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Original Research Article

The Effect of Saltpeter and Angkak on Total Cholesterol, Nitrite, and Color of Traditionally Smoked Curing "Se'i" Meat

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Abstract: Saltpeter has been traditionally used as a curing agent for meat in East Nusa Tenggara Province of Indonesia. It creates a good red color for meat; however, it has carcinogen potency. *Angkak* (red yeast rice) may be able to replace the coloring role of saltpeter it can reduce the cholesterol of meat. This research has been designed to evaluate the effect of reducing saltpeter in combination with different concentrations of *angkak* on total cholesterol, nitrite content, color, and organoleptic of traditional smoked beef "Se'i". The research has been designed using Simple Randomized Design with a 3x3 factorial pattern, i.e. Three levels of *angkak* (2%, 2.5%, and 3%) and saltpeter levels (100 ppm, 200 ppm, and 300 ppm). For control, a combination of 500 ppm saltpeter was only applied to Se'i. All treatments were replicated 4 times. The results show no interaction effect of treatments on total cholesterol, nitrite, Hunter value of L*, a* and b* of Se'i. Total cholesterol and lightness (L*) values of combination-treated se'i were lower than the control. The optimal values of L* (lightness) and a* (red or positive) were obtained at se'i with 200 ppm saltpeter and 2.5% angkak combination with good taste and low nitrite level (<0.25 ppm); while angkak note is still noticeable. Therefore, it is recommended to use this combination to reduce the use of saltpeter in se'i.

Keyword: Beef se'i, *angkak*-saltpeter combination, cholesterol, nitrite, color, organoleptic.

INTRODUCTION

Se'i is a traditional smoked meat, which previously has been cured with saltpeter and other seasonings. It is produced by people living on Timor Island and around it since hundreds of years ago as a preservation meat technique. Se'i has a typical flavor and taste that is significantly different from other known processed meat. The weakness of this preservation technique is the use of saltpeter for developing purplish red color which is sometime in uncontrolled concentration. Nitrite residue from saltpeter has a negative effect due to its carcinogenic potency. (Corpet *et al.*, 2012) explained that nitrite from saltpeter reacts with the oxymyoglobin of meat to produce nitrosyl heme-chromogen with the help of heat and the presence of oxygen. Furthermore, heme-nitrosyl reacts with Fe ions from hemoglobin to form nitrosamine, a reported carcinogen and toxic compound. Exceeding the use of saltpeter will increase nitrosamine production. The tolerable maximum nitrite residue in meat is 30 mg/kg according to the regulation of food safety standards in Indonesia (Ulfa *et al.*, 2020). (Honikel, 2008) reported that curing meat with 150 ppm of saltpeter is not left nitrite residue. De González *et al.*, (2012) found low levels of nitrite in some conventional curing meat. Malelak *et al.*, (2015), on the other hand, reported that using saltpeter at a concentration of 500 mg/kg of meat during the processing of se'i resulted in nitrite residue found was 427 ppm, which is 10 times higher than tolerable one.

All reports above indicate that the use of non-natural chemicals in curing meat, especially for coloring may lead to negative side effects. Therefore, it is advised to use natural colors for safety purpose. *Angkak* is one of the natural colors produced by *Monascus purpureus* that has been used in many kinds of food. In Asia, angkak has been applied in cheese, wine and sausage (Romulo *et al.*, 2017). Angkak contains mono-choline K or lovastatin, a compound that can reduce

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cholesterol. It can also improve food flavor, and preserve the food (Nguyen, *et al.,* 2017) On the other hand, angkak may contain citrinin which has a toxic effect. If lovastatin production is high, the production of citrinin becomes low, which depends on the type of rice used as a substrate for *Monascus purpureus* growth (Pengnoi *et al.,* 2017).

Both angkak and saltpeter contribute to the red color of cured meat, but each of them has different mechanism. It has been reported that both ingredients synergistically work if they are applied together (Seafast center, 2012). This research aims to know how the coloring mechanism of angkak and saltpeter work in se'i, the traditional smoked beef.

MATERIALS AND METHODS

Materials

Beef used for the experiment taken form thigh muscle of Bali cow, approximately 40 kg. Angkak was purchased form commercial company (Sahabat, Indonesia). Saltpeter (KNO_3), NaCl were obtained from local store; while *Schleichera oleosa* fire-wood was collected from around Kupang city, Indonesia.

Methods

Experiment was designed using Completed Randomized Design with two factors, i.e., level of angkak (2%, 2.5%, and 3%), and level of saltpeter (100 ppm, 200 ppm, and 300 ppm) replicated four times. The combination of treatments were P1 (2% angkak+100 ppm saltpeter), P2 (2% angkak+200 ppm saltpeter), P3 (2% angkak+300 ppm saltpeter), P4 (2.5% angkak+100 ppm saltpeter), P5 (2.5% angkak+200 ppm saltpeter), P6 (2.5% angkak+300 ppm saltpeter), P7 (3% angkak+100 ppm saltpeter), P8 (3% angkak+300 ppm saltpeter), and P9 (3% angkak+300 ppm saltpeter). As control, 1 kg of se'i was prepared with traditional way (500 ppm saltpeter).

Angkak solution was prepared according to the procedure developed by Ramadhan *et al.,* (2005) with small modification (Sabtu and Suryatni. 2015). To make se'i, the meat free from connective tissue and fat was cut for about 3x3 cm^2 thick and 35 cm long. Meat was then treated with 2% NaCl and further applied with saltpeter and angkak according to experimental design. All treated meats were marinated in refrigerator (4^oC) for about 12 hours (Malelak *et al.,* 2015). The marinated meats were than smoked in a drum at temperature about 80-90 ^oC covering with *Schleichera oleosa* leaves for about 30 minutes (Sabtu and Suryatni, 2015). Resulted Se'i was cooled and packed for analyses.

Parameters measured were total cholesterol, nitrite content, color (L*, a*, b* values), and organoleptic aspects.

a. Total Cholesterol

Five grams of crash sample were added with 25 mL of acetone-ethanol mixture (1:1) and heated at 80^oC for 30 minutes. After cooling, the mixture was centrifuged. One milliliter of clear supernatant was heated again at 100^oC for 30 minutes, cooled, and added with 2 mL of chloroform followed by the addition of 2 mL of acetate anhydrate-sulfate acid (30:1). The mixture was incubated in a dark room for 20 minutes, and then read its absorption at the wavelength of 680 nm using a spectrophotometer. The cholesterol content was calculated using a standard curve of pure cholesterol.

b. Nitrite Content

The nitrite content in Se'i was measured according to the method described in SNI 01-2894-1992. Five grams of sample were put in a 50 mL beaker glass, added with 40 mL of free nitrite water at the temperature of 80^oC. After stirring for a while, the mixture was transferred to the measuring flask and made up with water to the volume of 300 mL. The flask was put in the water bath for about two hours, cooled, and filtered. The filtrate was added with sulfanilamide reagent and NED reagent stirred until a color appeared, then the nitrite of the solution was measured at 540 nm using a spectrophotometer, which was calculated using the standard curve.

c. Lab Color of Se'i

The color of se'i in Lab Hunter value was analyzed using Hunter Hutching 1999 Chromameter (Konica Minolta CR 400)**.** The measurement was done in three replications.

d. Aroma and taste of Se'i

The aroma and taste of se'i were organoleptically analyzed using 15 trained panelists who are used to consuming se'i. Five hedonic scales were used, i.e. 1 (very strong angkak scent) to 5 (very strong se'i scent) for aroma, and 1 (do not like very much) to 5 (like very much) for taste.

Data Analysis

Parametric data, except for nitrite, were analyzed using Analysis of Variance (ANOVA) followed by a multiple range test. Non-parametric data were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test. All analyses were performed using SPSS software version 21.

RESULTS AND DISCUSSION

1. Physio-chemical Properties

The data of physio-chemical parameters of treated se'i were shown in Table 1.

Table 1: The average value of cholesterol, nitrite and Lab Hunter of treated se'i

Parameter	Treatment									K	P-value		
	P1	P2	P3	P4	P5	P6	P7	P8	P9		Int	A	S
Total Cholesterol (g/dl)	32.4± 6.72	42.8± 9.45	35.7± 7.86	41.3± 12.53	37.9± 14.70	42.5± 12.64	34.3± 14.72	35.3± 5.40	34.1± 3.10	73.03	.716	.328	.828
Nitrite (mg/kg)	<0,25	<0,25	<0,25	<0,25	<0,25	<0,25	<0,25	<0,25	<0,25	<0,25			
L value	24.5± 3.40	22.7± 1.14	22.9± 3.24	24.3± 0.97	28.2± 3.60	21.7± 2.54	26.4± 3.01	26.5± 1.96	23.1± 3.60	22.42	.171	.236	.020*
a value	7.33± 1.74	7.17± 1.31	7.78± 0.36	8.14± 0.60	8.90± 0.93	8.11± 1.56	6.37± 1.02	7.78± 1.27	6.52± 0.99	7.06	.537	.014*	.356
b value	12.1± 1.40	12.0± 0.49	13.3± 1.42	12.8± 0.56	14.1± 0.97	11.6± 1.87	12.7± 1.54	13.0± 2.07	11.9± 1.54	12.14	.131	.783	.421

P1=2% angkak+100 ppm saltpeter; P2= 2% angkak+200 ppm saltpeter; P3=2% angkak+300 ppm saltpeter; P4=2,5% angkak+100 ppm saltpeter; P5=2,5% angkak+200 ppm saltpeter; P6=2,5% angkak+300 ppm saltpeter; P7=3% angkak+100 ppm saltpeter; P8=3% angkak+200 ppm saltpeter; P9=3% angkak+300 ppm saltpeter; control = withoutangkak + 500 ppm saltpeter. K = control, Int=interaction, A=angkak, S=Saltpeter. *= significantly different.

a. Total Cholesterol of Se'i

Table 1 show that the total cholesterol of all se'i varies from32.49% to 42.85%. There is no statistical difference (P>0,05) in the interaction of angkak and saltpeter treatments on the total cholesterol of se'i. Angkak and saltpeter treatment at several concentrations did not significantly affect the cholesterol content of se'i. levels of angkak treated in se'i are a very small difference. It may not cause a big difference in the content of lovastatin (monacolin K), which reduces cholesterol content. Kasim *et al.,* (2005) reported that lovastatin in angkak resulted from nine rice varieties around 0.05% - 0.92%, while Wiyoto *et al.,* (2011) reported in other rice was 0.0266%.

In average, the total cholesterol content in treated se'i was lower than that in the control (untreated) se'i (73.03 g/dL). Lovastatin in angkak may cause this reduction. Vendruscolo *et al.,* (2016) have reported that lovastatin could inhibit the synthesis of cholesterol by obstructing Hydroxymethyl glutaryl coenzyme-A (HMG-CoA) reductase. *Monascus spp* produced some secondary metabolites including monacolin, ɤ-butyric acid, and pigment (Lachenmeier *et al.,* 2012; Chen and Wu 2016). Kawuri (2013) found that oral treatment of red mold rice (RMR) at 1.2 g/day reduced 30.9% of the blood cholesterol (LDL) of mice.

Saltpeter is used for curing and does not have the potency in reducing or inhibiting the synthesis of cholesterol. Therefore, increasing the level of saltpeter was not able to reduce the content of cholesterol in se'i. Saltpeter or potassium nitrate contains nitrite and nitrate that may affect each other concentration (Tonder, 2017). Nitrite inhibits the growth of *Clostridium botulinum*, gives a bright red color, and increases the flavor of meat (Anggresani, 2018).

b. Nitrite Content of Se'i

Adding saltpeter significantly affect the content of nitrite in se'i, while adding angkak to the se'i did not affect the content of nitrite (Table 1). The residue of nitrite in all treated as well as control se'i was lower than 0.25 ppm. It is categorized as safe to be consumed (Badan Pengawas Obat dan Makanan RI. 2013). The authority of food and drug commission allows daily consuming any food with nitrite content below 0.37 mg/kg body weight.

Application of saltpeter as curing agent is important to give bright red color as well as to preserve meat (Honikel, 2008). The bright red color occurs as a result of reaction between saltpeter and myoglobin of the meat (Corpet *et al.,* 2012). The excessive use of saltpeter will result in the increase of nitrite and or nitrate residue in meat. The reaction of nitrite or nitrate in meat will produce nitric oxide, nitroxide or nitrosamine. Nitrosamine is known as carcinogen (Sindelar & Milkowski, 2012). On the other hand, Honikel (2008) reported that using potassium nitrate for curing meat without heating will note left the residue. (Sebranek & Bacus, 2007) recommended to use sodium or potassium nitrate in the range of 200 ppm to 700 ppm.

c. Lab Hunter Color of Se'i

Lightness (L) of se'I was found in the range of 21,69 – 28,20 (Table 1) indicating it was a bit dark. Interaction between angkak and saltpeter treatment did not show significant difference (P>0.5). There was a trend that increasing saltpeter application reduces the lightness of se'i. Statistically, it was significant different between 100 ppm and 200 ppm with 300 ppm of saltpeter treatment of se'i.

The high concentration of saltpeter applied in meat increases nitroxide link to myoglobin. (Shakil *et al.,* 2022)explained that the presence of nitrite affects

pH value of meat and forms dark red color on meat. Firstly, nitrite oxide forms bright color and then change to brown after linking to myoglobin. Heating the myoglobin nitrosil complex will turn to pink color (Nitroso hemochrom) (Carvalho *et al.*, 2003).

The value of a indicates the green (negative) to red (positive) color. Results in Table 1 show that a value in the range of 6.37 to 890 or red, indicating the red color of se'i. Statistically, there was no significant (P>0.05) interaction effect of angkak and saltpeter on a value of se'i. However, there was a trend that increasing concentration of angkak causes more bright red color of se'i. Angkak is natural red color (*rubropunctamin)*, therefore, it directly gives the red color on surface of meat, while saltpeter has to link with myoglobin to get the red color (Zubaidah *et al.*, 2015). Yuliana (2018) explained that the red color compound from *Monascus purpureus* is *monascorubramin*. Torres *et al.*, (2016) identified that monascin has yellow and red colors, while *monascorubin* performs red and orange colors, and *rubropunctamin* gives orange color. Rumondor *et al.*, (2016) reported that adding 2% angkak to beef sausage increased the red color of it. The same result has also been reported in sausage of duck meat (Atma, 2015).

The value of b as indicated by yellow (negative) to blue (positive) color was found at the range of 11.64 to 14.17 (Table 1), which tends to yellow color. Statistically, there was no significant (P>0.05) effect of interaction between level of angkak and saltpeter on b value of se'i. Combination of 2% angkak and 200 ppm saltpeter gives more yellow color than higher concentration of saltpeter. Purwanto (2011) reported that *Monascus purpureus* produces color of angkak depending on the proportion of amylose and amylopectin.

2. Organoleptic Properties

Organoleptically, there was significant interaction effect between angkak and saltpeter on flavor and taste of se'i. Preferences of panelists on these two organoleptic properties of se'i were shown in Table 2.

Table 2: Panelist preference on flavor and taste of se'i

Parameters	Treatment										
	P1	P2	P3	P4	P5	P6	P7	P8	P9	K	P-value
Aroma	$3.6^{a}\pm$ 1.21	$3.8^{a}\pm$ 1.12	$3.9^{a}\pm$ 1.14	$3.7^{a}\pm$ 0.96	$3.7^{a}\pm$ 1.17	$3.4^{a}\pm$ 1.12	$3.1^{ab}\pm$ 1.03	$3.0^{b}\pm$ 1.10	$3.0^{b}\pm$ 1.26	4.5	.000
Taste	$2.5^{de}\pm$ 0.72	$3.1^{ab}\pm$ 0.70	$3.1^{abc}\pm$ 0.67	$2.4^{e}\pm$ 0.85	$3.0^{bc}\pm$ 0.96	$3.4^{a}\pm$ 0.92	$2.4^{e}\pm$ 0.86	$2.8^{c}\pm$ 0.96	$2.8^{c}\pm$ 0.86	3.3	.000

Superscript a,b,c, or d which follow numbers in similar column indicates significant difference (P<0,05). P1=2% angkak+100 ppm saltpeter; P2= 2% angkak+200 ppm saltpeter; P3=2% angkak+300 ppm saltpeter; P4=2,5% angkak+100 ppm saltpeter; P5=2,5% angkak+200 ppm saltpeter; P6=2,5% angkak+300 ppm saltpeter; P7=3% angkak+100 ppm saltpeter; P8=3% angkak+200 ppm saltpeter; P9=3% angkak+300 ppm saltpeter; control = withoutangkak + 500 ppm saltpeter. Flavor hedonic scale 1= very strong angkak scent, 2= medium angkak scent, 3= low angkak scent, 4= low se'i scent, 5= strong se'i scent. Taste hedonic scale 1= not tasty, 2= a bit tasty, 3=tasty, 4= nice tasty, 5= very nice tasty. K.= Control

a. Flavor of se'i

Statistical analysis of se'i flavor using Kruskal Wallis shown significantly (P<0.01) different in the interaction effect of angkak and saltpeter (Table 2). Response of panelists on se'i treated with 3% angkak in combination with 100 ppm, 200 ppm and 300 ppm saltpeter tended to be low score on flavor, which means they are having more angkak scent. Adding 2% or 2.5% of angkak on se'I tends to have more se'i scant at all combinations with saltpeter. Saltpeter itself does not have scent of se'i. Therefore, at se'i treated with 3% angkak, the scent of angkak starts appearing and tended to be dominant. Kawuri (2013) explains that the role of *Monascus spp.* in producing some odor compounds during was detected as some organic acids, and lipid degraded compounds.

b. Taste of Se'i

Panelist's judging on the taste of se'i is at the range of a bit tasty (like) to medium tasty (like). Statical analysis results show that interaction of angkak and saltpeter was significantly affected the taste of se'i (Table 2). Response panelist tends to be low on se'i treated with angkak (2% and 2.5%) in combination with 100 ppm saltpeter; however, it is increase at 3%of angkak treated se'i. The taste of se'i as well as any other cured meat has been reported to be related with juiciness of the meat, which is high at more juiciness meat (Soeparno *et al.*, 2011). Sabtu *et al.*, (2022) found that 2% up to 2,5% of angkak treated se'i are more have positive response from panelist. Adding angkak at the concentration of 0,5 – 1% beside inhibits the microbe growth, it gives more tasty and sweet on shrimp paste (Indriati and Andayani, 2012).

CONCLUSION

The combination of angkak and saltpeter in se'i did not affect its total cholesterol, nitrite and Lab Hunter color scales. However, it affected the flavor and taste of se'i. Comparing with control treatment, adding angkak in se'i reduced cholesterol and nitrite content in

se'i.The lightness (L) red color (a value) of se'I were better at 2,5% angkak in combination with 200 ppm saltpeter. Flavor and taste of se'i were low at the treatment of 3% angkak. The taste of se'i itselfwas more acceptable when it treated with 2% and 2,5% of angkak in combination with 200 ppm and 300 ppm of saltpeter. Adding 2,5% of angkak and 200 ppm of saltpeter is recommended to be used in processing beef se'i.

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