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THE EFFECTS OF NATURAL CURING ON SENSORY AND PHYSICO-CHEMICAL QUALITY ATTRIBUTES OF DENDENG SAPI (INDONESIAN DRIED BEEF)

E. Saputro[1*], V.P. Bintoro[2], Y.B. Pramono[2]

[1]National Animal Husbandry Training Center, Ministry of Agriculture, Republic of Indonesia, Batu, Indonesia
[2]Faculty of Animal and Agriculture Sciences, Diponegoro University, Tembalang Campus, Semarang, Indonesia

Abstract. This study investigated the effects of natural curing that used various levels of fresh celery leaves (FCL) and incubation temperature for 2 hours there were room temperature = RT and temperature of 40,6^{0}C = 40.6. The effects in the quality of sensory and physico-chemical attributes of naturally cured dendeng sapi were expected to be similar to the control or conventionally cured dendeng sapi that used 50 ppm of sodium nitrite (NaNO2). Four naturally cured dendeng sapi treatments (A) (A_1 = 22 g FCL/kg of beef, RT; A_2 = 36 g FCL/kg of beef, RT; A_3 = 22 g FCL/kg of beef, 40,6; A_4 = 36 g FCL/kg of beef, 40,6) and a treatment of conventionally cured dendeng sapi that used 50 ppm sodium nitrite as a control (A0) were used for this study. All the sensory quality attributes (cured aroma, cured color, color uniformity, firmness, celery aroma and celery flavor) among all treatments were different ($P<0.05$) except cured flavor ($P>0.05$). Cured flavor of all naturally cured dendeng sapi (A_1-A_4) showed no significant difference ($P>0.05$) which compared by the dendeng sapi control (A_0). The A0 received the highest score for cured aroma ($P<0.05$), cured color ($P<0.05$), color uniformity ($P<0.05$) and the softest ($P<0.05$) of all naturally cured dendeng sapi (A_1-A_4). All naturally cured dendeng sapi (A_1-A_4) showed similar measurement result values to the control (A_0) for the measurements of water activity, cook yield, proximate (moisture, fat and protein) and also more effectively suppressed lipid oxidation compared to the control (A_0). These results indicated that the natural curing treatment using 22 g FCL/kg of beef that incubated at room temperature (A1) was the best natural curing formulation. It was effectively replaced conventional curing using 50 ppm $NaNO_2$. A1 was fewer FCL levels and more practical of the incubation way. This resulted more effectively in naturally cured dendeng sapi that was comparable to conventionally cured dendeng sapi using 50 ppm $NaNO_2$.

Keywords: *natural curing, fresh celery leaves, sensory quality attributes of dendeng sapi, physico-chemical quality attributes of dendeng sapi, dendeng sapi.*

Corresponding Author: *Y.B. Pramono, Faculty of Animal and Agriculture Sciences, Diponegoro University, Tembalang Campus, Semarang 50274 – Indonesia, Tel: +628157932269, e-mail: yok_b_p@yahoo.com*

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1. Introduction

Curing process is defined as the use of cooking salt (NaCl) and nitrite (NO_2-) (the reduced form of nitrate, NO_3-) to change chemically the properties of physical, chemical and microbiological meat products [11]. Nitrite has positive effects that are responsible for cured color and flavor development. This source of powerful antioxidants protects the flavor from rancidity and acts as a strong antimicrobial to control the growth of Clostridium botulinum that produces of deadly botulism toxin [17]. Nitrite also has the negative effect that has invited food safety concerns. The toxic components development of carcinogenic N-nitroso such as N-nitrosamines become our concerns about the intake of nitrate and nitrite in humans that focused on the possibility. These two compounds can be source of nitrosating compounds. Nitrite which will be reduced to NO (nitric oxide), can bind a secondary amine under acidic condition or under heating too high temperatures to form N-nitrosamines, it's also called nitrosodietilamin (NDEA) which is carcinogenic, mutagenic and teratogenic. N-nitrosamines is formed faster in gastric fluid of humans and rabbits (pH 1-2) as compared to the rat gastric fluid (pH 4-5) [14]. The NO also can bind to haemoglobin to form nitroso-haemoglobin, called methaemoglobin that is not able to bind oxygen to be distributed to all body tissues called methaemoglobinemia. Death in people could occur if the content of methaemoglobin higher than ±70% [8].

The very dangerous negative effect of the use of sodium nitrite as a curing agent has been encouraged the efforts of many researchers of meat curing in order to get a replacement that is safer and healthier. However, there is no compounds have been found to date that can effectively replace all the functions of the role of nitrites in meat curing. The results and information collected from various efforts of nitrite replacement have increased knowledge about the importance of sodium nitrite and difficulty removing it from the meat curing process. The only one effort that can be taken until now is to replace sources of synthetic nitrate/nitrite with sources of natural nitrate/nitrite that is referred to as natural curing agents [10].

Some vegetables, such as celery, have been established to have high levels of nitrate in natural curing [4] which has the potential to be used as a source of natural nitrate. The effectiveness of the natural curing agent form of celery juice powder is combined with starter cultures of reducing nitrate (Staphylococcus carnosus) to replace conventional curing agent form of NaNO2. These have been proved by many [13,15-16]. Reduction of nitrate to nitrite by the starter culture is done through the incubation phase at a temperature that meets the specific requirements of the growth of Staphylococcus carnosus and performed for at least 2 hours of incubation before the cooking/thermal processing step. The results show the final product of naturally cured meat characteristics are similar to the final product of conventionally cured meat using $NaNO_2$.

Staphylococcus carnosus is the only nitrate reducing starter culture that is commercially available and has been proven safely for being used in naturally cured meat products. Availability in Indonesia arguably does not exist and not yet collected almost all the existing research institutions, including the National Sciences Institute of Indonesia. Availability has to be imported from abroad such as from the United States or Europe that there are commercially available. Surely, this will be very costly to procure, as result is not practical for producers of cured meat products, such as sausages, dendeng sapi and corned beef. The authors attempt to solve this problem by using only natural curing agent in the form of fresh celery leaves without the addition of nitrate-reducing bacteria. It is based on [8] reported that fresh celery leaves naturally contain high nitrate and nitrate reductase enzyme. The authors suggest that the reduction of nitrate to nitrite will rely on nitrate reductase enzyme contained in fresh celery leaves and produced by the nitrate-reducing bacteria according to [7] is naturally present in fresh meat.

This study aimed to investigate the influence of natural curing using various levels of fresh celery leaves (FCL) and incubation temperature for 2 hours in manufacturing of dendeng sapi to characterize some of the sensory and physico-chemical quality attributes which were similar to the control or conventional curing added 50 ppm synthetic sodium nitrite ($NaNO_2$) during product manufacture and during the storage period of finished products.

2. Materials and methods

Manufacturing materials of dendeng sapi used include: ground fresh beef (bottom round) from several local bulls of ongole crossbreed (OC) about three years old from the Penggaron abattoir of Semarang City, sugar, spices and fresh celery leaves (FCL) from Bandungan, Semarang Regency used to produce naturally cured dendeng sapi. The experiment used the experimental design of split-plot using a randomized complete block design consists of 3 replication groups. The main plot was curing treatments (A) consisted of 4 natural curing treatments by various levels of FCL and incubation temperature for 2 hours (room temperature = RT and temperature of 40.6^0C = 40.6) (A_1 = 22 g FCL/kg of beef, RT; A_2 = 36 g FCL/kg of beef, RT; A_3 = 22 g FCL/kg of beef, 40,6; A_4 = 36 g FCL/kg of beef, 40,6) and a treatment of conventional curing by 50 ppm sodium nitrite-added as a control (A_0). Subplot was 3 periods of storage or 0, 14th and 28^{th} observation days with the sample of their own. The replicate groups were the origin of bottom round 1, 2 and 3. For more details about the treatments of this study are presented on Table 1.

The predecessor studies about natural curing by Sindelar et al. (2010) [16] apply incubation temperature of 40.6^0C which provides optimal conditions for activity of the nitrate-reducing bacterial to produce nitrite. Sebranek (2009) [11] has reported the nitrite that is actually a curing agent in the meat curing process. The use of room temperature incubation is according to [16]. It says that nitrate-reducing bacteria has the minimal growth temperature at 10°C and the optimal growth temperature for activity at 30°C. Choosing of the amount of celery (22 or 36 g/kg of beef) is based on a study report by Saputro (2016) [9] reported that the activity nitrate reductase enzyme of 22 or 36 g fresh celery leaves for 2 hours incubation at room temperature has been calculated to be able to produces nitrite equivalent to 30 ppm and 50 ppm nitrite, respectively (or equivalent 8.26 µmol or 0.38 mg NO_2/g /h, n = 3).

Dendeng sapi formulation based Suryati et al. (2014) [18] which consisted of the following materials: 1 kg of ground fresh beef (bottom round), 16.5% brown sugar, white sugar 16.5%, 10% garlic, 8.5%, galangal, 2.5% salt, 2.0% coriander, 0.3% white pepper, 0.3% tamarind, 0.3% lime and 0.3% ice/water (based on the weight of the fresh beef (1 kg)). Treatment of A_0 was added 50 ppm $NaNO_2$; treatments of A1 and A3 were added 22 g of FCL; and treatment of A_2 and A_4 were added 36 g of FCL. All ingredients every treatment thoroughly mixed using a meat cutter bowl until dendeng sapi dough was evenly mixed about 5 minutes. The dendeng sapi dough of A_1 or A_2 was incubated for 2 hours at room temperature. The dendeng sapi dough of A_3 or A_4 was placed in an incubator and incubated for 2 hours at a temperature of 40.6°C. The control (A_0) was dried directly in the oven without incubation step after being flaked by a thin around 0.2 cm (measured using a board with a thickness of 0.2 cm). All treatments were dried by using a drying oven at 63°C. After 2 hours, dendeng sapi sheets were turned over in turns to reach a water activity ≤ 0.85. After drying, dendeng sapi pieces of each treatment for each sample of 0, 14^{th} and 28^{th} day were placed in a plastic barrier bag polypropylene (PP) and packaged vacuum for storage. They were stored at room temperature in a tightly closed, opaque container until to be done in sensory and physico-chemical quality analysis.

Tabel 1. List of the materials and the treatments of study

	Conventional Curing	Natural Curing			
	A_0	A_1	A_2	A_3	A_4
	..........(g)..........				
material					
fresh beef (bottom round)	1000	1000	1000	1000	1000
brown sugar	165	165	165	165	165
white sugar	165	165	165	165	165
garlic	100	100	100	100	100
galangal	85	85	85	85	85
salt	25	25	25	25	25
coriander	20	20	20	20	20
white pepper	3	3	3	3	3
tamarind	3	3	3	3	3
lime	3	3	3	3	3
ice/water	3	3	3	3	3
treatment					
fresh celery leaves (FCL)	no FCL	22	36	22	36
Sodium nitrite ($NaNO_2$)	50 mg	replaced with FCL			
incubation temperature	no incubation	RT	RT	40.6°C	40.6°C

RT = room temperature

Cook yield of each treatment was expressed in percent and determined by the dry weight of dendeng sapi divided by the total weight of raw materials and multiply to 100. Water activity (a_w) was measured using a water activity meter from two different dendeng sapi slices for each treatment. The average was used for statistical analysis. Proximate composition (AOAC, 1990) which was measured included: crude fat, moisture and crude protein. The pH levels of dendeng sapi samples were determined according to the methods of [12]. The temperature of the dendeng sapi dough was measured when preincubation using meat thermometer. Measurement of each treatment was performed in duplicate and the average was used in data analysis. Lipid oxidation was measured by peroxide value test (AOAC, 1990) [1] on the 28th day storage of dendeng sapi. Peroxide value was reported as mg equivalent O_2/kg sample of dendeng sapi. Trained sensory analysis was carried out by the method according to Sindelar et al. (2007a) [13] by 14 trained panelists that was performed on the 14th day after the manufacture of the product in order to mimic the approximate time period of initial product availability in a commercial distribution chain. Panelists were trained 3 times before the sensory analysis conducted using 3 commercial dendeng sapi products on the 1st training and 5 dendeng sapi products of treatments on the 2nd and 3rd training. For each session sensory analysis was used 14 trained panelists. They consisted of Food

Technology Major students of 5th semester who were receiving Sensory Course, Faculty of Animal and Agriculture Sciences in Diponegoro University students. Attributes of sensory quality were measured using a scale line (numerical value of 15 units) with graduation from 0 to 15 where 0 represented none (aroma and flavor), not uniform (color), low (color) and soft (firmness) and 15 represented intense (aroma and taste), uniform (color), high (color) and hard (firmness).

Statistical analysis was performed for all measurements that used the SPSS procedures (version 17.0) by analysis of variance (ANOVA). The differences between treatments were tested further using the Tukey test. Especially for sensory dates were tested by non-parametric test (the Kruskal Wallis test) and the differences between treatments were tested further using the Mann Whitney test. This were was used to test further the differences between treatments. Significance level was determined at $P<0.05$.

2. Results and discussions

The pH Values and Temperature

Various products processing parameters were measured and recorded during the manufacture of dendeng sapi. They are presented on Table 2. Preincubation pH ranged from 6.03 to 6.08 couldn't be found differences ($P>0.05$) from those the treatments. And also postincubation pH ranged from 6.12 to 6.27 couldn't be found differences ($P>0.05$) from those the treatments. Postincubation pH for control (A_0) was not measured because there was no incubation step was applied.

Table 2. Means for product processing attributes and peroxide value

Treatments	Preincubation		Postincubation	a_w	Cook Yield (%)	Peroxide Values (mg/kg)
	pH	Temperature (°C)	pH			
A_0	6.05	27.83 ^{q}	not measured	0.68	46.8	3.891 ^{p}
A_1	6.04	28.33 ^{q}	6.19	0.61	46.7	2,594 ^{rs}
A_2	6.08	29.00 ^{p}	6.12	0.57	45.5	3,597 ^{pq}
A_3	6.07	28.33 ^{q}	6.27	0.60	46.8	2,928 ^{qr}
A_4	6.03	29.00 ^{p}	6.15	0.68	47.9	1,998 ^{s}
Standar Error	0.05	0.15	0.05	0.02	0.93	0,201

$^{p-s}$ *Means within same column with different superscripts are significantly different (P<0.05); Means within same column without the superscripts are not different; n = 30 except n for Postincubation pH levels = 24.*

The range of preincubation pH become the ideal conditions for the formation of nitric acid (HNO_2) as reported [2, 6]. When nitric acid is in equilibrium with dissociates N_2O_3. They form nitric oxide (NO) and nitrogen dioxide (NO_2). Then the NO can react with the myoglobin to produce dark red NO-myoglobin, after that, this is converted to pink stable nitrosylhemochrome upon heating [11].

The temperature of the dendeng sapi dough was measured when preincubation ranged from 27.83 to 29.00°C. There were significant differences ($P<0.05$) of preincubation temperature of the dough between treatments. The dough temperature of

A_2 and A_4 significantly higher ($P<0.05$) than the dough temperature of A_0, A_1 and A_3. This can be presumed because A_2 and A_4 used more fresh celery leaves.

The temperature range was the ideal temperature range for optimal growth and activity nitrate-reducing bacteria that are naturally present in the fresh beef which used in the dendeng sapi manufacture. According to [16], nitrate-reducing bacteria that are naturally present in fresh beef and nitrate-reducing starter culture, such as Staphylococcus carnosus strains have the minimal growth temperature at 10°C and the optimal growth temperature for activity at 30°C. Sebranek et al. (2001) [12] had reported that the nitrous acid (HNO_2) is generated during the curing process depends on pH, temperature and time incubation.

Cook Yield and Water Activity (aw)

Cook yield of dendeng sapi treatments ranged from 45.47% to 47.88% showed no significant differences ($P>0.05$), that were found between every treatments for cook yields. This showed that uniform drying took place equally. Finishing product (oven dried dendeng sapi) water activities (aw) of all treatments ranged between 0.57 and 0.68 could inhibit the growth of spoilage and pathogenic bacteria. Most spoilage bacteria will not grow below aw of 0.91 and pathogenic bacteria like Staphylococcus aureus are limited by below aw of 0.86. [3]. No significant differences ($P>0.05$) were found between treatments for water activity. This indicated that all batches of dendeng sapi were produced in a uniform condition.

Proximate Composition

Proximate composition was represented by the sample of conventional curing treatment A_0 or the control with a curing agent in the form of sodium nitrite and by the sample of natural curing treatment with a curing agent in the form of 22 g (A_1 or A_3) or 36 g (A_2 or A_4) of fresh celery leaves. The sample of natural curing treatments was randomly selected among incubations at room temperature or at a temperature of 40.6^0C. The sample of natural curing treatment used 22 g (A_1 or A_3) or 36 g (A_2 or A_4) FCL that was measured in its proximate composition respectively without mixing. The proximate composition measurement results of the natural curing treatment sample used 22 g (A_1 or A_3) FCL was combined and the average was used in data analysis. Likewise for the natural curing treatment sample used 36 g (A_2 or A_4) FCL. These were done to determine whether the FCL levels affected the proximate composition of naturally cured dendeng sapi.

Table 3. The Means for Proximate Composition of *Dendeng Sapi* (n = 18)

Treatments	Moisture	Fat	Protein
	.... (%)	....	
A_0	19.9	5.2	29.2
$A_{1/3}$	22.1	5.5	28.1
$A_{2/4}$	20.7	4.8	28.4
Standard Error	0.57	0.50	0.99

Proximate composition is presented on Table 3. The proximate composition of oven dried/raw dendeng sapi was treated as follow; for moisture ranged from 19.92 to 22.11%; for fat ranged from 4.75 to 5.54% and for protein ranged from 28.08 to 29.22%. There were no significant differences (P>0.05) for proximate composition between all treatments. It showed that concentration which added by fresh celery leaves, did not affect proximate composition and all treatments equally in the proximate composition.

Peroxide Values

Table 2 shows that there were significant differences (P<0.05) for lipid oxidation that were measured by peroxide value at day 28 of storage. The peroxide value mean ranged between 1.998 and 3.891 mg equivalent of O_2/kg. The lowest peroxide values significantly (P<0.05) were in the A_4 and A_1. The highest peroxide values significantly (P<0.05) were in the A_0 and A_2. It showed that the 22 g of fresh celery leaves which used as an agent of natural curing was more effective as an antioxidant of lipid oxidation suppressing during storage time than an agent of conventional curing in the form of synthetic sodium nitrite (50 ppm). A natural curing by incubation at room temperature for 2 hours with 22 g of fresh celery leaves (A_1) significantly produced lower peroxide values (P<0.05) than with 36 g of fresh celery leaves (A_3 or A_4). It showed that the natural curing by incubation at room temperature for 2 hours with 22 g of fresh celery leaves (A_1) was more effective as an antioxidant of fat oxidation suppressing during storage time than the others. Instead of a natural curing by incubation at a temperature of 40.6°C for 2 hours with 36 g of fresh celery leaves (A_4) significantly produced lower peroxide values (P<0.05) than the others. It showed that 36 g of fresh celery leaves used as an agent of natural curing would be more effective as an antioxidant of lipid oxidation suppressing during storage time if it was incubated at a temperature of 40.6^0C. If the natural curing using 36 g of fresh celery leaves which was incubated at room temperature would produce peroxide value equivalent to conventional curing using synthetic sodium nitrite (50 ppm).

Sensory Quality Attributes

The score mean for all sensory quality attributes are presented on Table 4. All sensory quality attributes treatments: cured aroma, cured color, color uniformity, firmness, celery aroma and celery flavor were different (P<0.05) except for the cured flavor (P>0.05). There was no different cured flavor allegedly because the nitrate reductase enzyme activity during 2 hours of incubation at room temperature or 40.6°C of 22 g and 36 g of fresh celery leaves had been calculated to be able to produces nitrite equivalent to 30 ppm and 50 ppm nitrite, respectively (8.26 μmol or equivalent to 0.38 mg NO_2-/g/h, n=3) [9]. MacDonald et al. (1980) [5] found that nitrite levels of 50 ppm were sufficient to induce cured flavor as identified by the consumer sensory test.

The control or A_0 received the higher score for cured aroma (P<0.05), cured flavor (not significant), cured color (P<0.05) and color uniformity (P<0.05) than all treatments without the addition of $NaNO_2$ (A_1 – A_4). The lowest of firmness scores (P<0.05) were received by the control or A0 and the highest (P <0.05) were received by treatment of A4. It was the opposite from [6]. They had reported that the nitrite curing reaction increases firmness. Sebranek & Bacus (2007) [10] reported that the nitrite is reduced from FCL nitrate or added (form NaNO2) would react during curing. Not only to produce NO, but this has also been reported as a partial explanation for the change in the cured meat texture. In cross linking between protein, if it is significant, it can contribute to more compact product texture.

Table 4. Means for Sensory Quality Attributes (n = 210)

Treatments	Cured Aroma	Cured Flavor	Cured Color	Color Uniformity	Firm-ness	Celery Aroma	Celery flavor
A_0	10.76^{a}	10.61	8.91 ^{a}	10.96 ^{a}	5.48 ^{e}	-	-
A_1	8.67 ^{d}	10.03	7.77 ^{c}	8.75 ^{d}	6.15 ^{d}	1.60 ^{d}	3.10 ^{c}
A_2	8.12 ^{e}	8.47	6.70 ^{d}	8.58 ^{e}	6.81 ^{b}	3.64 ^{b}	4.15 ^{a}
A_3	9.34 ^{b}	9.49	8.21 ^{b}	10,11 ^{c}	6.81 ^{c}	1.72 ^{c}	2.54 ^{d}
A_4	8.84 ^{c}	9.19	5.72 ^{e}	10.50 ^{b}	7.99 ^{a}	3.19 ^{a}	3.48 ^{b}
SEM ^{f}	0.30	0.29	0.26	0.24	0.25	0.24	0.25

$^{a-e}$ Means on the same column with different superscripts are significant different (P<0.05).

^{f} SEM = *standard error of the means*

The lowest of celery aroma scores (P<0.05) were received by treatment of A_1 and the highest (P<0.05) were received by treatment of A_4. The lowest of celery flavor scores (P<0.05) were received by treatment of A_3 and the highest (P<0.05) were received by treatment of A_2. The results of the subjective assessment from trained panelists could be assumed that the natural curing using 22 g FCL and the low incubation temperature (room temperature) could minimize the appearance of celery aroma but this improved the appearance of celery flavor. It was the other way in the natural curing using 36 g FCL. The results of the trained panelists assessment had to be proved by more objective assay in the celery aroma and celery flavor of naturally cured dendeng sapi.

Trained sensory analysis showed the celery aroma and flavor which tended to approach did not exist in the product. The average of the trained panelists assessment for celery aroma and celery flavor was in the range from 1.60 to 3.64 and 2.54 to 4.15, respectively. The mean closed to zero or none at all was not detected for celery aroma and celery flavor. In naturally cured dendeng sapi of A_1 showed that the assessment results of trained panelists the closest for celery aroma was undetectable and A_3 was also undetected for celery flavor. Due to the dendeng sapi spices used, this might provide a predominant aroma and flavor and resulted masking in aroma and flavor of celery in dendeng sapi.

3. Conclusions

All sensory quality attributes (cured aroma, cured color, color uniformity, firmness, celery aroma and celery flavor) in among all treatments were significantly different except cured flavor. Conventional curing treatment or the control using the $NaNO_2$ addition (50 ppm) (A_0) received the highest scores for cured aroma, cured color, color uniformity and the most tender which were compared to all natural curing treatments without the addition of $NaNO_2$ (A_1 – A_4). Sensory analysis showed that celery aroma and flavor scores tended to approach undetected score as a result of the masking by spices used. Natural curing treatments without the addition of $NaNO_2$ (A_1 – A_4) showed simile to the controls for the measurements of water activity, cook yield, proximate and cured flavor. They showed more effective suppress the lipid oxidation compared to the control. Natural curing treatment by 22 g of fresh celery leaves and incubation at room temperature were the best of natural curing formulation. Further research regarding the effect of this natural curing method on microbiological and shelf life control; and carcinogenic nitrosamines formation of dendeng sapi at the concentrations tested in this study is needed.

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