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Formulation of Natural Curing in the Manufacturing of *Dendeng Sapi* – Indonesian Dried Beef from Local Beef Cattle

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ABSTRACT

This study was conducted to investigate the formulation of natural curing by various levels of fresh celery leaves (FCL) and incubation temperature. The incubation temperature was room temperature (RT) and temperature of 40.6°C (40.6) for 2 hours. These incubation temperatures for manufacturing *dendeng sapi* were designed to provide the similar curing characteristics of the control (conventional curing by addition of 50 ppm of $NaNO_2$). Four naturally cured *dendeng sapi* treatments were 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°C; A4 = 36 g FCL/kg of beef, 40.6°C and a treatment of conventionally cured *dendeng sapi* by addition of 50 ppm $NaNO_2$ as a control (A_0). All sensory quality attributes (cured aroma, cured color, color uniformity, firmness, celery aroma and celery flavor among all treatments were significantly different ($P<0.05$) except cured flavor ($P>0.05$). The control (A_0) received the highest scores for cured aroma ($P<0.05$), cured flavor (not significant), cured color ($P<0.05$), color uniformity ($P<0.05$) and the most tender ($P<0.05$) than all natural curing treatments without the addition of $NaNO_2$ (A_1 - A_4). Sensory analysis showed celery aroma and flavor scores which tend to approach undetectable as a result of the closure of (masking) by spices used. Treatments of A_1 - A_4 showed similar to the control for the measurements of proximate, water activity, cook yield, color, cured pigment, total pigment, residual nitrite and cured flavor. They showed more effectively suppress the lipid oxidation compared to the control. These results indicated that natural curing treatment by 22 g of FCL and incubated at room temperature (A_1) is the best of natural curing formulation.

Key Words: Natural Curing, Fresh Celery Leaves, Sensory Quality, Chemical Quality, *Dendeng Sapi*

INTRODUCTION

Dendeng sapi is Indonesian preserved beef by chemically and physically preservation. *Dendeng sapi* is made with the addition of kitchen salt (NaCl), saltpetre (sodium nitrate/nitrite), sugar and Indonesian typical spices as a seasoning on thin slices of meat or ground meat then dried using sunlight or using a drying oven. *Dendeng sapi* is included conventional curing meat products because of use of saltpeter (sodium nitrate/nitrite) in the composition of raw material. The negative effects of nitrite have invited concerns through food safety which can cause methaemoglobinemia and the formation of carcinogenic N-nitrosamine have become the reason many researchers to find alternative healthier and safer natural curing.

Sebranek & Bacus (2007) had described the process of meat curing called natural curing. This process uses natural ingredients that contain relatively high levels of nitrate naturally combined with a starter culture of bacteria that produce enzyme of nitrate reductase to reduce nitrate to nitrite. Some vegetables, such as celery, have been shown to have high level of natural nitrate (Walker 1990; Fujihara et al. 2001). Reduction of nitrate to nitrite by the starter culture is done through the incubation step at a temperature that meets the specific requirements of the growth of the microorganism used and carried over a certain period before the cooking/thermal processing step. Previous researches have

recommended a minimum of 2 hours incubation for the generation of enough nitrite then to develop the characteristics of cured meat (Sindelar et al. 2007a; Sindelar et al. 2007b).

Previous researches by Sindelar et al. (2007a); Sindelar et al. (2007[b]); Sindelar et al. (2010); Terns et al. (2011a) and Terns et al. (2011b) used the natural curing agents in the form of vegetables or fruit juice powdered and *Staphylococcus carnosus* that produce nitrate reductase enzyme that is able to reduce nitrate to nitrite. The authors did study by trying to use only natural curing agent in the form of fresh celery leaves (FCL) that naturally contains high nitrate and enzyme of nitrate reductase without the addition of nitrate-reducing bacteria. Nitrate reduction relied on enzyme of nitrate reductase contained in the leaves of fresh celery and produced by the nitrate-reducing bacteria according to McDougall et al. (1975); Sebranek (1979); Sanz et al. (1997) and Pinotti et al. (2001) naturally found in fresh meat. This study aimed to investigate the formulation of various concentrations of fresh celery leaves and incubation temperatures that affect both the quality of some chemical and sensory characteristics of *dendeng sapi* during product manufacture and then during storage times of finished products that is similar to the control added with synthetic sodium nitrite.

MATERIAL AND METHODS

Materials and experimental design

Materials of *dendeng sapi* used included: ground of fresh bottom round from local bulls of ongole crossbreed (OC) about 3 years from Penggaron abattoir (slaughterhouse) Semarang City, sugar, spices and fresh celery leaves from Bandungan, Semarang Regency were used to produce *dendeng sapi* natural curing.

The study used the experimental design of split-plot on the basis of a randomized complete block design consisting 3 replicate groups of each. The main plot was curing treatments (A) consisting 4 natural curing treatments by various levels of FCL and incubation temperature for 2 hours (room temperature = RT and temperature of 40.6°C). The levels of FCL were 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°C; A_4 = 36 g FCL/kg of beef, 40.6°C) and a treatment of conventional curing by 50 ppm sodium nitrite-added as a control (A_0). The subplot was 3 periods of storage or 0, 14^{th} and 28^{th} observation days with the sample of their own. The replicate groups were the origin of bottom round 1, 2 and 3.

Bottom round was trimmed in order to clean of external fat and ground, then was randomly placed in a container (each 1 kg for each treatment). *Dendeng sapi* formulation based Suryati et al. (2014) consisted of the following materials: 1 kg of ground bottom round, 16.5% brown sugar, 16.5%white sugar, 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 beef). Treatment of A_0 was addition of 50 ppm of sodium nitrite; treatment of A_1 and A_3 were added 22 g of fresh celery leaves; and treatment of A_2 and A_4 were added 36 g of FCL. The mixture of *dendeng sapi* A_1 and A_2 were incubated for 2 hours at room temperature. The mixture of *dendeng sapi* A_3 and A_4 were 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 after flaked by a thin 0.1 to 0.2 cm without incubation step. All treatments were dried using a drying oven for 2 hours at 63°C and reversed such that the bottom side was at the top, and then the drying was continued at 63°C for 2 hours to reach a water activity $\leq$ 0.85. After the drying was complete, *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. They were stored at room temperature in a tightly closed container and opaque until chemical and trained sensory quality analysis.

Research parameters

Cook yield of each treatment was expressed in percent and determined by dividing the dry weight by the total weight of raw materials and multiplying by 100. Water activity (a_w) was measured using a water activity meter from two different *dendeng sapi* slices from each treatment. The average was used for statistical analysis. Commission International d'Eclairage (CIE) *L** (lightness), *a** redness) and *b** (yellowness) were determined using a digital colorimeter for Macintosh. Measurements of surface color *dendeng sapi* were taken after the products were removed from vacuum packaging. Measurements were taken at 6 randomly selected areas on the sample and the average was used in data analysis. Proximate composition measured include: crude fat (AOAC 1990a), moisture (AOAC 1990b) and crude protein (AOAC 1993). The pH of *dendeng sapi* samples were determined according to the methods of Sebranek et al. (2001). 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 1997) on the 28th day storage of *dendeng sapi*. Peroxide value was reported as mg equivalent O_2/kg sample of *dendeng sapi*. Mononitrosylhemochrome pigment (cured meat pigment) and total pigment concentrations were measured using a method of Hornsey (1956), after extraction in 80% acetone and acidified acetone, respectively. Residual nitrite was determined before incubation (preincubate), after incubation (postincubate), after drying (day 0) and continuing throughout a 28-day storage times. Residual nitrite were determined by the AOAC method (AOAC 1990c). All residual nitrite assays were done in duplicate and all treatments within a replication were analyzed at the same time to minimize the variation in the analysis due to time. Sensory test carried out by the method according Sindelar et al. (2007a) 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. 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).

Data analysis

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

RESULTS AND DISCUSSION

Product processing attributes

Various products processing parameters were measured and recorded during the manafacture of *dendeng sapi* are presented in Table 1. Preincubated pH ranged from 6.03 to 6.08 and no differences ($P>0.05$) were found between the treatments. Postincubate pH

ranged from 6.12 to 6.27 and no differences (P>0.05) were found between the treatments. Postincubate pH for control (A_0) was not measured because there was no incubation step applied to the control.

Table 1. Means for product processing attributes and peroxide value

Treatments	Preincubate		Postincubate	a_w	Cook yield (%)	Peroxide values (mg/kg)
	pH	Temperature (°C)	pH			
A_0	6.05	27.83^{q}	not measured	0.68	46.82	3.891^{p}
A_1	6.04	28.33^{q}	6.19	0.61	46.66	2.594^{rs}
A_2	6.08	29.00^{p}	6.12	0.57	45.47	3.597^{pq}
A_3	6.07	28.33^{q}	6.27	0.60	46.81	2.928^{qr}
A_4	6.03	29.00^{p}	6.15	0.68	47.88	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 different (P<0.05)

The Preincubated pH becomes the ideal conditions for the formation of nitric acid (HNO_2) as reported Honikel (2004); Pegg & Shahidi (2000). Nitric acid is in equilibrium with N_2O_3, which dissociates to form nitric oxide (NO) and nitrogen dioxide (NO_2). The NO then can react with the myoglobin to produce dark red NO-myoglobin, then converted to pink stable nitrosylhemochrome upon heating (Sebranek 2009).

The temperature of the mixture was measured only when pre-incubate ranged from 27.83 to 29.00°C. There were significant differences (P<0.05) of pre-incubate temperature of the mixture between treatments. The temperature of the mixture of A_2 and A_4 significantly higher (P<0.05) than the temperature of the mixture of A_0, A_1 and A_3. It can be presumed because A_2 and A_4 using fresh celery leaves more. Cook yield of all treatments ranged from 45.47% to 47.88% and no significant differences (P>0.05) were found between any treatments for product yields. It suggests that uniform drying took place. Finished product (oven dried *dendeng sapi*) water activities of the all treatments ranged between 0.57 and 0.68 that were expected to inhibit the growth of spoilage and pathogenic bacteria. Most spoilage bacteria will not grow under a_w of 0.91 and pathogenic bacteria such as *Staphylococcus aureus* are limited by an a_w of 0.86 (Jay et al. 2005). No significant differences (P>0.05) were found between treatments for water activity indicating that all batches of *dendeng sapi* were produced in a uniform condition.

Color measurement

CIE *a** (redness) values of no-sodium nitrite *dendeng sapi* (A_1 - A_4) and sodium nitrite-added (A_0) indicated that the color was not red because all values were under zero. It was presumed pink cured color produced by the curing reaction covered by a brown color resulted from the browning reaction due to heating temperature of 63°C for 4 hours to the brown sugar and the white sugar added to the product which totals 33% of the meat used weight.

No significant interactions (P>0.05) were found between treatments and storage times for CIE *a** values as reported in Table 2, but the main effect of storage times (time of observation) significantly different (P<0.05) as reported in Table 3. CIE *a** values significant (P<0.05) increased throughout storage times toward the red color values (zero). The nitrate concentration in fresh celery leaves as a reservoir for curing reactions may

explain this occurence. According to Keeton et al. (2009), the nitrate concentration of celery average of 1496 ppm with the range of 20-4269 ppm and the nitrite concentration of celery average of 0.1 ppm with the range from 0.02 to 0.5 ppm. According to Sebranek (2009), nitrite is highly reactive as a curing agent so that it will run out relatively quick from cured meat. Nitrate serves as an important reservoir in cured meat products to maintain an effective concentration of nitrite during heating/drying or storage in the long term.

Table 2. The means for residual nitrite, cured pigment, total pigment and CIE *L**, *a**, *b** values at various storage times

Treatments	Storage times			Treatments	Storage times		
	0	14	28		0	14	28
Residual nitrite (ppm)				CIE *L** (lightness) values			
A_0	0.060	0,084	0.050	A_0	46.762	41.666	41.992
A_1	0.048	0.073	0.054	A_1	40.807	41.851	42.477
A_2	0.053	0.089	0.035	A_2	44.196	43.349	44.303
A_3	0.038	0.083	0.045	A_3	42.080	40.496	41.978
A_4	0.061	0.072	0.083	A_4	40.140	40.655	38.148
SEM [a]	0.006			SEM [a]	0.542		
Cured pigment (nitrosylhemochrome)				CIE *a** (redness) values			
A_0	57.855	113.293	189.853	A_0	-3.177	-2.505	-2.603
A_1	37.072	141.230	139.683	A_1	-5.093	-5.605	-4.197
A_2	99.470	155.150	186.180	A_2	-6.066	-5.139	-4.971
A_3	41.180	146.933	211.217	A_3	-5.912	-4.540	-4.207
A_4	35.090	88.063	185.987	A_4	-5.931	-5.504	-4.929
SEM [a]	13.353			SEM [a]	0.205		
Total pigment				CIE *b** (yellowness) values			
A_0	444.040	260.893	244.120	A_0	18.297	15.613	16.496
A_1	577.433	248.653	424.547	A_1	16.642	12.110	13.988
A_2	340.567	384.427	493.227	A_2	17.187	13.552	16.140
A_3	371.280	264.293	338.867	A_3	18.528	13.276	16.121
A_4	247.633	259.080	471.240	A_4	18.035	14,500	14.514
SEM [a]	42.901			SEM [a]	0.527		

[a] SEM: standard error of the means

Table 3. The means for the main effect of storage times (days 0, 14, 28) for CIE *a** and *b** values and cured pigment

Research parameter	Storage times			Standard error
	0	14	28	
CIE *a** (redness)	-5.236^{q}	-4.658^{pq}	-4.181^{p}	0.205
CIE *b** (yellowness)	17.738^{p}	13.810^{q}	15.452^{pq}	0.527
Cured pigment (ppm)	54.133^{q}	128.934^{pq}	182.584^{p}	13.353

$^{p-q}$ Means within same row with different superscripts are significant different (P<0.05)

Table 4. The means for the main effect of curing treatment

Research parameter	Treatments					Standard error
	A_0	A_1	A_2	A_3	A_4	
a* (redness)	-2.762 p	-4.965 q	-5.392 q	-4.886 q	-5.454 q	0.205

p-q Means within same row with different superscripts are significant different (P<0.05)

Table 5. The means for proximate composition of *dendeng sapi*

Treatments	Moisture	Fat	Protein
		(%)	
A_0	19.92	5,20	29.22
$A_{1/3}$	22.11	5.54	28.08
$A_{2/4}$	20.66	4.75	28.37
Standard error	0.57	0.50	0.99

The mean for CIE *L** (lightness) values of *dendeng sapi* in various storage times (days 0, 14 and 28) are reported in Table 2. No interaction was presented for treatment x storage times for CIE *L** values of *dendeng sapi*. CIE *L** values generally remained similiar throughout storage times which showed cured color not fading throughout storage times. Although no significant difference, A_0 or the control revealed a lighter color than all other treatments at day 0. According to Sindelar et al. (2010) a lighter color suggests the presence of more darkish-colored metmyoglobin pigment and less reddish-colored nitrosylhemochrome pigment. According to Pegg & Shahidi (2000), when nitrite is added to ground beef, browning effect occurs because nitrite is so a strong oxidant to the heme in myoglobin that myoglobin and oxymyoglobin were oxidised to metmyoglobin. Reduction of nitrite into nitric acid make an unstable intermediate pigment (nitrosylmetmyoglobin) formed. Presume due to absence of adequate endogenous and exogenous reductants, nitrosylmetmyoglobin pigments can not autoreduct to form more stable dark red NO-myoglobin or nitrosylmyoglobin.

The mean for CIE *L** values of A_0 or the control showed higher (not significant) at day 0 than at 14th and 28th storage day due to the decreasing of nitrosylhemochrome pigment or fading cured color throughout storage times. This can be explained that A_0 is only added sodium nitrite and no nitrates added as a backup source of nitrite to the regeneration reaction curing color development and stability throughout the storage times.

The mean for CIE *b** (yellowness) values of *dendeng sapi* from all treatments at various storage times (days 0, 14, 28) are reported in Table 2. No interaction was present for treatment x storage times for CIE *b** values but the main effect of storage times (time observation) was significantly different ($P<0.05$) as reported in Table 3. CIE *b** values were generally significantly decreased ($P<0.05$) at day14 of storage compared with day 0 of storage and significantly increased ($P<0.05$) or remained at day 28 compared with day 14 of storage. CIE *b** values were generally significantly decreased ($P<0.05$) or remained at day 28 compared with day 0 of storage.

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 the sample of natural curing treatment with a curing agent in the form of 22 g or 36 g of fresh celery leaves which were randomly selected among incubated at room temperature or at a temperature of 40.6°C. Proximate composition is presented in Table 5. The proximate composition of oven dried/raw *dendeng sapi* all treatments 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%. No significant difference ($P>0.05$) were found for proximate composition between all treatments. It showed that concentration of fresh celery leaves added did not affect proximate composition and all treatments were uniformly in the proximate composition.

Peroxide values

A significant difference ($P<0.05$) was observed for lipid oxidation measured by peroxide value at day 28 of storage as reported in Table 1. The mean for peroxide values ranged between 1.998 and 3.891 mg equivalent of O_2/kg. Peroxide values were significant ($P<0.05$) the lowest in the A_4 and significan ($P<0.05$) the highest in the A_0. It suggests that the 22 g or 36 g of fresh celery leaves used as an agent of natural curing was more effective as an antioxidant of lipid oxidation suppressing during storage times compared with an agents 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 significantly produces lower peroxide values ($P<0.05$) than with 36 g of fresh celery leaves. It suggests that the natural curing by incubation at room temperature for 2 hours with 22 g of fresh celery leaves was more effective as an antioxidant of fat oxidation suppressing during storage times. Instead of a natural curing by incubation at a temperature of 40.6°C for 2 hours with 36 g of fresh celery leaves significantly produces lower peroxide values ($P<0.05$) than with 22 g of fresh celery leaves. It showed that 36 g of fresh celery leaves used as an agent of natural curing will be more effective as an antioxidant of lipid oxidation suppressing during storage times if it was incubated at a temperature of 40.6°C.

Cured pigment and total pigment

No significant differences ($P>0.05$) were observed for the treatment x storage times interaction for cured pigment concentration of each treatment at various periods of storage but the main effect of storage times was different significantly ($P<0.05$) as presented in Table 3. Trends indicated that cured pigment concentration generally increased significantly ($P<0.05$) over time of storage regardless of treatment of fresh celery leaves

concentration and applied incubation temperature. It showed that the level of formulated fresh celery leaves and applied incubation temperature did not appear to be as important as the amount of time required for enzyme of nitrate reductase of fresh celery leaves or fresh beef to convert nitrate to nitrite and then into nitric oxide (NO) to result in cured pigment development. Lee & Cassens (1976) concluded that a minimum of two hours of incubation is required to convert 90% of nitrite to nitric oxide (NO) and bound with myoglobin to nitrosylmyoglobin formation. According to Cassens et al. (1979) and Shahidi & Pegg (1992), after heating, the globin portion of nitrosylmyoglobin were denatured and escaped from the iron atom to form stable nitrosylmyochrome or nitrosylhemochrome.

Although it was not significantly different, trends at any time observations showed that incubation temperature of 40.6°C in natural curing with 22 g of fresh celery leaves was capable of producing nitric oxide (NO) for cured pigment formation more resulting from conversion of nitrate of fresh celery leaves to nitrite compared with incubation at room temperature. It did not occur in natural curing with 36 g of fresh celery leaves, opposite happened where incubation temperature of 40.6°C produce less of nitric oxyde compared with incubation at room temperature.

Means for total pigment concentration of treatments over storage times are reported in Table 2. No significant differences ($P>0.05$) for treatment x storage times interaction were observed for total pigment concentration of each treatment at various storage times. It suggested that bottom round differences from different local beef cattle carcasses utilized randomly for *dendeng sapi* mixture of each treatment did not cause variation in total pigments. Although no significant differences, total pigment analysis revealed that total pigment concentration for A_0 decreased over storage times. This may be explained because of depleting amounts of nitrite reservoir in A_0 over storage times . Total pigment for A_2 and A_4 showed an increasing trend over storage times although no significant difference. Total pigment for A_1 and A_3 showed a decreasing trend at day 14 and then increased at day 28 of storage although no significant difference.

Residual nitrite

Residual nitrite at pre- and post-incubation of all treatments was very slightly under 0.1 ppm or nearly undetectable. It suggested that nitrite available had been exhausted in the quick curing reactions during chopping and mixing *dendeng sapi* mixture. No significant differences ($P>0.05$) were found for residual nitrite at pre- or post-incubate in any treatments (Table 6). Residual nitrite at post-incubate of all treatments is not significant compared with pre-incubate. It showed the importance of the incubation time required for the conversion of nitrate to nitrite. According Sindelar et al. (2007a) as incubation time increased, level of nitrite was also increased.

All treatments contain residual nitrite very slightly under 0.1 ppm or nearly undetectable during the storage times as at pre- and post-incubation of *dendeng sapi* mixtures. This suggested that available nitrite were used in the regeneration of the rapid curing reactions during the storage times. No significant interaction ($P>0.05$) of treatment x storage times for residual nitrite between all treatments (Table 2). The residual nitrite of all treatments increased at day 14 of storage and then decreased at day 28 of storage (not significant). A decreasing in the residual nitrite although it was not significantly different at day 28 of storage was as expected, the levels of residual nitrite diminished over time for all treatments. This observation has been well documented by Jantawat et al. (1993), who found a decreasing residual nitrite level with increasing storage times relationship, and by Hustad et al. (1973), who reported that nitrite concentration was affected by storage times and storage temperature. Another explanation was suggested by Ahn et al. (2002), who

noted packaging effect in sausage samples stored in vacuum packages compared with aerobic packages. These authors reported that vacuum-packaged sausages had lower residual nitrite than samples stored in aerobic conditions. The authors suggested that this phenomenon was caused by the product environment being in the reduced state thus allowing the conversion of nitrite to nitric oxide and resulting in the lower residual nitrite levels found.

Table 6. Means for residual nitrite at pre and post-incubate

Treatments	Residual nitrite (ppm)	
	Pre-incubate	Post-incubate
A_0	0.075	not measured
A_1	0.053	0.046
A_2	0.048	0.061
A_3	0.062	0.073
A_4	0.042	0.043
Standard error	0.006	0.010

Each pair of treatment combinations (A_1 and A_3; A_2 and A_4) where celery leaves level held was constant, residual nitrite levels were not significantly different ($P>0.05$) when the incubation temperatures increased or were differentiated between room temperature and temperature of 40.6°C (Table 3). This pattern occured at all days over the 28-d storage times. It showed that the celery leaves levels of 22 g or 36 g produced the same levels of residual nitrite converted from nitrates during the 120 minutes of incubation at room temperature or at temperature of 40.6°C compared with A_0 or the control of sodium nitrite-added (50 ppm). Available nitrite were reacted through curing reactions result in low amount of nitrite of all treatments and over storage times.

The residual nitrite of oven dried *dendeng sapi* of all treatments at days 0, 14 and 28 storage still showed safe limits for consumption related methaemoglobinemia and or the formation of carcinogenic nitrosamines in the human body. BPOM RI has set the daily consumption which does not pose a danger to health (Acceptable daily intake, ADI) for potassium or sodium nitrite by 0 to 0.06 ppm or mg/kg body weight (BPOM 2013). The lethal doses of sodium nitrite for humans has been reported to be approximately 1 g (Ellenhorn & Barceloux 1988).

Sensory quality

The means score for all sensory quality attributes are presented in Table 7. All sensory quality attributes (cured aroma, celery aroma, cured color, color uniformity, firmness and celery flavor among all treatments were different ($P<0.05$) except for the cured flavor ($P>0.05$). No different cured flavor guessed because the enzyme of nitrate reductase from 22 g and 36 g of fresh celery leaves incubated for 2 hours either at room temperature or at temperature of 40.6°C used in this study has reckoned capable of producing the equivalent of 30 ppm and 50 ppm nitrite (n = 3). MacDonald et al. (1980) found that nitrite levels of 50 ppm were sufficient to induce cured flavor as identified by the consumer sensory test.

A_1 had no significant differences ($P>0.05$) for sensory quality attributes of cured color, firmness and cured flavor the same curing but significantly different ($P<0.05$) for cured aroma and color uniformity compared with A_0. A_2 had significant differences

($P<0.05$) for all sensory quality attributes compared with A_0. A_3 had no significant differences ($P>0.05$) for all sensory quality attributes compared with A_0. A_4 had no significant differences ($P>0.05$) for sensory quality attributes of color uniformity and cured flavor but significantly different ($P<0.05$) for cured aroma, cured color and firmness compared with A_0.

Table 7. Means for sensory quality attributes

Treatments	Cured aroma	Cured flavor	Cured color	Color uniformity	Firmness	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

A_0 or the control received the highest score for all sensory attributes except firmness. A_0 had a significantly ($P<0.05$) higher score for cured aroma than A_1, A_2, A_3 and A_4. No differences ($P>0.05$) were found between all treatments for cured flavor but A_0 or the control had a significantly ($P<0.05$) higher score for cured flavor than A_1, A_2, A_3 and A_4. A_0 had a significantly ($P<0.05$) higher score for cured color and color uniformity than A_1, A_2, A_3 and A_4. A_4 had a significantly ($P<0.05$) lower score for cured color than A_0, A_1, A_2, and A_3. A_2 had a significantly ($P<0.05$) lower score for color uniformity than A_0, A_1, A_3 and A_4. A_0 had a significantly ($P<0.05$) lower score for firmness than A_1, A_2, A_3 and A_4. It did not agree with Pegg & Shahidi (2000) reported that the curing reactions of nitrite increase firmness.

Trained sensory analysis showed the aroma and flavor of celery which tend to approach did not exist in the product. This may have been due to the *dendeng sapi* spices used, which could provide a predominant aroma and flavor and result in aroma and flavor of celery masking in *dendeng sapi*.

CONCLUSION

The natural curing treatment by fresh celery leaves in this study was comparable to the conventional curing treatment by sodium nitrite-added (50 ppm). Sensory analysis showed celery aroma and celery flavor scores tend to approach undetectable as a result of the masking by spices used. The results indicated that the natural curing treatment by 22 g of fresh celery leaves and incubated at room temperature was the best of natural curing formulation.

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REFERENCES

Ahn HJ, Kim JH, Jo C, Lee CH, Byun MW. 2002. Reduction of carcinogenic nnitrosamines and residual nitrite in model system sausage by irradiation. J Food Sci. 67:1370-1373.

AOAC. 1990a. Fat (crude) or ether extract in meat (AOAC 960.39). In: Official Methods of Analysis. 15th Ed. Arlington (USA): Association of Official Analytical Chemists Inc. p. 2:931-948.

AOAC. 1990b. Moisture in meat (AOAC 950.46). In: Official Methods of Analysis. 15th Ed. Arlington (USA): Association of Official Analytical Chemists Inc. p. 2:931-948.

AOAC. 1990c. Nitrite in cured meat. In: Official Methods of Analysis. 15th Ed. Arlington (USA): Association of Official Analytical Chemists Inc. p. 973.31.

AOAC. 1993. Crude protein in meat and meat products (AOAC 992.15). In: Official Methods of Analysis. 15th Ed., 4th suppl. Arlington (USA): Association of Official Analytical Chemists Inc. p. 197-198.

AOAC. 1997. Offical Method of Peroxide Value of Oils and Fats. The Association of Analytical Chemists Inc. Arlington (USA): Association of Official Analytical Chemists Inc.

BPOM. 2013. Peraturan Kepala BPOM RI Nomor 36 Tahun 2013 tentang Batas Maksimum Penggunaan Bahan Tambahan Pangan Pengawet. Jakarta (Indonesia): Badan Pengawas Obat dan Makanan.

Cassens RG, Greaser ML, Lee M. 1979. Reactions of nitrite in meat. Food Tech. 33:46-57.

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology. New York (USA): Elsevier.

Fujihara SA, Kasuga, Aoyagi Y. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. J Food Sci. 66:412-415.

Honikel KO. 2004. Curing agents. In: Jensen WK, Devine C, Dikeman M, editors. Encyclopedia of meat sciences. Oxford (UK): Elsevier. p. 195-201.

Hustad GO, Cerveny JH, Trenk H, Deibel RH, Kautter DA, Fazio T, Johnston RW, Kolari OE. 1973. Effect of sodium nitrite and sodium nitrate on botulinal toxin production and nitrosamine formation in wieners. Appl Microbiol. 26:22-6.

Jantawat P, Runglerdkriangkrai J, Thunpithayakul C, Sanguandeekul R. 1993. Effects of initial nitrite level on residual nitrite and spoilage of canned ham roll. J Food Qual. 16:1-11.

Jay JM, Loessner MJ, Golden DA. 2005. Modern food microbiology (7th ed.). New York (USA): Springer.

Keeton JT, Osburn WN, Hardin MD, Bryan NS, Longnecker MT. 2009. A national survey of the nitrite/nitrate concentrations in cured meat products and nonmeat foods available at retail, American meat institute foundation report [Internet]. [cited August 11, 2015]. Available from: http://www.amif.org/ht/a/GetDocument Action/i/52741.

Lee SH, Cassens RG. 1976. Nitrite binding sites on myoglobin. J Food Sci. 41:969-970.

MacDonald B, Gray JI, Stanley DW, Usborne WR. 1980. Role of nitrite in cured meat favor: Sensory analysis. J Food Sci. 45:885-904.

McDougall DB, Mottram DS, Rhodes DN. 1975. Contribution of nitrite and nitrate to the colour and flavour of cured meats. J Sci Food Agric. 26:1743-1754.

Pegg RB, Shahidi F. 2000. Nitrite curing of meat. Trumbull (USA): Food & Nutrition Press Inc. p. 7-66.

Pinotti A, Graiver N, Califano A, Zaritzky N. 2001. Diffusion of nitrite and nitrate salts in pork tissue in the presence of natrium chloride. J Food Sci. 67:2165-2171.

Sanz Y, Vila R, Toldrá F, Nieto P, Flores J. 1997. Effect of nitrate and nitrite curing salts on microbial changes and sensory quality of rapid ripened sausages. Int J Food Microbiol. 37:225-229.

Sebranek JG, Bacus JN. 2007. Natural and organic cured meat products: Regulatory, manufacturing, marketing, quality and safety issues (American Meat Science Association White Paper Series No. 1) [Internet]. [cited August 11, 2015]. Available from: American Meat Science Association Web site: http://www.meatscience.org/Pubs/White%20Papers/wp_001_2007_Natural_Organic_Cured_Meat.pdf.

Sebranek JG. 1979. Advances in the technology of nitrite use and consideration of alternatives. Food Tech. 33:58-62.

Sebranek JG. 2009. Basic curing ingredients. In: Tarte R, editor. Ingredients in Meat Products. New York (USA): Springer Science+Business Media LLC.

Sebranek JG, Lonergan SM, King-Brink M, Larson E. 2001. Meat science and processing. 3rd Ed. Virginia (USA): Peerage Press. p. 141.

Shahidi F, Pegg RB. 1992. Nitrite-free meat curing systems: update and review. Food Chem. 43:185-191.

Shahidi F and Pegg RB. 1994. Absence of volatile N-nitrosamines in cooked nitrite-free cured muscle foods. Meat Sci. 37:327-336.

Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. 2007a. Effects of vegetable juice powder concentration and storage times on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. J Food Sci. 72:S324-S332.

Sindelar JJ, Cordray JC, Sebranek JG, Love JA, Ahn DU. 2007b. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. J Food Sci. 72:S388-S395.

Sindelar JJ, Terns MJ, Meyn E, Boles JA. 2010. Development of a method to manufacture uncured, no-nitrate/nitrite-added whole muscle jerky. Meat Sci. 86:298-303.

Suryati T, Astawan M, Lioe HN, Wresdiyati T, Usmiati S. 2014. Nitrite residue and malonaldehyde reduction in dendeng-Indonesian dried meat-influenced by spices, curing methods and precooking preparation. Meat Sci. 96:1403-1408.

Terns MJ, Milkowski AL, Claus JR, Sindelar JJ. 2011a. Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages. Meat Sci. 88:454-461.

Terns MJ, Milkowski AL, Rankin SA, Sindelar JJ. 2011b. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. Meat Sci. 88:311-318.

Walker R. 1990. Nitrates, nitrite and N-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. Food Addit Contam. 7:717-768.