Study of utilization liquid smoke and carrageenan as a natural antibacterial in manufacturing beef meatballs

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Abstract. This research have observed liquid smoke and carrageenan ability to obstruct microbe activity. Phenol and sulfate ester in liquid smoke and carrageenan, give preservation effect by obstruct microorganisms growth on beef meatballs. Liquid smoke and carrageenan was added on a particular variation into meatballs dough. Liquid smoke variation was 0.5%-1%, and carrageenan variation was 0.5%-1.5%. Meatballs then stored up to 36 hours at room temperature and performed TPC test in every 12 hours. The results indicates that in 0-12 hours carrageenan has significant effect to obstruct microorganism growth with the percentage reduction in total bacteria was 13.54% and 93.73%. In 24-36 hours liquid smoke effected to the significant effect obstruction of microorganism growth with the percentage reduction in total bacteria was 98.99% and 99.93%. The addition of liquid smoke and carrageenan did not give a significant effect on the lightness of beef meatballs produced, but provided significant effect on the storage time and the lightness of beef meatballs.

Keywords : liquid smoke, carrageenan, utilization, antibacterial, beef meatball

1. Introduction
Meatballs has a high nutrition, pH 6.0-6.5 and high aw (> 0.9), were the maximum storage period is 1 day (12-24 hours) at room temperature [11]. Foodstuffs that have a high nutritional value in the pH range of neutral pH and high water content such as meatballs is a good medium for microbial growth. Damage caused by the emergence of microbes in foods are mucus, discoloration, mildew, the incidence of irregularities aroma, fermentation damage and decay protein ingredients. Therefore, meatballs sold in the market are usually added preservatives to extend the shelf life. However, it is still widely used preservatives are not recommended for foods such as formaldehyde and sodium borate.

Liquid smoke is derived from natural ingredients that is hemicellulose burning, cellulose and lignin from hardwoods and coconut shell to produce compounds that have antimicrobial properties, antibacterial and antioxidant such as acidic compounds and their derivatives, alcohols, phenols, aldehydes, carbonyls, ketones and pyridine [7]. Liquid smoke has several advantages over the traditional fumigation techniques in terms of ease of application, speed, uniformity of product, good characteristics desired in the final product, as well as reduce the danger of polycyclic aromatic hydrocarbons and resulting a chewy texture in processed meat products [6].

Carrageenan is a polysaccharide that soluble in water and extracted from red seaweed (Rhodophyceae). Carrageenan is a hydrocolloid consisting of potassium, sodium, magnesium and calcium sulphate esters of galactose and 3,6-anhydrogalactosa (AG) copolymer [13]. Carrageenan has function as an emulsifiers, stabilizers, gelling, and coagulant. Carrageenan also has a water binding

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properties that will affect the yield and chewy texture in processed meat products. Carrageenan is used as a gelling material to replace the use of borax that harmful to health because of its ability to bind water.

The addition of liquid smoke in manufacturing meatballs have been done by [1] in which the liquid smoke has function as a water bounding agents on the meatballs in order to increase of quality and durability of the meatballs. [2] Have been doing experiments using liquid smoke in the preservation of meatballs with 0-7% concentration of liquid smoke. [3] Observed changes the quality of beef meatballs with the addition of liquid smoke which indicates that liquid smoke has significant effect on the level of preference and flavour, optimal results obtained on the addition of liquid smoke 1%. [5] Also have been studied the effect of using carrageenan in the manufacture of beef meatballs.

2. Materials and methods
2.1. Material
Materials in this study was beef, tapioca starch 15% of the meat weight, salt 2%, garlic 2.5%, pepper 0.8%, ice cubes, 20% MSG 1%, carrageenan, and liquid smoke [4]. Tools set for making meatballs, a set for total microbial test equipment and Digital Color Meter were used in this experiment.

2.2. Making meatballs
Making meatballs were started with cutting beef into small pieces and ground in a grinding machine. There were two stages of milling in order to obtain soft dough. Garlic 2.5%, salt 2% and pepper 0.8% which has been mashed and mixed with tapioca starch 15% and ice cubes 20% in second milling process. All compound which has been formed from mixing process, divided into 10 samples and then added liquid smoke and carrageenan with concentrations varied according to Table 1. The mixture was crushed for a half minute and then released for forming meatballs by put in warm water with a temperature of 60°C to 80°C and allowed to float. After float, meatballs were moved into the boiling water and heated until cooked, about 10 minutes. Furthermore, meatballs were stored for 36 hours at room temperature, sample were obtained for every 12 hours and total bacteria were tested by TPC. Lightness was measured using Digital Colorimeter Software by Macintosh.

2.3. Total bacteria test with TPC (Total Plate Count) method
According to Indonesian National Standard (SNI) limit of total bacteria in food, especially in the processed meat product is 1x10^5 colonies/g (ISO 7388; 2009). To calculate total bacteria in beef meatballs used Total Plate Count (TPC) method. Storage time was 0, 12, 24, 36 hours. As much as 15 ml Plate Count Agar (PCA) was poured into the sterile petri dish and chill. One gram of meatballs sample was crushed and dissolved in distilled water until 10 mL, then homogenized the sample through shaking until the sample was well diluted in solvent. The series of dilutions were 10^1, 10^2, 10^3, 10^4, 10^5. As much as 0.5 mL solution from each dilutions were put in petri dish containing PCA (Plate Count Agar) and trim using rod bent glass to form “eight” symbol. Samples were allowed to stand for 1 hour then incubated in 48 ± 2 hours at 35°C. The number of colonies was calculated by colony counter. Calculation of Total Plate Count as follows:

\[
\frac{\sum C}{[(1 \times n1) + (0.1 \times n2)] \times (d)} = N
\]

N is total colonies product, expressed in colonies per g; \(\Sigma C\) is total colonies in all counted plate; n1 is total plate on the first dilution; n2 is total plate in second dilution; d is first calculated dilution (SNI 01-2332.3-2006).

3. Results and discussion
3.1 Beef meatballs total bacteria analysis
Table 1 showed that at a range of 0-12 hours, sample code V6 provided highest total bacterial concentration. This is because V6 has the smallest concentration of carrageenan compared to other samples. In this condition, carrageenan has a significant influence into sample because of the
carrageenan has a hydrophilic properties so that if the number of carrageenan less than the amount of water tied too little and allowing more bacteria to grow.

**Table 1. Beef meatballs total bacteria analysis**

| Sample Code | Liquid smoke concentration (%) | Carrageenan concentration (%) | 0 hours     | 12 hours    | 24 hours    | 36 hours    |
|-------------|--------------------------------|------------------------------|-------------|-------------|-------------|-------------|
| V0          | 0                              | 0                            | 5.76 x 10^4 | 1.33 x 10^6 | 1.66 x 10^6 | 3.94 x 10^6 |
| V1          | 0.5                            | 0.5                          | 3.84 x 10^4 | 4.25 x 10^4 | 5.52 x 10^5 | 3.1 x 10^6  |
| V2          | 1                              | 0.5                          | 4.58 x 10^4 | 4.99 x 10^4 | 8.32 x 10^5 | 2.28 x 10^6 |
| V3          | 0.5                            | 1.5                          | 3.75 x 10^4 | 3.06 x 10^4 | 5.31 x 10^5 | 1.41 x 10^6 |
| V4          | 1                              | 1.5                          | 4.3 x 10^4  | 1.04 x 10^5 | 3.09 x 10^5 | 4.43 x 10^6 |
| V5          | 0.75                           | 1.707                        | 5.33 x 10^4 | 1.34 x 10^5 | 6.23 x 10^5 | 1.02 x 10^6 |
| V6          | 0.75                           | 0.293                        | 7.71 x 10^4 | 2.83 x 10^5 | 2.8 x 10^6  | 2.28 x 10^6 |
| V7          | 1.103                          | 1                            | 6.67 x 10^4 | 6.81 x 10^4 | 5.19 x 10^5 | 9 x 10^5    |
| V8          | 0.3965                         | 1                            | 3.35 x 10^4 | 4.5 x 10^4  | 4.65 x 10^6 | 7.33 x 10^6 |
| V9          | 0.75                           | 1                            | 6.94 x 10^4 | 5.01 x 10^4 | 2.9 x 10^6  | 1 x 10^6    |
| V10         | 0.75                           | 1                            | 3.33 x 10^4 | 2.64 x 10^4 | 3.08 x 10^6 | 2.59 x 10^6 |

At a range of 24-36 hours, V8 was highest in total bacteria value. This is because V8 had the smallest concentration of liquid smoke. The longer storage time the more yield of free water resulting in the more bacteria to grow. Carrageenan had no significant effect on the sample since the acidic conditions by more bacteria was appeared, and carrageenan would undergo the hydrolysis. Therefore, if the sample has a low concentration of liquid smoke, amount of water bound was also low thus allowing more bacteria to grow.

TPC meatballs value up to 12 hours storage still below the maximum limit specified number of microbes (1x10^5 CFU/g) based on SNI 01-2729-2006. The percentage reduction in total bacteria against storage time can be illustrated in Figure 1.

![Figure 1. Percentage bacteria decrease in each storage time](image-url)
Liquid smoke and carrageenan were able to inhibit bacteria growth on beef meatballs as can be seen by reduction percentage of bacteria to 13.54%, 93.73%, 98.99%, 99.93% for 0, 12, 24, and 36 hours of storage time, respectively. This is because liquid smoke contains phenols which serves to inhibit bacteria growth. As antibacterial, phenolic compound has a working mechanism by damaging bacterial cells structure and inhibits cell wall formation process that causes the lysis of a bacterial cell wall [12]. Carrageenan has sulfate ester groups that have ability to bind water. Carragenan also have a good ability in water binding and also to form three dimensional mesh that can trap water [8].

### Table 2. Beef meatballs color analysis

| Liquid Smoke (%) | Carrageenan (%) | Lightness (0) | Lightness (12) | Lightness (24) | Lightness (36) |
|-----------------|-----------------|---------------|----------------|----------------|----------------|
| 0 | 0 | 64.81 | 67.77 | 54.67 | 53.13 |
| 0.5 | 0.5 | 68.42 | 57.53 | 53.08 | 61.88 |
| 1 | 0.5 | 64.86 | 61.77 | 56.26 | 57.03 |
| 0.5 | 1.5 | 67.41 | 63.12 | 54.46 | 57.52 |
| 1 | 1.5 | 64.93 | 59.77 | 50.01 | 50.60 |
| 0.75 | 1.707 | 65.23 | 62.98 | 52.01 | 53.98 |
| 0.75 | 0.293 | 65.10 | 59.55 | 50.87 | 53.75 |
| 1.103 | 1 | 64.42 | 65.49 | 50.68 | 56.68 |
| 0.3965 | 1 | 64.55 | 65.55 | 48.01 | 56.21 |
| 0.75 | 1 | 64.06 | 65.88 | 62.30 | 59.86 |
| 0.75 | 1 | 63.15 | 71.27 | 62.44 | 50.20 |

#### 3.2 Beef meatballs color anaysis

Color is one of visual nature of the first seen by the consumer. Color has meaning and a very important role in food product since it can give the impression of like and dislike. Signification and role of color in food products, depends on types, signs of damage, and the manual processing [10].

An objective color measurements were performed using an Digital Colourmeter. L is a color parameter value (lightness/brightness level) stating dark and bright of sample. The greater the value of L, the more the light or bright sample. L value ranges between 0 (black) to 100 (white). L value beef meatballs can be seen in Table 2.

Based on Table 2, the addition of liquid smoke and carrageenan did not give significant effect on the lightness of beef meatballs. This is because the carrageenan has white color powder and liquid smoke in form of a clear liquid so the additional of these two components does not effect to the L value of meatballs. However, the storage time effected the L value. The longer of storage time, L value decreased and turned into darker color.

### 4.Conclusions

Liquid smoke and carrageenan could be used as a natural preservative in manufacturing beef meatballs because it could inhibit bacteria growth resulting the extension in the storage time of meatballs. By liquid smoke and carrageenan application in meatballs, the decrease in bacteria could be achieved more than 90% and did not significantly effect to the lightness of meatballs.

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