0 days because antioxidant activity is able to counteract the oxidation of fat that occurre on the 10th day. This is regarding to the opinion of Rachmawati *et al.* (2009) that the temperature has a significant effect on the content of the material. The higher the storage temperature, the active content of food decreases.

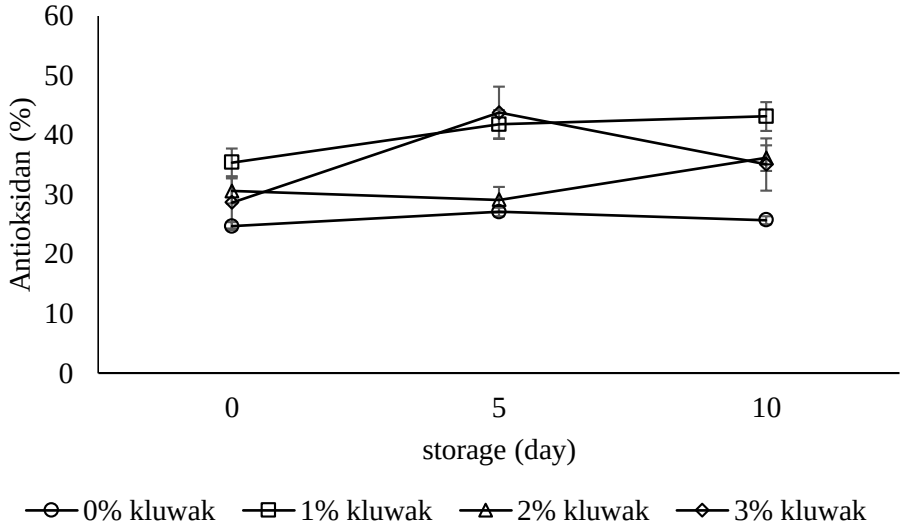

Figure 3. The antioxidant value of% sausage using raw kluwak and fermentation 40 days at the level of kluwak at each storage time

In figure 3, level 1% experiences a significant increase from day 0 to day 10 and interaction between levels 1% and 3% occurre. Level 2% declines on the 5th day and increases on the 10th day but is inversely proportional to the level of 3% and there is an interaction between the levels of 2% and 3%. This is in accordance with the opinion of Windono (2004) that there is a high correlation between total phenol and antioxidant activity, total phenol which plays a major role in contributing to antioxidant activity. High phenol levels will increase the availability of hydroxyl groups capable of trapping radicals.

The value of sausage fat oxidation rate by kluwak fermentation was 0.09 ± 0.05 and 0.11 ± 0.05%. Besides containing antioxidants, kluwak contains fat and free fatty acids will increase during the fermentation of kluwak seeds, but the dominant fatty acids are oleic acid and linoleic acid, do not change levels. We recommend that the kluwak seed fermentation process does not change the amino acid composition of the kluwak produced (Romlah 1992). This value difference is due to the ability of kluwak as an antioxidant to reduce or prevent fat oxidation. This research is the lowest level, which is 2% this is in accordance with the opinion of Rina (2013) that the addition of ingredients that act as antioxidants capable of inhibiting the oxidation of fat by the oxygen on the sausage so that the value of TBA produced does not exceed the specified threshold is 3 mgMDA/kg of material

The existence of differences during storage due to the time interval the old and the storage method that uses the temperature *refrigerator* (5^0C). Where the growth of microorganisms can accelerate fat oxidation in this study a 5-day storage had the lowest value. This is in accordance with the opinion of Rachmawati *et al.* (2009) that temperature has a significant effect on the content of the material. The higher the storage temperature, the active content of food decreases. The level of fat oxidation is very dependent on temperature. Increased storage stability can be obtained by lowering the storage temperature. For example, the storage time for raw meat can be extended to a shelf life of about 3 times by lowering the temperature from -15 to -25 ° C. It can be seen in Figures 4, 5 and 6.

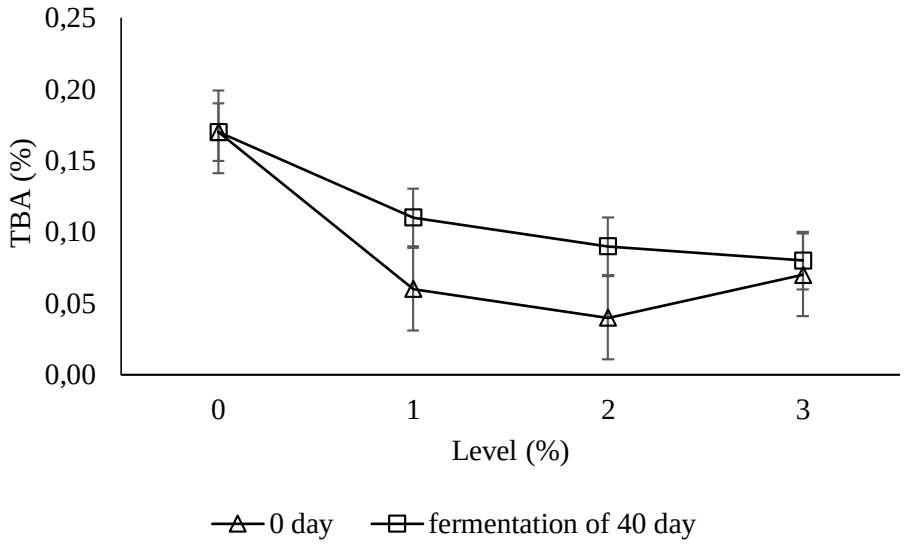

Figure 4. TBA (Thiobarbituric-Acid%) value using raw kluwak and fermentation 40 days at each level of kluwak

In figure 4, level 0 kluwak 0 days (raw) and fermentation 40 days has the same fat oxidation value. 40 days of fermented kluwak decreases significantly at each level compared to 0 days which experienced an increase in the value of fat oxidation at the level of 3%. This result means that the addition of different levels and levels influences the oxidation of lipid. The level oxidation of lipid decreases with increasing levels. Antioxidants have a variety of mechanisms which are radical scavenging, connecting metals, enzyme inhibition, lipid oxidation and single oxygen attraction (Pratt, 1992; Suratmo, 2012).

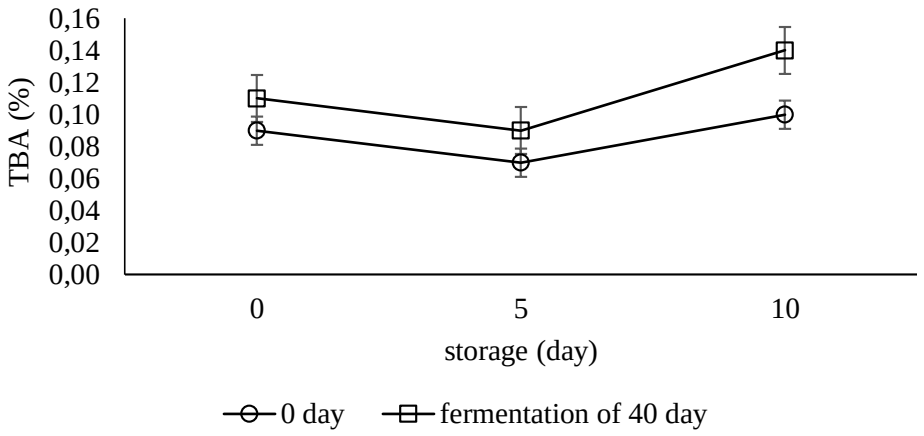

Figure 5. TBA (Thiobarbituric-Acid %) value of raw sausage using kluwak and fermentation 40 days at each storage time

In figure 5, the value of fat oxidation has a difference in value between 0 days and 40 days fermentation. 0 day had a lower fat oxidation value compared to 40 days fermentation. Storage on the 5 day decreased during fermentation time and experienced an increase on the 10 day because this antioxidant activity did not work optimally on the 10 day resulting in an increase, indicating that the ascorbic acid content at the end of the storage process was higher than initially, because the possibility of generation or recycling (of dehydroaskorbic acid into ascorbic acid) when it is being reduced by enzymes or other substances with the same properties can be found in sausages (Padayatty, 2002; Guija, 2005).

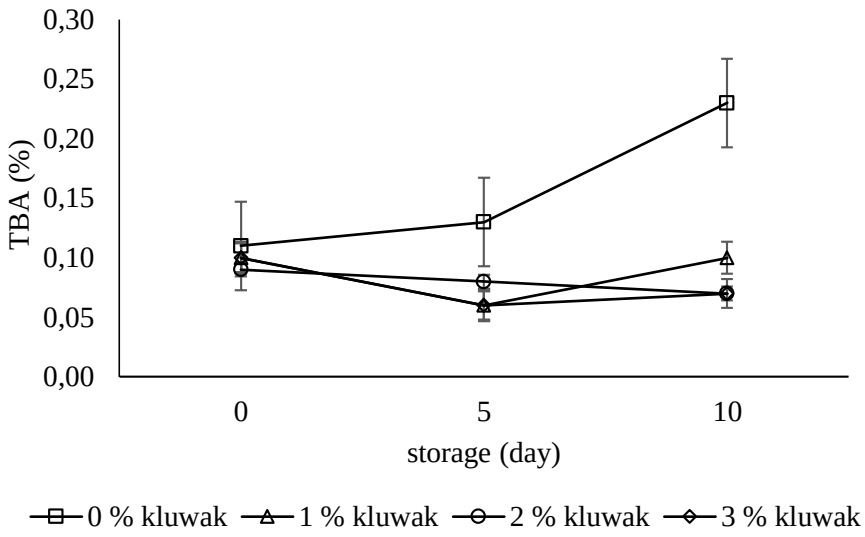

Figure 6. TBA (Thiobarbituric-Acid %) value using raw kluwak and fermentation 40 days at kluwak level every time of storage

In figure 6,the storage on day 0 level 1% and 3% occur different interactions, with level 0 and 2% do not experience interaction but at level 2% there is an interaction with levels of 1% and 3%. The storage on the 5th day there was a decrease in oxidation lipid at the level of 2% and 3% compared to the level of 1% which experienced an increase and interaction occurred. The level of fat oxidation decreases with increasing levels. This is in accordance with the opinion of Gordon (1990) the mechanism oxidation of lipid is influenced by oxidation conditions, namely temperature, catalyst, type of fatty acid, distribution and form of double bonds, and the amount of oxygen available.

CONCLUSION

The addition of kluwak can increase antioxidant activity characterized by high DPPH values and low sausage TBA values.

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