The Effect of Edible Coating Enriched With Kaffir Lime Leaf Essential Oil (Citrus hystrix DC) on Beef Sausage Quality During Frozen Storage (-18°±2°C)

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Abstract. The aim of this study was to determine the effect of edible coating enriched with kaffir lime (Citrus hystrix DC) leaves essential oil at various concentration on beef sausage quality during frozen storage (-18°±2°C). The concentration of kaffir lime leaves essential oil enriched in edible coating were varied at 0%; 0.2%; 1.4%. Microbiological, physical and chemical characteristics (TPC, color, TBA, TVB, and pH) were investigated at 0, 1, 2, 3, and 4 months of storage. The result showed that edible coating with the addition of kaffir lime leaves essential oils decreased the microbial growth, TVB value, and TBA value of beef sausage. The color and pH of samples can be stabilized during storage. The selected kaffir lime leaves essential oil concentrations based on microbial, physical, and chemical characteristics of beef sausages during frozen storage at -18°C was 0.2%.

1. Introduction
Meat is one of livestock foodstuffs production which has good nutritional content for human consumption. Nutritional content of meat are water (66%), protein (18.8%), and fat (14%) [1]. In food industries, meat also processed into food product such as sausage. According to SNI 01-3020-1995, sausage is a food produced from the mixture of ground meat (not less than 75% of meat) with flour or starch, and with the addition or no addition of spices and other food ingredients that permitted and put into a sausage casing.

Beef sausage has high enough of nutrient content and food processed practically so many people like to consume it. Nutrient content of 100 gram of beef sausages ingredients is 27.98 g of fat, 0.35 gram of carbohydrate, and 18.21 g of protein [2]. Fat and protein content of beef sausages were high enough easier to spoil during storage. The sausages damaged due to microbial growth and lipid oxidation. Beef sausage was suitable media for pathogenic microbial growth and decaying that caused the decreasing of nutritional content. Lipid oxidation will cause the beef sausage flavor damaged as rancidity. Therefore, preservation is needed to inhibit the spoilage.

Beef sausages shelf life was extended by low temperature or frozen storage. According to United States Department of Agriculture [3], the shelf life of the uncooked and cooked beef sausage was one up to two months at frozen storage (-18°C). However, chemical changes will occur during frozen storage such as protein denaturation, lipid oxidation, flavor decreased and degradation of pigments and vitamins [4]. Pseudomonas often resulted in mucus and pigment formation on frozen meat surface [5]. Therefore, alternative preservation is needed to inhibit other frozen resistant microorganisms growth.
especially for *Pseudomonas* genus and chemical changes especially lipid oxidation during frozen storage, by using an antimicrobial edible coating.

The edible coating is thin layer coating foodstuffs and safe to eat. Edible film or edible coating show ability to inhibit the moisture, oxygen, aroma and solute transportation [6]. To improve edible coating function, the addition of antimicrobial compound in the packaging materials is needed. The combination of edible coating and antimicrobial could inhibit the growth of pathogenic and spoilage microbial that could extend the shelf life of food.

The spices contain some substances that could inhibit the microbial growth. One of those spices is kaffir lime leaf essential oil. The main compounds of kaffir lime leaf essential oil (*Citrus hystrix* DC) were *Citronellal* (64.15%), *Beta-citronellol* (10.71%), *Trans-caryophyllene* (5.54%), and *Linalool* (5.31%) [7]. *Citronellal* is monoterpenic compounds that showed high antifungal properties [8]. Those compounds as an active compound that could inhibit and destruct the microbial cell.

Previous research has been utilized extract or essential oil of plant as a beef sausage natural preservative agent. Garlic essential oil [9], galangal essential oil, and lemongrass essential oil [10] were the essential oils have been used as a sausage preservative agent. However, no research has been studied about edible coating enriched with kaffir lime leaf essential oil as frozen sausage preservative agent. Furthermore to investigate the effect of edible coating enriched with kaffir lime leaf essential oil on the quality of beef sausage, the microbiological, physical and chemical characteristics of beef sausage during frozen storage were determined.

2. Experimental

2.1. Preparation of kaffir lime leaf essential oil

Kaffir lime leaf (obtained from a local market in Surakarta, Indonesia) was stored for one day [7]. Before storage stage, the leaf was kneaded and tored to break the leaf cells, sprinkled by water (200ml of water for 3 kg of material), and covered with a wet cloth. Essential oil of kaffir lime leaf was produced by water vapor distillation. Distillation conducted for 3h after the first oil drop.

2.2. Preparation of edible coating solution enriched with kaffir lime leaf essential oil

The edible coating was made by dissolved 5 g tapioca flour in 100 ml distilled water. Therefore flour solution was heated at 60°C until gelatinized. The gelatinized solution was mixed with a plasticizer (2 ml glycerol (Merck)) and heated at 60°C for 30 minutes. The essential oil was added and mixed in edible coating solution when the solution cooled [11].

2.3. Application of edible coating enriched with kaffir lime leaf essential oil on beef sausage

Edible coating solution poured into beef sausages “Besto”. Beef sausages with coating were hanged and dried by a dryer. Beef sausages were packaged in polypropylene plastic 0.88 mm of thickness. The coated Beef sausages were stored in the freezer (-18 ± 2°C) and analyzed for chemical (TVB, TBA, pH, and color) and microbiology properties (TPC). The value of TVB, TBA, and TPC were determined by followed the methods of Kasmadiharja *et al* [12], Apiyanto *et al* [13] and Faridiaz [14] respectively. pH analysis used pH meter (Eutech Instrument Handheld Series). The color analysis used Chromameter Konica Minolta CR-400/410. Each analysis was done at 0, 1st, 2nd, 3rd, and 4th month. The analysis of the 0-month sample was done after the sample was stored for 2 days at -18 ± 2°C and the next month analysis were done after 30 days of storage.

2.4. Statistical analysis

The statistical analysis which used this research was randomized design (RAL) with one factor (concentration of kaffir lime leaf essential oil variation (0%, 0.2%, 1.4%)). Each sample prepared in duplicate. Obtained data were analyzed by ANOVA (Analysis Of Variance). If there were the difference, it will continue by Duncan Multiple Range Test (DMRT) analysis to know whether there was a difference of each sample at 0.05 of significant.
3. Result and Discussion

3.1. Total Plate Count (TPC)

| Table 1. Total Plate Count (TPC) of beef sausages coated with edible coating enriched with kaffir lime leaf essential oil during frozen storage -18±2°C (log CFU/g) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Oleoresin concentration | 0 | 1 | 2 | 3 | 4 |
| 0% | 4.21±0.38\textsuperscript{Aa} | 4.08±0.31\textsuperscript{Aa} | 4.48±0.57\textsuperscript{ABa} | 4.76±0.19\textsuperscript{BAa} | 5.16±0.17\textsuperscript{Ba} |
| 0.2% | 3.72±0.07\textsuperscript{Aa} | 3.88±0.41\textsuperscript{ABa} | 4.45±0.58\textsuperscript{ABa} | 4.63±0.46\textsuperscript{ABa} | 4.91±0.23\textsuperscript{Ba} |
| 1.4% | 3.15±0.73\textsuperscript{Aa} | 3.88±0.49\textsuperscript{ABa} | 4.19±0.16\textsuperscript{ABa} | 4.31±1.08\textsuperscript{ABa} | 4.93±0.38\textsuperscript{Ba} |

Note: Notation with same letters in the same column (lowercase) and same row (upper case) showed no significant difference at a significance level of 5%.

During storage, the TPC value of all samples increased (Table 1). The increasing of TPC value related to the previous study that reported that beef sausage coated with edible coating enriched with kaffir lime leaf oleoresin also increased during frozen storage [15]. This indicated that at frozen storage still allowed bacterial growth. However, kaffir lime leaf essential oil more able to inhibit the microbial growth on beef sausage during storage. At 4\textsuperscript{th} month storage, the TPC value of beef sausage coated with edible coating enriched with 0.2% and 1.4% of kaffir lime leaf essential oil were 4.91 CFU/g and 4.93 CFU/g, respectively. These values were lower than that of beef sausage coated with edible coating enriched with kaffir lime leaf oleoresin at 4\textsuperscript{th}-month storage (6.52 CFU/g; 6.60 log CFU/g). This due to citronellal content of kaffir lime leaf essential oil was higher than that of kaffir lime leaf oleoresin. The citronellal content of kaffir lime leaf essential oil was 64.15% [7] while citronellal content of kaffir lime leaf oleoresin was 25.66% [16]. The kaffir lime leaf essential content consists of citronellal, beta-citronellol, trans-caryophyllene, and linalool. Citronellal showed an antimicrobial activity [17]. Citronellal was terpenoid compound that could inhibit the microbial growth by disrupting the cell membrane and cell wall formation [18]. The strong antifungal activity of C. nardus essential oil associated with synergy effect both of citronellal and linalool [19].

According to previous report [20], the requirement level of microbial content of ready to eat food product for cooked meat product was 10\textsuperscript{4} colony/g for satisfied level, 10\textsuperscript{4}-10\textsuperscript{6} colony/g for rather satisfied level, 10\textsuperscript{6}-10\textsuperscript{8} colony/g for unsatisfied (danger potential) level, and more than 10\textsuperscript{8} colony/g for not accepted product. While the maximum level of TPC by Indonesian National Standard SNI 01-3820-1995 was 1 x 10\textsuperscript{8} colony/g or 5 log CFU/g. The study result showed that the TPC value of 4\textsuperscript{th}-month storage control sample has been passed the SNI standard (5.16 log CFU/g), while 0.2% and 1.4% of kaffir lime leaf essential oil treatment samples still on SNI standard until four months storage. This indicated that kaffir lime leaf essential oil inhibited the microbial growth and maintain the beef sausage quality during frozen storage.

3.2. Total Volatile Base value (TVB)

| Table 2. Total Volatile Base Value (TVB) of beef sausages coated with edible coating enriched with kaffir lime leaf essential oil during frozen storage -18±2°C (mg N/100g) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Essential oil concentration | 0 | 1 | 2 | 3 | 4 |
| 0% | 2.51±0.01\textsuperscript{Aa} | 10.94±1.25\textsuperscript{Bc} | 23.89±1.74\textsuperscript{Bc} | 45.18±1.47\textsuperscript{Bc} | 52.12±0.64\textsuperscript{Bc} |
| 0.2% | 2.48±0.06\textsuperscript{Aa} | 7.56 ±0.01\textsuperscript{Bc} | 17.63±1.80\textsuperscript{Bc} | 38.32±0.92\textsuperscript{Bc} | 42.51±3.33\textsuperscript{Bc} |
| 1.4% | 2.50±0.03\textsuperscript{Aa} | 8.78 ±1.80\textsuperscript{Bc} | 15.10±0.04\textsuperscript{Bc} | 31.50±0.08\textsuperscript{Bc} | 35.89±0.98\textsuperscript{Bc} |

Note: Notation with same letters in the same column (lowercase) and same row (upper case) showed no significant difference at a significance level of 5%.
Table 2 showed that TVB value of all beef sausage samples significantly increased during 4 months frozen storage. TVB value of control samples increased from 2.52 mg N/100g to 52.12 mg N/100g. TVB value of beef sausage coated with edible coating enriched by 0.2% of kaffir lime leaf essential oil increased from 2.48 mg N/100g to 42.51 mg N/100g. TVB value of beef sausage coated with edible coating enriched by 1.4% of kaffir lime leaf essential oil increased from 2.50 mg N/100g to 89 mg N/100g.

TVB value related to the bacterial growth on samples. TVB is resulted from protein decomposition by bacterial activities and autolytic enzyme. Resulted protein breakdown was volatile and caused off-flavor compounds such as ammonia, H2S, mercaptans, phenol, cresol, indole, and skatole [21]. Dimethyl amine and trimethylamine also included as TVB compounds [22]. Trimethylamine was produced by decomposition bacteria, dimethyl amine was produced by autolytic enzyme during frozen storage, while ammonia was produced by amino acid deamination and catabolite of nucleotides [23]. Proteolytic enzymes produced by microorganisms will degrade protein of beef sausage to amino acids and further will degrade into alkali compound (indole or trimethylamine) [24].

Limit TVB value of fresh meat and processed products were 10 mg N/100 g for fresh meats, 20-30 mg N/100 g for the early stage of spoilage meat, and 30 mg N/100 g for spoilage meat [12]. After three month storage, TVB value of all samples exceeded the limit value. However, TVB value of control sample significantly higher than that of treatment samples. The higher of kaffir lime leaf essential oil enriched on edible coating solution the lower TVB value of beef sausage.

The result was similar to the previous study that incorporated the kaffir lime leaf oleoresin on edible coating solution as the coating material of beef sausage. TVB value of control samples also higher than that of treatment samples [15]. Inhibitory effect of kaffir lime leaf essential oil was caused by disturbing of microbial cell wall formation [25]. Essential oil could inhibit the microbial growth by disturbing the membrane or cell wall formation [18].

3.3. Acidity (pH)

Table 3. Acidity (pH) of beef sausages coated with edible coating enriched with kaffir lime leaf essential oil during frozen storage -18±2°C

| Essential oil concentration | Storage time (month) |
|-----------------------------|----------------------|
|                             | 0                    | 1                    | 2                    | 3                    | 4                    |
| 0%                          | 6.58±0.00b           | 6.24±0.15AB          | 5.77±0.21A           | 6.22±0.35AB          | 6.36±0.36AB          |
| 0.2%                        | 6.21±0.33A           | 5.92±0.23A           | 6.05±0.35A           | 6.28±0.38A           | 6.43±0.05A           |
| 1.4%                        | 6.37±0.01b           | 6.31±0.19b           | 5.96±0.07A           | 6.36±0.14b           | 6.38±0.07b           |

Note: Notation with same letters in the same column (lowercase) and same row (upper case) showed no significant difference at a significance level of 5%

The concentration of kaffir lime leaf essential oil enriched on edible solution had no significant effect on pH of samples. The initial pH of beef sausage ranged from 6.21-6.58. During storage, there were slightly increasing and decreasing of pH value. However, pH value of beef sausage coated with edible coating enriched by 0.2% of the kaffir lime leaf essential oil was stable during 4 months of storage. At the end of storage, the pH values of samples ranged from 6.36-6.43. This pH value similar to a previous study [26] that reported the pH value of cooked beef sausage between 6 and 6.5. However, if the pH value under 4.6 to 5.5, indicating the sausage was spoilage.

Increasing and decreasing of pH value of beef sausage were related to the bacterial growth. Decreasing of pH value related to lactic acid bacteria contamination. Decreasing of pH value during storage due to psychrophilic bacteria activity which degraded the carbohydrate and spices which produced organic acid especially lactic acid during carbohydrate fermentation [9]. Bacterial which usually contaminated foodstuffs and produced decreasing of acidity related to various bacteria such as Lactobacillus, Acinetobacter, Bacillus, Proteus, Micrococcii, Clostridium, and Enterococci [22].
Increasing of pH of beef sausage during storage was affected by the activity of decomposition bacteria which produced more alkali metabolite. The pH value of anchovy also increased during vacuum frozen storage [27]. Increasing of pH value was affected by the producing of alkali compound such as ammonia, dimethyl amine, trimethyl amine by decomposition bacterial activity.

3.4. Color

Table 4. Color of beef sausages coated with edible coating enriched with kaffir lime leaf essential oil during frozen storage -18±2°C

| Essential oil concentration | Storage time (month) | 0  | 1  | 2  | 3  | 4  |
|-----------------------------|----------------------|----|----|----|----|----|
|                             |                      |    |    |    |    |    |
| L*                         | 0%                   | 55.7±2.86<sup>a</sup> | 54.11±2.02<sup>a</sup> | 52.79±0.52<sup>a</sup> | 54.75±1.24<sup>a</sup> | 54.66±0.04<sup>a</sup> |
|                             | 0.2%                 | 53.08±1.30<sup>a</sup> | 54.62±0.87<sup>a</sup> | 52.67±0.91<sup>a</sup> | 54.59±2.14<sup>a</sup> | 56.15±2.21<sup>a</sup> |
|                             | 1.4%                 | 53.50±1.03<sup>a</sup> | 54.68±1.50<sup>a</sup> | 53.67±0.38<sup>a</sup> | 55.33±0.77<sup>a</sup> | 55.28±1.19<sup>a</sup> |
| a*                         | 0%                   | 22.88±0.40<sup>b</sup> | 23.35±0.27<sup>b</sup> | 23.26±1.15<sup>b</sup> | 23.58±0.59<sup>b</sup> | 22.95±0.55<sup>b</sup> |
|                             | 0.2%                 | 24.38±1.16<sup>b</sup> | 23.90±0.40<sup>b</sup> | 22.14±0.88<sup>b</sup> | 23.61±1.16<sup>b</sup> | 22.47±0.97<sup>b</sup> |
|                             | 1.4%                 | 23.61±0.75<sup>b</sup> | 22.78±0.42<sup>b</sup> | 22.06±1.41<sup>b</sup> | 22.56±0.88<sup>b</sup> | 21.59±0.85<sup>b</sup> |
| b*                         | 0%                   | 17.99±0.41<sup>c</sup> | 18.03±0.18<sup>c</sup> | 17.96±0.73<sup>c</sup> | 19.98±0.33<sup>c</sup> | 19.33±0.15<sup>c</sup> |
|                             | 0.2%                 | 19.56±0.78<sup>c</sup> | 18.82±0.81<sup>c</sup> | 17.76±0.74<sup>c</sup> | 19.40±1.27<sup>c</sup> | 18.72±0.42<sup>c</sup> |
|                             | 1.4%                 | 17.96±0.79<sup>c</sup> | 17.57±0.33<sup>c</sup> | 17.53±0.91<sup>c</sup> | 18.14±0.52<sup>c</sup> | 17.56±0.01<sup>c</sup> |

Note: Notation with same letters in the same column (lowercase) and same row (upper case) showed no significant difference at a significance level of 5%

During frozen storage for 4 months, the L* (Lightness) value of beef sausage was not affected by the concentration of kaffir lime leaf essential oil enriched on an edible solution (Table 4). The L* (Lightness) value of beef sausage ranged from 52.67 to 56.15. The lightness of beef related to the oxidation reaction of sausage which produced metmyoglobin compound. Pseudomonas growth on meat will reduce the partial pressure of oxygen and support metmyoglobin (metMb) formation which resulted in gradual discoloration of meat product [28]. The a* (redness) color intensity of beef sausage samples were stable ranged from 21.59 to 24.38. The edible coating application could maintain the redness of beef sausage during frozen storage. The b* (yellowness) value of control sample increased during frozen storage for four months. Yellowness degree on meat increased with increasing of storage time [29]. This was due to the lower content of metmyoglobin pigment on sausage. Decreasing of myoglobin content into metmyoglobin can cause the pale brown color of meat.

Kaffir lime leaf essential oil contains citronella and beta-citronellol. Both of components were can inhibit the oxidative damaged on beef sausage during storage. The main components of essential oil of Artemisia scoparia waste were Citronellal and Beta-citronellol [30]. The essential oil can be a powerful antioxidant and radical activity against hydroxyl (OH) ions and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

3.5. Thiobarbituric Acid Value (TBA)

Table 5. Thiobarbituric Acid value (TBA) of beef sausages coated with edible coating enriched with kaffir lime leaf essential oil during frozen storage -18±2°C (mg malonaldehyde/kg)

| Essential oil concentration | Storage time (month) | 0  | 1  | 2  | 3  | 4  |
|-----------------------------|----------------------|----|----|----|----|----|
|                             |                      |    |    |    |    |    |
| 0%                          | 0.25±0.05<sup>a</sup> | 0.11±0.02<sup>a</sup> | 0.13±0.07<sup>a</sup> | 0.13±0.04<sup>a</sup> | 0.18±0.08<sup>a</sup> |
| 0.2%                        | 0.32±0.01<sup>a</sup> | 0.09±0.01<sup>a</sup> | 0.13±0.04<sup>a</sup> | 0.10±0.04<sup>a</sup> | 0.16±0.04<sup>a</sup> |
| 1.4%                        | 0.25±0.04<sup>a</sup> | 0.08±0.01<sup>a</sup> | 0.12±0.04<sup>a</sup> | 0.11±0.01<sup>a</sup> | 0.11±0.01<sup>a</sup> |

Note: Notation with same letters in the same column (lowercase) and same row (upper case) showed no significant difference at a significance level of 5%
The TBA value of all samples ranged from 0.25 to 0.32 at initial storage and 0.11 to 0.18 mg malonaldehyde/kg at the end of storage. The concentration of kaffir lime essential oil enriched on an edible solution has no significant effect on TBA value of the sample. However, the kaffir lime leaf essential oil has high antioxidant activity with Inhibitory Concentration value were IC<sub>50</sub> 59.98 mg/mL [31]. IC<sub>50</sub> is number of extract concentration (microgram/milliliter) capable inhibiting 50% oxidation. This is due to kaffir lime leaf essential oil contained citronellal and beta-citronellol [7]. The compound can show the antioxidant and radical activity of hydroxyl ions (OH) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) [30]. Kaffir lime leaf essential oil also contained Linalool compound [7]. Linalool compound was estimated can trigger to increasing of singlet oxygen. Linalool compound was a compound which caused singlet oxygen increased [32]. When singlet oxygen developed and reacted with unsaturated lipid so the hydroperoxide formed (ROOH) [33].

Based on the TBA value of samples, all samples still acceptable to consume because of the TBA value less than 2 mg malonaldehyde/kg of the sample [34] and have not reached the rancidity level (0.5 mg malonaldehyde/kg of the sample).

4. Conclusion
The result showed that edible coating with the addition of kaffir lime leaves essential oils decreased the microbial growth, TVB value, and TBA value of beef sausage. The color and pH of samples can be stabilized during storage. The selected kaffir lime leaves essential oil concentrations based on microbial, physical, and chemical characteristics of beef sausages during frozen storage at -18°C was 0.2%.

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