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BEEF SOUSAGE TRAITS ENHANCED WITH CELERY LEAF POWDER (*APIUM GRAVEOLENS* L.) AS ANTIOXIDANT SOURCE AND NATURAL PRESERVATIVES

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ABSTRACT: The study aims to determine the effect of celery leaf powder as antioxidant source and natural preservative on the raits of beef sausages; and to analyze the Nitrite residues in beef sausage during storage. An experimental Laboratory was used in this study. It was carried out at the Laboratory of Animal Products processing Technology, Faculty of Animal Science, and the Analytical Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University. The research was conducted based on a Randomized Block Design (RCBD) with two factors such as celery powder and storage period, each treatment level was repeated 3 times. Data were analyzed using SAS software based on analysis of variance in significant level of 5%. During storage bacterial contamination tends to increase. Generally, it was indicate that at the level of celery was not effective to inhibit bacterial growth. Whereas Nitrate and Nitrite residues tend to decrease significantly during the storage period. Celery leaf powder which contains both Nitrate and Nitrite compounds can only inhibit the growth of acid-forming bacteria, it was reflected in the pH value which was increasing. In addition to Nitrate and Nitrite compounds, celery leaf powder can also be used as a source of antioxidant compounds, so that the possibility of fat damage in sausages can be inhibited.

KEYWORDS: Antioxidant, natural preservatives, celery, sausage

I. INTRODUCTION

Postharvest handling for improving the quality of livestock products can be done by preservation or processing. Meat is one of the main results of livestock has a complete nutritional content but has a low durability. So it needs an effort to handle, preserve, and process it to maintain the nutritional content. Nowadays food processing naturally and or organically becomes a very significant part in market growth. Consumers consider that natural or organic food processing is healthier than conventionally produced food products. To qualify as a natural or organic product, food must be produced and processed in accordance with the regulations of the United States Department of Agriculture (USDA) that sets the product. In some cases, natural and organic food products are very similar to conventional products and do not differ in characteristics expected by consumers. However, some types of processed meat products such as sausages, corned meat, burger meat and others, are usually cured with the addition of sodium nitrite, and sometimes sodium nitrate. In its requirements, the addition of natural or organic food products is not permitted the addition inorganic compound of nitrites or nitrates.

The role of nitrates and/ or nitrites in meat processing (curing meat) has yet to be replaced by other ingredients, both in terms of the level of color development of the curing meat products, and their characteristics as bacteriostatic products. Nitrites play an important role in meat that is meaty as a bacteriostatic and bactericidal agent. Nitrite strongly inhibits the growth of anaerobic bacteria, especially Clostridium botulinum, and also contributes to controlling other microorganisms such as Listeria monocytogenes. The two types of pathogen microbes often contaminate meat and processed meat products. However nitrite effects and inhibitory mechanisms will be different for several different bacterial species [1]. It was further reported, that the effectiveness of nitrite as an antibotulinal agent depends on several factors including pH, the concentration of sodium chloride, the presence of reductants such as iron content.

The USDA requires a minimum nitrite concentration of 120 ppm for products that are cured and stored in a refrigerator [2], implies that the presence of nitrite is very important. However, it is possible that the presence of nitrite is important because the nitrite content of the material affects the nitrite residue in the product.

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Several studies have documented consumer preferences for organic and natural foods based on concerns about antibiotics, pesticides, hormones, genetic modification in plants and animals, and chemical additives consumed by consumers regarding conventionally produced foods [3-6]. Vegetables are known as sources of nitrates with concentrations as high as 1500 ppm to 2800 ppm [7], in celery, lettuce and beets. Vegetable juices and vegetable powders are commercially available and can be used as natural and organic food ingredients. Analysis of some commercially available vegetable juices shows that carrots, celery, beets and spinach juice each contain 171; 2114; 2273 and 3227 ppm nitrate [8]. After 10 days of storage at room temperature, nitrate levels in this juice decreased by 14-22%. Nitrite was not detected initially but a concentration of 128-189 ppm nitrite was found after 10 days at room temperature, possibly caused by the reduction of nitrate bacteria. Analysis of commercial celery juice powder showed nitrate content on the order of 2750 ppm or around 2.75%, reflecting an increase in concentration after drying [9]. It appears that vegetable products are the greatest potential as a source of natural nitrate for meat processing.

Based on previous research, the concentration and duration and method of storage greatly influence the characteristics and residue of nitrite in meat products. Different studies on the use of natural curing ingredients in several conditions and levels, as well as the time and storage conditions for beef products need to be done, and therefore, substitute for conventional $NaNO_2$ as a natural curing agent and supporting technology needs to be found.

II. RESEARCH METHOD

The method used in this research was an experimental laboratory, which was carried out at the Laboratory of Animal Product Processing Technology, the Laboratory of Microbiology and Biotechnology, Faculty of Animal Science, and the Laboratory of Chemical Analytic, Faculty of Mathematics and Natural Sciences, Mataram University.

2.1 Experimental Design

The research was conducted based on a Randomized Block Design (RCBD) with two factors such as four levels of celery powder (0; 0.4; 0.8; and 1.2) percent, and three different of storage period, each treatment level was repeated 3 times. Data were analyzed using SAS software based on analysis of variance in significant level of 5%, following Duncan's Multiple Range Test.

2.2 Materials and Research Tools

Research material. Ingredients used in this study include: six kilograms of Bali Cattlle meat; 300 g of celery powder; Nutrient Agar (NA); plastic wrapping; N- (1-naphthyl) ethylene diamin dihydroxide (NED); KI in acetic acid; chloroform; sulfanylic acid; ferrous sulfate in concentrated sulfuric acid; petroleum benzene, concentrated H_2SO_4, K_2SO_4, 0.1N H_2SO_4, aquadest, H_3BO_3 3%, NaOH 40%, $CuSO_4$, boiling stones, BCG and MM indicators and sterilized aquadest.

The tools used in this study include: analytical scales, spoons, porcelain dishes, desiccators, tongs, ovens, soxhlet extraction unit, spectrophotometers; water bath, flask, flask pliers, erlenmeyer, bekker glass, measuring pipette, burette, fume hood, pH meter, pan, stove, knife, meat grinder, sausage filler and refrigerator.

2.3 Research Implementation

The implementation of this research includes the preparation of processing of the celery powder and preliminary research for the processing of sausages, followed by the implementation of the research covering the processing of sausages and observing/analyzing several parameters namely: a) physical characteristics; b) total bacterial contamination; c) Nitrite and itrate residues; e) total Antioxidants; and f) physical characteristics of sausage.

Celery powder was made in two forms: a) celery leaf powder, and b) celery stem/stalk powder. Celery leaves and stems were dried up for 6 hours at 50 ^{o}C, then mashed/ground until smooth and sieved/filtered.
According to [10] complete steps of work on making sausages are as follows:
a. Meat was cooled at a temperature of 1 to 4 °C.
b. Meat was cleaned from bones and veins or connective tissue.
c. Weigh 1 kg of meat, then cut into small blocks.
d. Pieces of meat were ground in a meat grinder while adding 100 grams of ice, 500 mg of vitamin C. Milling was done 2 times so that the meat was smooth. During grinding, the temperature of the mixture was not exceeded 22 °C.
e. Milled meat plus 10 grams of sugar, 7 grams of sodium tripolyphosphate, 250 grams of corn oil, 200 grams of ice, pepper, nutmeg, have been mashed to taste. The mixture was stirred in a listening container using a mixer for about 3 minutes.

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f. The mixture was then added with about 100 grams of tapioca flour as a binder.
g. Stirring was continued for 10 minutes. During the stirring the temperature of the dough was sought not to exceed 22 °C.
h. Stirring sausage results put into a filler (stuffer).
i. With the filling tool (stuffer) the dough was put into the wrapper (casing).
j. After filling the sausage wrapper was tied at the ends and at every 15 cm.
k. Provide hot water temperature of 70 to 80 °C.
l. Sausage was cooked in hot water for about 40 minutes.
m. After cooking, sausages were immediately cooled to a temperature of 25 °C and then hung for further packaging and storing.

2.4 Total Bacterial Count

The method used to calculate the total bacterial contamination in this study was the Pour Plate method. The ingredients used in the Pour Plate method were the Nutrien Agar (NA) media, as a medium for culture and physiological sodium chloride (physiological NaCl), as a sausage sample diluents solution.
The sampling method for calculating total bacteria in sausages was as follows:
a. Weighed 10 grams of sausage sample, then put into a stomacher to be crushed/ mashed so that it was easy to be dissolved/ diluted. Then 90 mL of physiological NaCl was added (dilution 10^{-1}).
b. Take 1 mL of solution a) into the test tube and add 9 ml of physiological NaCl solution, then homogeneous using vortex for 1 minute, this was a 10^{-2} dilution.
c. Subsequently a dilution of 10^{-3}, 10^{-4}, 10^{-5} was made in the same manner as in the 10^{-2} dilution process.
d. Take 1 ml of suspension from each dilution then put it in a Petri dish.
e. Added 15 mL to 20 mL NA (Nutrient Agar) to each cup that contains suspension. So that the sample solution and media can be mixed evenly, the cup was turned slowly and then settles it until it becomes solid.
f. Incubated at 37 °C for 24 hours to 48 hours by placing the cup in an upside down position.
g. Method for calculating total bacteria. Calculation of total microbes can be done using the Equation (1):

$$Total\ Bacteria = Total\ colony \times \frac{1}{\text{Dilution Factor}} \qquad (1)$$

To report the results of microbiological analysis as a Standard Plate Count (SPC) the following procedures were performed according to [11]:
a. The number of colonies that meet the counting requirements was between 30 to 300 per dish.
b. Several colonies that merge into one was a large collection of colonies, can be counted as one colony.
c. A row of colonies that appear as a thick line can be counted as one colony.

2.5 Fat Content

Determination of fat content was carried out using the Soxhlet method. The procedure works as follows:
Weighed the filter paper that had been dried in an oven at 105 ºC. Two grams of sample was weighed and then wrapped using filter paper with known weight. Samples that have been wrapped using filter paper were then dried up at 105 ºC, and cooled in a desiccator for 30 minutes and weighed. 75 mL petroleum benzene was poured through a sample of material in a Soxhlet tube. Water was flowed through the condenser and the temperature of the water bath was regulated in such a way that evaporation and condensation and the discussion of petroleum benzene continue. The extraction process was stopped when the solvent flask is clear. Samples were extracted from the extractor and the remaining benzene petroleum was evaporated then put into the oven at 105 ºC for 1 hour and cooled in a desiccator for 1 hour then weighed.
Calculation of fat content as shown in Equation (2):

$$\text{Crude fat (\%)} = \text{"C - D" / "B - A"} \times 100\% \qquad (2)$$

Where A is weight of filter paper that has been dried (g), B is sample weight + filter paper (g), C is weight of sample and filter paper which has been roasted (g), D is weight of sample that has been extracted (g).

2.6 Nitrate and Nitrite Residues

Identification of nitrites and nitrates. Nitrite residues were analyzed using sulfanylic acid reagents, N-(1-naphthyl) ethylene diamin dihydroxide (NED) and KI in acetic acid and chloroform. Nitrate identification was carried out using Zn metal reagents, sulfanylic acid and N- (1-naphthyl) ethylene diamin dihydroxide (NED) and ferrous sulfate in concentrated sulfuric acid. Determination of nitrite and nitrate levels was carried out by

visible light spectrophotometry using N-(1-naphthyl) ethylene diamin dihydroxide (NED) color reagents at a maximum wavelength of 540 nm [12].

2.7 Total Antioxidant

Determination of total antioxidants in organic sausage products was determined according to Joudalová, K., and Réblová [13], 0.1 mL sausage extract solution was added with FRAP reagent (2.5 mL acetate buffer; 2.5 mL 2,4,6-tripyridyl-striazine (TPTZ) solution and 2.5 mL FeCl3 6H2O solution) as much as 3 mL in the tube reaction. Then the absorbance solution was read with a spectrophotometer at a wavelength of 596 nm. The total antioxidant content was expressed as equivalent to Fe3+ to Fe2+ in μmol/ L extract. Calibration curves were prepared in the same way using Fe2+ as standard. Total antioxidants were calculated by the Equation (3):

$$\text{Total Antioxidant} = (\text{FRAP})\ (\text{Abs}-0.0692)/0.0081 \qquad (3)$$

III. RESULTS AND DISCUSSION

The results obtained from the analysis of natural Nitrite and Nitrate content in celery leaf powder (*Apium graveolens* L.) as shown in Table 1, while moisture content of sausage in Table 2, bacterial contamination in Table 3, Nitrate and Nitrite residues in sausages were listed in Tables 4 and 5, the pH values of stored sausage in Table 6, and the antioxidant activities was listed in Table 7.

As seen in Table 1, that celery leaves contain higher Nitrate compounds than celery leaves, while the content of Nitrite compounds in both leaves and stems was relatively the same. WHO (World Health Organization) sets the amount of daily intake/ ADI (Acceptable Daily Intake) for nitrite is 0 - 0.07 mg/ kg for 60 kg of human body weight, so based on WHO standards the nitrite content of celery leaf flour was still below ADI - WHO. So that the use of celery leaf flour level in this study is still possible to be improved [14].

Table 1. Nitrate and Nitrite Content of Celery Leaf Powder (*Apium graveolens* L.)

Sample	Nitrate Content (mg/L)	Nitrite Content (mg/L)
Celery leaf powder (*Apium graveolens* L.)	2.1401	0.012
	2.0596	0.015
Average	**2.1001**	**0.0135**
Celery stem powder (*Apium graveolens* L.)	1.8093	0.019
	1.7504	0.027
Average	**1.7798**	**0.0228**

3.1 Moisture Content of Sausage during Storage

Water content can be seen clearly in Table 2, that the average moisture content of various treatments, during storage did not change significantly (P> 0.05). This was caused by several things, among others: all components of the sausage dough were the same in all treatments except celery leaf powder, during storage packaged in plastic and carried out in a refrigerator, as well as sausage casings that were relatively airtight. This situation causes evaporation to be inhibited during storage, so that the moisture content was relatively less. The moisture content of sausage in all treatments during storage still meets the standards set by the National Standardization Agency.

Table 2. Moisture Content of Sausage during Storage (%)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	63.45 ± 0.89	63.08 ± 0.82	63.56 ± 0.36	**63.36 ± 0.25**
0.4 %	63.44 ± 0.88	62.99 ± 0.51	63.84 ± 1.32	**63.42 ± 0.43**
0.8 %	63.93 ± 0.45	63.42 ± 0.54	63.42 ± 0.19	**63.59 ± 0.29**
1.2 %	63.85 ± 0.66	63.20 ± 0.22	63.90 ± 1.07	**63.65 ± 0.39**
Average (B)	**63.67 ± 0.26**	**63.17 ± 0.19**	**63.68 ± 0.23**	-

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3.2 Total Bacterial Count of Sausage

Storage conditions, degree of acidity, salt content, and physical condition of food, greatly affect the development and viability of bacteria in food, especially processed meat products. The results of the observations on bacterial content can be seen in Table 3. It was indicated that during storage there has been a development in the number of bacteria, especially from phychrophylic bacteria groups, while acid-forming bacteria were relatively decreased reflected by a decrease in acidity or increase in pH value during storage (Figure 1).

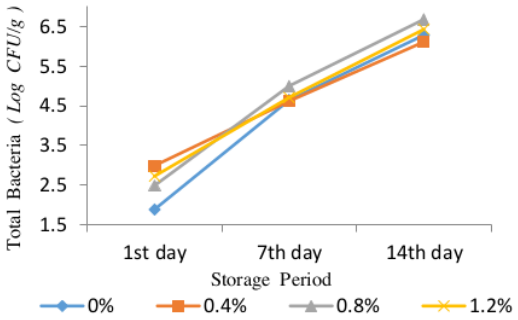

Figure 1. Total Bacteria Count of Sausage during Storage

Table 3. Total Bacterial Count of Sausages during Storage (Log. CFU/ g)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	1.89 ± 1.72	4.65 ± 0.39	6.28 ± 0.55	**4.27 ± 2.22**
0.4 %	2.99 ± 0.85	4.62 ± 0.27	6.11 ± 0.57	**4.57 ± 1.56**
0.8 %	2.49 ± 2.16	5.00 ± 0.07	6.68 ± 0.74	**4.72 ± 2.11**
1.2 %	2.73 ± 0.63	4.71 ± 0.25	6.43 ± 0.91	**4.62 ± 1.85**
Average (B)	**(2.53 ± 0.47) [c]**	**(4.75 ± 0.17) [b]**	**(6.43 ± 0.33) [a]**	-

Note: Different superscripts between columns show significantly difference (P <0.05)

Celery contains both Nitrate and Nitrite compounds, during the storage of sausages which was added by celery leaf powder, showed that Nitrate and Nitrite residues were still detected even in small concentrations, this can be seen in the following tables. The graph above shows an increase in the number of bacteria during storage. It was clear in the graph that the sharpest increase occurred in the 0% treatment of celery leaf powder, while the treatment of adding celery leaf powder was relatively lower. Indicates that there was an effect of inhibiting bacterial growth, although not significantly. According to SNI no: 01-3020-1995, the maximum total bacterial contamination is 105 or (5 log CFU/g), in Table 3 it was appears that on the 7th day storage the average bacterial contamination was 4.75 log CFU/g or (104) and still meets the SNI requirements, but on the 14th day storage the total bacteria has exceeded the standard, so it was not suitable for consumption. Other study by Majidah [15] show that celery has an antibacterial against the growth of Streptococcus mutans as an alternative mouthwash. In addition, Patricia et al. [16] also investigated celery as an antibacterial by testing the antibacterial power of celery essential oil hand sanitizer gel. Rusdiana [17] has examined celery plants as a source of high-potential natural ingredients in health promotion efforts and stated some celery-containing compounds have pharmacological properties that are very beneficial both in the curative process, prevention and maintenance (promotive) of human health. Among the uses of celery that have been scientifically researched and can be developed into promising health products are anticancer, antihypertensive, antibacterial, antifungal, antiinflammatory, antioxidant, and anticancer.

3.3 Residues of Nitrate and Nitrite Compounds

Celery contains both Nitrate and Nitrite compounds, during the storage of sausages added celery leaf powder in this study showed that Nitrate and Nitrite residues were still detected even in small concentrations, this can be seen in Tables 4 and 5. During storage of residues Nitrates in sausages tend to be decreased, the decrease was

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due to the Nitrate compounds can turn into Nitrites during storage. However, the concentration of Nitrite compounds also decreased during storage. Consider to bacterial contamination data, these two types of compounds can inhibit the growth of bacteria, especially acid-forming bacteria (Lactic Acid Bacteria). This is supported by data on decreased in pH values, which indicate acid-forming bacteria were experiencing growth retardation (Table 4). In general the celery leaf powder can not be said to inhibit bacterial growth, because the content of both nitrate and nitrite in celery leaf powder used was very low. So that at the level of 1.2 % (the highest) in this study has not shown good ability as a natural preservative.

Table 4. Nitrate Residues in Sausages during Storage Period (mg/ L)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	1.25 ± 0.10	0.82 ± 0.33	0.67 ± 0.29	**(0.91 ± 0.30) [f]**
0.4 %	1.89 ± 0.58	1.09 ± 0.50	0.55 ± 0.27	**(1.18 ± 0.67) [d]**
0.8 %	1.41 ± 0.54	1.10 ± 0.91	0.68 ± 0.20	**(1.06 ± 0.37) [e]**
1.2 %	0.93 ± 0.16	1.28 ± 0.81	0.94 ± 0.51	**(1.05 ± 0.20) [e]**
Average (B)	**(1.37 ± 0.40) [a]**	**(1.07 ± 0.19) [b]**	**(0.71 ± 0.16) [c]**	-

Nitrates can be converted into nitrites by bacteria in their metabolism, whereas nitrites when reacting with myoglobin and hemoglobin will turn into nitricoxide. Nitrate and nitrite have function as an anti-bacterial/ bactericidal compound if the pH is between 4.5 - 5.5, while the sausage pH was apparently not supported in this study (Table 5).

Table 5. Nitrite Residues in Sausages during Storage Period (mg/ L)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	0.08 ± 0.003	0.04 ± 0.014	0.05 ± 0.012	**(0.06 ± 0.02) [e]**
0.4 %	0.09 ± 0.003	0.05 ± 0.021	0.04 ± 0.006	**(0.06 ± 0.03) [e]**
0.8 %	0.10 ± 0.015	0.06 ± 0.039	0.06 ± 0.017	**(0.07 ± 0.02) [d]**
1.2 %	0.07 ± 0.007	0.07 ± 0.029	0.03 ± 0.008	**(0.06 ± 0.02) [e]**
Average (B)	**(0.09 ± 0.01) [a]**	**(0.06 ± 0.01) [b]**	**(0.05 ± 0.01) [c]**	-

Note: Different superscripts between columns or rows, show significantly difference (P <0.05)

Some researchers report that the use of celery powder (0.2 and 0.4) %, and stored for 30 and 120 minutes, respectively, did not show the characteristics of significant meat products [9]. The remaining nitrite after storage differed dramatically, with levels of 5.6 and 7.7 ppm found for celery powder levels of 0.2% and 0.4%, after 30 minutes of storage time, but 24.5 ppm and 46 respectively. 0 ppm was observed after 120 minutes of storage.

3.4 Degree of Acidity (pH) of Sausages

During storage the degree of acidity (pH) of sausages underwent a marked change (P <0.05). As can be seen in the Table 6, that during storage the sausage pH has increased from 5.69 + 0.03 on the first day shortly after sausage production to 5.98 + 0.06 after seven days of storage in the refrigerator and increased again to 6.27 + 0.08 after the 14th day storage.

Table 6. Degree of Acidity (pH) of Sausages during Storage Period

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	5.67 ± 0.15	5.90 ± 0.36	6.07 ± 0.12	**5.88 ± 0.20**
0.4 %	5.67 ± 0.21	6.00 ± 0.42	6.17 ± 0.15	**5.95 ± 0.25**
0.8 %	5.70 ± 0.26	6.03 ± 0.38	6.17 ± 0.06	**5.97 ± 0.24**
1.2 %	5.73 ± 0.25	6.00 ± 0.44	6.27 ± 0.32	**6.00 ± 0.27**
Average (B)	**(5.69 ± 0.03) [c]**	**(5.98 ± 0.06) [b]**	**(6.17 ± 0.08) [a]**	-

Note: Different superscripts between columns show significantly difference (P <0.05)

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3.5 Antioxidant Content of Sausage

Celery contains antioxidant compounds, it can be seen in the Table 7 that the higher the level of celery leaf powder, the higher the antioxidant content, seen at the level of 0% on average antioxidant content of (54.01 + 3.53)% it is a contribution from components of ingredients other than celery that are in sausage dough. While the highest is (69.92 + 5.85)% at the level of celery leaf powder 15%. It can be said that there is a contribution of celery leaf powder in raising antioxidant compounds. Furthermore, the antioxidant effect of celery was demonstrated from research conducted by Uddin et.al. [18], using the FRAP method of celery methanol fraction having antioxidant activity at concentrations equivalent to 12.48 mmol Fe_2SO_4/ Liter extract. Mean while, Li et el. [19] showed that flavonoids taken and purified from celery leaf ethanol extracts had antioxidant activity both in vitro (DPPH-, O_{2^-}, OH-) tests and in vivo in mice. The IC50H value was 68 g / mL on determination using DPPH, 0.39 mg/ mL at O_2 and 48 g/ mL at OH.

Antioxidant compounds during storage period tend to decrease (Figure 2), it can believed that there has been antioxidant activity in relation to the binding of free radicals contained in the sausages. Moreover, Kusnadi and Devi [20] conducted a research on isolation and identification of flavonoid compounds in celery leaf extract by reflux method and concluded that there were flavonoid compounds in celery leaf extract (*Apium graveolens* L.) from reflux results. The average yield of flavonoid compounds obtained in sample A was 16.58 mg/ 100 g, in sample B 20.79 mg/ 100 g, in sample C 22.47 mg/ 100 g, and in the D sample 24.71 mg/ 100 g. Natural antioxidants in addition to protecting the body from free radical attack are also able to slow the occurrence of chronic diseases caused by a decrease in reactive oxygen species (ROS), especially hydroxyl radicals and superoxide radicals. Natural antioxidants also function to inhibit lipid oxidation which causes rancidity and damage to closed maceration vessels (jars), buchner funnels, stirrers, rotary evaporators, erlenmayer flasks, silica gel aluminum plates F254, glass plates, separating funnels, glass columns for vacuum liquid chromatography (stirring), rotary evaporators, erlenmayer flasks, silica gel aluminum plates F254, glass plates, separating funnels, glass columns for vacuum liquid chromatography, pipette drops, capillary pipes and others [21].

Table 7. Antioxidant Content of Sausages during Storage Period (%)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	57.29 ± 1.87	54.47 ± 2.18	50.27 ± 4.48	**(54.01 ± 3.53) ^{g}**
0.4 %	63.62 ± 1.52	58.99 ± 2.08	56.20 ± 2.17	**(59.60 ± 3.75) ^{f}**
0.8 %	69.80 ± 5.86	62.61 ± 1.68	59.96 ± 2.43	**(64.12 ± 5.09) ^{e}**
1.2 %	76.49 ± 6.54	67.99 ± 6.77	65.27 ± 5.80	**(69.92 ± 5.85) ^{d}**
Average (B)	**(66.80 ± 8.24) ^{a}**	**(61.02 ± 5.72) ^{b}**	**(57.93 ± 6.32) ^{c}**	-

Note: Different superscripts between columns or rows show significantly difference (P <0.01)

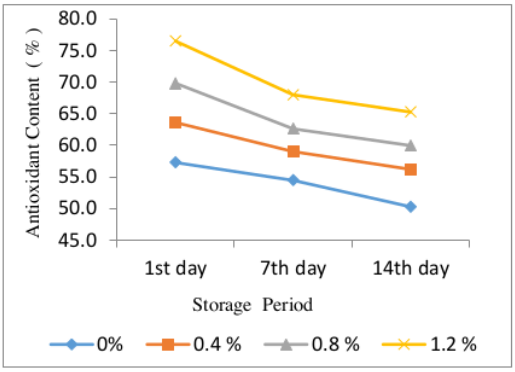

Figure 2. Antioxidant Content of Sausages during Storage Period

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Apigenin is the main flavonoid component of celery which belongs to the flavon group. Figure 3 shows an image of the chemical structure of apigenin. The molecular formula is $C_{15}H_{10}O_5$ with a molecular weight of 270.23 g/mol. The name of the International Union of Pure and Applied Chemistry of apigenin is 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopiran -4-one. Melting point of apigenin (345–350) ^{o}C [22]. Apigenin compounds have 3 aromatic rings depicted with ring A, ring B and ring C. Based on the results of Vidic et el. [23] prediction value of IC50 in the equation of quantitative relation to the structure of antioxidant activity of apigenin derivative compounds the most effective position to increase antioxidant activity is found in ring B with add electron donor groups. Antioxidant activity will increase when the C and B rings are added to the electron donor group in accordance with previous studies conducted [24]. The results also concluded that the methoxy group (OCH_3) had better antioxidant activity compared to the compound substituted for the ethoxy group (OC_2H_5). The antioxidant activity of a compound can be classified based on the value of Inhibition Concentration (IC50), based on Silvia's research [25], it states that celery leaf extract has antioxidant activity based on IC50 value of 98.24 µg/ml, including the strong category. Celery contains flavonoids as antioxidants which have the potential to prevent the formation of free radicals [26]. Apigenin is the main flavonoid component of celery which belongs to the flavon group. In addition, the content of vitamin C, tannin and essential oils in celery can also act as natural antioxidants.

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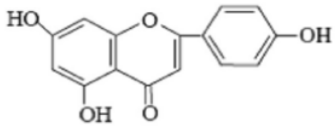

Figure 3. The Chemical Structure of Apigenin [27]

3.6 Sausage Fat Content

Fat is an important component in sausage products, because one of them is to determine the consistency or texture of sausages. The use of fat in making sausages is useful for producing sausages that are compact and soft, but also can improve the taste and aroma of sausages. The addition of fat components should not be more than 30% by weight of the meat used, it is intended to maintain texture during processing and handling. If too much fat results in sausage products becoming wrinkled. However, if the addition is too little, the sausage products will become hard and dry [10]. This research produces sausages with fat content that are still far below the standard (SNI: 01-3020-1995), which is a maximum of 25%. It can be seen in the Table 8, that the highest sausage fat content is 0.61% that is in control/ without the addition of celery powder and the fat content decreases significantly in line with the level of celery powder addition. In terms of percentage, sausage fat content has decreased, but has no significant effect on aroma, taste or texture

Table 8. Sausage Fat Content during Storage (%)

Level of Celery leaf powder (%) = B	Storage Period (day) = A			
	1 (A1)	7 (A2)	14 (A2)	Average (A)
0 %	0.64 ± 0.07	0.61 ± 0.11	0.59 ± 0.10	**(0.61 ± 0.03)** ^{d}
0.4 %	0.54 ± 0.04	0.52 ± 0.02	0.49 ± 0.07	**(0.52 ± 0.03)** ^{e}
0.8 %	0.54 ± 0.02	0.53 ± 0.03	0.52 ± 0.04	**(0.53 ± 0.01)** ^{e}
1.2 %	0.47 ± 0.06	0.49 ± 0.05	0.50 ± 0.07	**(0.49 ± 0.02)** ^{f}
Average (B)	**(0.55 ± 0.07)**	**(0.54 ± 0.05)**	**(0.53 ± 0.05)**	-

Note: Different superscripts between columns show significantly difference (P <0.01)

IV. CONCLUSION AND RECOMMENDATION

The research on the addition of celery leaf powder in the production of sausages as a natural preservative can be concluded that:

a. Celery leaf powder which contains both Nitrate and Nitrite compounds can inhibit bacterial growth from acid-forming bacteria, reflected in the pH value which is increasing.

b. In addition to Nitrate and Nitrite compounds, celery leaf powder can also be used as a source of antioxidant compounds, so that the possibility of fat damage in sausages can be relatively inhibited.

c. The addition of celery powder tended to significantly reduce sausage fat levels ($P < 0.05$), but no changes occurred during the storage period.

d. The level of celery powder addition increases the antioxidant levels significantly ($P < 0.01$), and during storage the antioxidant levels of sausage have decreased significantly ($P < 0.01$).

As recommendation, the level of celery leaf powder in the sausage mix is still possible to be increased, in order to obtain the inhibition effect of the microbial contamination and antioxidant activity as well.

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GENERAL COMMENTS

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