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Effects of *Hibiscus sabdariffa* and *Schleichera oleosa* Liquid Smoke on Lipid Content, Lipid Oxidation and Residual Nitrite in *Se'i* (Rotenese Smoked Beef)

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ABSTRACT. *Hibiscus sabdariffa* calyces and liquid smoke are kinds of ingredients which are always used in some processed meat products. The objectives of this study were to determine the effect of Roselle calyces extract (*Hibiscus sabdariffa*) (RCE), *Schleichera oleosa* liquid smoke (SOLS) and a combination of the extract and the liquid smoke (RCSO) on lipid content, lipid oxidation and residual nitrite of *se'i* (Rotenese smoked beef). The experiment was assigned in a completely randomized design (CRD) with 6 treatments namely: control (C) = *se'i* traditional (without extract or liquid smoke), RCE_1 = roselle extract 4% (w/v), RCE_2= roselle extract 8% (w/v), SOLS= *Schleichera oleosa* liquid smoke (5%) (v/w), $RCSO_1$ = roselle extract 4% + liquid smoke 5%, $RCSO_2$ = roselle extract 8% + liquid smoke 5%. There were three replicates each treatment. Data were analyzed using Analysis of variance (ANOVA) and continued by Duncan multiple range tests to detect differences between means. Result showed that lipid content was significantly lower ($P<0.05$) in *se'i* giving RCE or SOLS compared to their combination (RCSO). Based on TBA numbers, RCSO1 was the most effective treatment compared to other treatments in inhibing lipid oxidation in *se'i*. Residual nitrite was significantly increase ($P<0.05$) in *se'i* adding RCE or SOLS. The experiment showed that combination of roselle (*Hibiscus sabdariffa*) extract and *Schleichera oleosa* liquid smoke ($RCSO_1$ or $RCSO_2$) was more effective in inhibiting lipid oxidation while roselle (*Hibiscus sabdariffa*) extract (RCE) or *Schleichera oleosa* liquid smoke (SOLS) alone was more effective in reducing lipid content. Addition of roselle (*Hibiscus sabdariffa*) extract (RCE) or *Schleichera oleosa* liquid smoke (SOLS) caused nitrite residual of *se'i* increasing.

Key words: Roselle, liquid smoke, *se'i*, lipid oxidation, lipid content, residual nitrite

INTRODUCTION

Se'i (Rotenesse smoked meat) is usually made from beef, slicing into rope-shape, spicing with salt and saltpeter (KNO_3), then cured and smoked. The traditional (vaporous) smoking of *se'i* is usually done by *Schleichera oleosa* wood smoke and above the meat surface is covered with *Schleichera oleosa* raw leaves.

Nowadays *se'i* has been produced in home and industry scale too. As a smoked meat product, the safety of *se'i* depends on level of carcinogenic compounds and residual nitrite which always contained in cured and smoked meat products. The carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs) are formed and released during pyrolysis of wood. In traditional smoking, *se'i* is placed directly over the smoking wood thus all components of smoke, include PAH, and adhere directly to meat surface. On the other hand, when *se'i* is processed with liquid smoke, level of the carcinogenic compounds is very low since before applied in food, the liquid smoke was distillated and filtrated.

Using of saltpeter (KNO_3) in *semi* plays an important role on enhancing red bright color as

well as preservative. However, a safety concern relating to the use of nitrate/ nitrite is leading to a formation of carcinogenic compounds such as nitrosamines and nitrosamines. Thus, residual nitrite concentration in food product should be low to avoid formation of the carcinogenic compounds.

Organic acids are good sources of bioactive compounds that could help to reduce the level of residual nitrite in meat products (Viuda-Martos *et al.,* 2009). Organic acids also contain in roselle calyces such as: oxalic dan succinic acids, malic dan tartaric acids and ascorbate acid. In liquid smoke also contains organic acids. It has not been reported effect of roselle and liquid smoke on reducing resdidual nitrite in meat products included in *se'i*.

Mainly in meat products included *se'i* contain high levels of lipids. Lipid content in *se'i* is a important point to be considered since it relates to cardiovascular diseases. Thus using of antioxidant in meat products may help to reduce the incidence of the diseases. In roselle calyxs and in liquid smoke contain phenols. Phenolic compounds are known for exhibiting antioxidant properties that could decrease the rate of lipid oxidation. Addition of roselle extract in *sucuk* dan *kavurma* could reduced TBA numbers (Bozkurt and Belibag, 2009). Giving liquid smoke in beef patties could reduced TBA numbers (Estrada-Munoz *et al.*, 1998). It has not been reported effect of roselle and liquid smoke on lipid oxidation of *se'i*. Therefore, this study aimed to investigate the effect using roselle extract, liquid smoke and the mixture of roselle and liquid smoke on lipid content, lipid oxidation and residual nitrite of *se'i* (Rotenese smoke beef).

MATERIALS AND METHODS

A total of 10 kg of beef was taken from butt and rump of Bali cattle, was purchased in the meat shop in Kupang. The beef was trimmed of fat and connective tissue, cut into rope-shaped with three cm in thickness. Addition 20 g of refined and ground table salt and 300 mg of saltpeter of kg^{-1} meat then mixed well. The beef divided into six groups namely: Control (C) = without adding roselle or liquid smoke, RCE1 = roselle 4% (w/v), RCE2= roselle 8% (w/v), SOLS= *Schleichera oleosa* liquid smoke (5%) (v/w), $RCSO_1$ = roselle 4% + liquid smoke 5%, $RCSO_2$ = roselle 8% + liquid smoke 5%.

Calyces of roselle was obtained from Oefafi village- Kupang- East Nusa Tenggara Province. The calyces were separated from seed and dried in oven at temperature of 60° C for 3 days, then blended with Philips blender to abtain the mass. To obtain 4% and 8% (w/v) of roselle extract, each of 4 and 8 g of roselle mass poured into volumetric glass and added distilled water up to 100 ml, stirred at 600° C for 5 min and filtered with Whatman (No.41) (Karabacak and Bozkurt, 2008) with modification. The filtrate then poured in the batches RCE_1 and RCE_2 respectively, mixed well and marinated for 12 h.

Schleichera oleosa liquid smoke was obtained from Department of Agricultural Technology, Gadjah Mada University. It was produced in 4000° C pyrolysis temperature, destilated and filtrated. To obtain SOLS, fvie ml of *Schleichera oleosa* liquid smoke poured in the batches and mixed well in batches (SOLS). To obtain $RCSO_1$ = roselle extract 4% + liquid smoke 5% and $RCSO_2$ = roselle extract 8% + liquid smoke 5%, roselle extract added first, followed by liquid smoke and then mixed well. All treatment then cured for ± 12 h and then smoked used *Schleichera oleosa* wood except for SOLS, $RCSO_1$ and $RCSO_2$ were smoked in oven at 100° C until well done. When the beef surface was dry, firmness and the color turned to bright red, the smoking was stopped. Triplicate pieces of meat, 100 g of each piece, were carried out for each group of *se'i* used as samples.

Lipid content was determined by Soxhlet extraction (AOAC, 1995). Residual nitrite level was determined as mg $NaNO_2$ / kg *se'i* by a spectrophotometer method at 540 nm (AOAC,

1995). Lipid oxidation was determined as 2-thiobarbituric acid-reactive substances (TBARS) in mg malondialdehyde (MDA)/kg *se'i* by a spectrophotometer method as described by Mohd-Esa *et al.* (2010).

All data obtained from the experiment were analyzed by analyses of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to determine differences among mean values SPSS 18.

RESULTS AND DISCUSSION

Addition of roselle, liquid smoke or mixture of roselle and liquid smoke affect lipid content, thiobarbituric acid reactive substances values (TBARS) and residual nitrite of *se'i* ($P<0.05$). *Se'i* treated with roselle extract (RCE_1 or RCE_2) or *Schleichera oleosa* liquid smoke (SOLS) had lower lipid content than *se'i* treated with combination of roselle and liquid smoke ($RCSO_1$ and $RCSO_2$). The lowest lipid content was found at *se'i* treated with *Schleichera oleosa* liquid smoke (SOLS) ($P<0.05$) (Table 1).

Table 1. Average of lipid content, lipid oxidation (TBARS), residual nitrite level (ppm) of *se'i* treated with roselle extract, *Schleichera oleosa* liquid smoke and their combination

Parameters	Control (C)	Roselle extract 4% (RCE_1)	Roselle extract 8% (RCE_2)	*Schleichera oleosa* liquid smoke 5% (SOLS)	Roselle extract 4% + liquid smoke5% ($RCSO_1$)	Roselle extract 8% + Liquid smoke 5% ($RCSO_2$)
Lipid (%) DM ± Std	6.39 ± 0.02[c]	5.60 ± 0.01[b]	5.67 ± 0.01[b]	5.13 ± 0.02[a]	6.02 ± 0.01[c]	6.56 ± 0.03[c]
TBARS (Mg. Malonaldehide/ Kg ± Std	0.85 ± 0.01[e]	0.64 ± 0.01[b]	0.68 ± 0.01[c]	0.68 ± 0.01[c]	0.40 ± 0.01a	0.71 ± 0.01[d]
Nitrite Residual (ppm) ± Std	29.01 ± 2.23[a]	51.32± 2.33[b]	50.86±2.21[b]	64.21 ± 2.21[c]	28.62 ±2.20[a]	28,83± 2.21[a]

[a, b] significantly difference at $P<0.05$. ± std (standard deviation). DM= dry matter. ppm = part per million.
TBARS= Thiobarbituric Acid Reactive Substance

Addition of roselle (RCE_1 or RCE_2), liquid smoke (SOLS) and combination of roselle and liquid smoke ($RCSO_1$ or $RCSO_2$) resulted in a significant reduction in the TBA values of *se'i* ($P<0.05$). The highest TBA values of **se'i** was in control (C) and the lowest was in roselle 4% + liquid smoke 5% (RCSO1).

Lipid oxidation occurred because of membranes of muscle destroyed by heating while smoking. As a result lipoprotein complexes were broken and lipids tissue was easily attacked by oxygen and catalysts, in consequence the smoked meat more sensitive to oxidation (Onibi, 2000). In roselle calyces contain antioxidant compounds such as vitamin C, anthocyanins, b-carotene and lycopene (Wong, Yusof, Ghazali, & Che Man, 2002). Whereas in liquid smoke contains aldehyde, carboxylic acids and phenols as antioxidant compounds (Rorvik, 2000). It could be suggested that in this experiment the roselle extract, the liquid smoke and their combination could slow the rate of lipoprotein rupture so the rate of oxygen attack the lipid tissue was slow, thereby the rate of lipid oxidation was low. However, the combination of roselle 4% and liquid smoke 5% are more powerful to inhibit the rate of lipid oxidation compared to other treatments.

The means residual nitrite permitted in processing meat is 30 mg/Kg (Indonesian Food and Drugs Board, 2013). It is interested that addition of roselle or liquid smoke caused the residual nitrite level significantly increased ($P<0.05$), and the level was higher than the level permitted by Indonesian Food and Drugs Board (2013). Meanwhile addition of roselle and liquid smoke together ($RCSO_1$ and $RCSO_2$) caused the level of nitrite residual was same with control and the nitrite level was lower than the level permitted by Indonesian Food and Drugs Board (2013).

Commonly in *se'i* processing, nitrate/saltpeter is added to form specific color of *se'i* and also as a preservative. When nitrate was added, it is converted to nitrite by nitrate – reducing bacteria and then it is reduced to nitric oxide (NO) that reacts with myoglobin to form nitric-oxymyoglobin, red in color but unstable. When the meat is smoked, nitric-oxymyoglobin is converted to Nitrosylhemochromagen that is responsible for stable cured-pink color (Sebranek and Bacus, 2007). That is the favorable color of *se'i*.

Nitrite, also can react with secondary and tertiary amines which then result in formation of carcinogenic n-nitrosamines in cured meat (Choi *et al* ., 2007). Nitrosamine formation in meat increases with higher cooking temperatures (Sen *et al.,* 1973). It was reported that in raw-cured sausages level of nitrite turn down as storage time increased and at 20 days the nitrite reduced quickly, after that became moderately stable (Moawad *et al.,* 2012). Thus in this experiment, residual nitrite level could decline further if the *se'i* was stored. However it should be proved by other experiment.

CONCLUSIONS

Based on the result above it could be concluded that combination of roselle (*Hibiscus sabdariffa*) extract and *Schleichera oleosa* liquid smoke ($RCSO_1$ or $RCSO_2$) was more effective in inhibiting lipid oxidation while roselle (*Hibiscus sabdariffa*) extract (RCE) or *Schleichera oleosa* liquid smoke (SOLS) alone was more effective in reducing lipid content. Addition of roselle (*Hibiscus sabdariffa*) extract (RCE) or *Schleichera oleosa* liquid smoke (SOLS) increased nitrite residual of *se'i*.

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