Effect of aging time on changes in smoke flour compounds on meatballs and fresh meat of Bali beef

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Abstract. The use of smoked flour in Balinese beef meatballs and fresh meat was expected to be able to work more effectively in preservation compared to the use of liquid smoke. Loss of liquid smoke compounds in the form of evaporation during the preservation and storage process resulted in a lack of maximum performance of liquid smoke as a preservative and aroma of smoke. This study aimed to see changes in smoke compounds during storage in fresh Bali beef meat and meatballs. Smoke was used in the form of smoke flour resulting from oven drying, freeze drying and spray drying. This research was carried out using 6 head of 3-year-old male Bali cattle, Longissimus dorsi (LD) muscle. The experimental design used a complete random factorial pattern in which factor 1 was a type of meat (meatballs and fresh meat), factor 2 was a type of smoke flour level 2% (oven dry, freeze-dried, and dry spray), and factor 3 was the aging time (0, 7, and 14 days). During observation, fresh meat and meatballs were aging at 2-5°C. Observations were made on water content, phenol, acetic acid, and carbonyl. The results showed that contents of phenol, acetic acid, and water were higher in fresh Balinese beef while carbonyl content was higher in Bali beef meatballs. Frozen dry smoke produced higher carbonyl content up to 0.46%. The maturation time produced approximately the same levels of phenol, acetic acid, and carbonyl. It could be concluded that a 2% freeze-dried flour could be added to fresh meat and processed meat products as natural and environmentally friendly preservatives.

1. Introduction
One of preservation technique that was often done on meat and processed meat products is smoking. Preserved of animal products can last a long time because of the role of the compounds found in smoke. Also, smoke will also give a distinctive aroma to smoked food ingredients and become a consideration for consumers in choosing smoked meat based on the type of firewood as a source of smoke used. There are more than 300 compounds found in the smoke from the combustion of firewood where three of them are mainly phenols, acids and carbonyls [1,2].

For health reasons for consumers, smoke has been purified by removing harmful compounds into smoke in liquid form. The pyrolysis process in the coconut shell will produce liquid smoke which still contains tar and concentrated will become clear after going through filtering several times and is safe for consumption [2].

The importance of fresh meat and meatballs as a raw material for smoking can be seen from the availability of these raw materials. According to [3] in 2017 the production of beef was obtained from 1,114,748 heads, with an estimated a 50 kg meat per a head of cattle, so the production of fresh meat
reached 55,737.4 tons/year or 152.71 tons/day. There was about 60% of cattle slaughtered in slaughterhouses was intended for making meatballs (personal communication). Beef meatball production reaches 33,442.4 tons/year or 91.62 tons/day in Indonesia.

The use of liquid smoke added to fresh meat or meatball dough is feared to experience evaporation of smoke compounds so that their effectiveness as preservatives will be reduced. Drying and flouring of liquid smoke are expected to be able to inhibit the evaporation of smoke compounds in smoke flour. There are three types of drying methods for food that are commonly carried out, namely oven drying [4], freeze drying [5] and spray drying [6].

Several studies on the use of liquid smoke in fresh meat and beef meatballs have been carried out [7-10]. As [11] concluded that the protein content of tilapia (Oreochromis niloticus) increased by 79% by adding liquid smoke of 5% concentration as a preservative. Meanwhile [12] has used liquid smoke as an antioxidant in catfish sausages. Similarly, the addition of liquid smoke in the feed of coconut water urea supplement multi-nutrient liquid smoke blocks (UKAMB) as feed for cattle has been carried out by [13]. The results showed that increasing the level of liquid smoke in UKAMB feed reduced the ability of meat to bind water, the shear force of raw meat was more or less the same, the level of fat oxidation increased, and antioxidant activity was more or less the same [13]. The use of smoked flour in Balinese beef meatballs and buffalo showed higher tenderness and elasticity of buffalo meatballs, while the score of meatball mastication residues was lower than that of Balinese beef meatballs. This type of smoke flour produced approximately the same sensory score [8].

2. Materials and methods
This study used fresh Bali beef (Longissimus dorsi) and beef meatballs from the same muscle, derived from 6 heads of male Bali cattle aged 3 years. Fresh Balinese beef and beef meatball products were added with smoke flour resulting from oven drying, freeze drying and liquid smoke spray drying at a concentration of 10%. Smoked flour was rubbed on fresh meat and mixing on the meatball dough for 2 hours. After that, storage (aging) was carried out at a temperature of 2-5°C for 14 days.

The research design used was a complete design of factorial patterns 2x3x3, in which a factor of 1 type of meat (fresh and meatballs), a factor of 2 types of smoke flour (oven drying, freeze drying, and spray drying), and a factor of 3 maturation times (0, 7, and 14 days). Replication of the research was carried out for three times. The parameters observed were the analysis of smoke compounds concerning the levels of water, phenol, acetic acid and carbonyl.

2.1. Making meatballs
The making of the dough begun with grinding the meat with salt and ice cubes to extract as much of myosin as possible so that the meatballs were springy, compact and solid. After that, the addition of tapioca flour and smoked flour while ground using a food processor until the mixture was evenly mixed. Meatball dough that has been made then made meatball spheres then boiled at a temperature of 80°C until the meatballs expanded the surface of the boiling water [7].

2.2. Determination of phenol content
Test of total phenol content was carried out by the Follin-Ciocalteu method. Phenol total contents were determined by a visible light spectrophotometer method. Amount of 5 g of pureed sample was diluted with distilled water to a volume of 100 ml. The solution was filtered to obtain a clear filtrate. Addition of 0.5 ml of Follin denis (Follin 1: 1) to 1 ml of filtrate then added 1 ml of Na2CO3 solution and allowed to stand for 10 minutes. Added 10 ml of distilled water and vortex solution until homogeneous. The sample absorbance was read using a spectrophotometer with a wavelength of 730 nm. The results of absorbance data were calculated using the standard phenol curve [14].
2.3. Determination of acetic acid content
The total acetic acid analysis was carried out using acid and base titration methods (acid-y-alkalimetry). A total of 10 g of the sample was diluted into a measuring flask to reach 250 ml. A total of 25 ml of the solution was put into an Erlenmeyer 100ml, then 3-5 drops of phenolphthalein (PP) indicator was then titrated with 0.1 Na NaOH standard solution until the color turned pink [14].

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\% \text{ Total Acid Content} = \frac{\text{Volume of titration} \times \text{fp} \times \text{normality NaOH} \times \text{MW Acetic Acid}}{\text{Sample weight (mg)}}
\]

2.4. Determination of carbonyl content
A total of 5 grams of sample were added with 100 ml of distilled water. As much as 1 ml of the solution was put into a test tube, added 1 ml of 2.4 Dinitrophenyl Hydroxyl reagent (1% in methanol). Then added three drops of concentrated HCl, then heated in a water bath at 50 °C for 30 minutes. After cold, add KOH 1 N to 10ml volume. The solution was distorted. And then the absorbance was read using a spectrophotometer at a wavelength of 480 nm. The absorbance reading results are calculated using standard curves [14].

2.5. Determination of water content
Moisture content was determined using the oven method [15].

2.6. Data Analysis
Data were calculated using analysis of variance of factorial pattern to find out the effects of treatment to compound smoke flour during maturation with the help of SPSS program (SPSS 16, SPSS Ltd., West Street Woking, Surrey, UK). If a significant effect was found, then it was continued with the least significant difference test [16].

3. Results and discussion
3.1. Phenol content
Changes in phenol content based on the type of flour and meat product types during storage can be seen in table 1.

| Treatments                      | Water (%) | Phenol (%) | Acetat (%) | Carbonyl (%) |
|--------------------------------|-----------|------------|------------|--------------|
| Meat type                      |           |            |            |              |
| - Meatball                     | Sig: 0.01 | Sig: 0.01  | Sig: 0.01  | Sig: 0.01    |
| - Fresh Meat                   | 68.93±1.37a | 0.029±0.03a | 0.351±0.06a | 0.603±0.09a  |
| Smoke flour type               | Sig: NS   | Sig: NS    | Sig: NS    | Sig: 0.05    |
| - Oven                         | 71.25±2.46 | 0.052±0.04 | 0.549±0.21 | 0.393±0.15   |
| - Freeze drying                | 70.64±2.21 | 0.062±0.04 | 0.555±0.21 | 0.458±0.22   |
| - Spray drying                 | 70.64±2.15 | 0.051±0.04 | 0.521±0.21 | 0.430±0.19   |
| Aging time                     |           |            |            |              |
| - 0 day                        | Sig: NS   | Sig: NS    | Sig: NS    | Sig: NS      |
| - 7 days                       | 70.85±2.54 | 0.064±0.04 | 0.542±0.22 | 0.433±0.19   |
| - 14 days                      | 70.82±2.06 | 0.054±0.03 | 0.540±0.21 | 0.423±0.20   |

Description: numbers with different letter notation on the same column stated a significant difference (P< 0.05) and highly significance (P< 0.01)

Analysis of variance showed that meat products had a very significant effect (P <0.01), the type of flour and aging times had no significant effect on phenol contents of meatballs and beef. Beef produces phenol contents higher than 179.31% of beef meatballs. That condition indicates that the
addition of smoke level 2% on fresh meat of Bali beef could maintain phenol levels much higher than that of Balinese beef meatballs. The process of grinding and mixing of ingredients during the manufacture of meatballs and then continued with cold storage at 2-5°C, most likely more evaporating contents levels of smoke flour than adding smoke flour to fresh meat accompanied by cold storage at 2-5°C. In previous studies using liquid smoke with a concentration of 5 and 10% in Bali beef, phenol contents were decreased with increasing concentration of liquid smoke by 11.88% lower than the concentration of 10%. Meanwhile, in the chicken breast, there was a decrease of 18.44% at a concentration of 10% lower than the concentration of 5% [17]. Study of [18] showed that an increase in the concentration of liquid smoke in soaking beef meatballs was able to increase the total phenol from the produced beef meatballs. At the level/concentration of 2%, the highest phenol contents reached 0.11%. Which conditions make it clear that phenol contents as volatile compounds evaporated during addition to fresh meat where this increased evaporation was greater in chicken breast meat compared to beef. The results of the present study show that addition in the form of smoke flour can maintain phenol levels in fresh Balinese beef.

Even though the type of flour produced phenol contents that are more or less the same as Bali beef meatballs and fresh Bali beef, there was a tendency for phenol contents from freeze-dried smoke flour to be slightly higher than phenol contents of the two other types of flour. Frozen drying techniques using freezing and vacuum temperatures in the drying and flouring of liquid smoke may be able to maintain or lose fewer phenol contents compared to the other two drying and flouring techniques.

The aging times provided approximately the same phenol contents in Balinese beef meatballs and fresh Bali beef, even though there was a tendency for phenol contents to decrease slightly during aging. The difference in phenol contents that were not significantly different between the treatment of aging could explain that phenol content of smoke flour could be maintained during aging, which is very significant in its use as an antioxidant and antimicrobial. Previous studies using liquid smoke showed that phenol contents increased with increasing storage time, but there was no significant difference between a 1st week and 3rd-week storage, as well as between the 2nd week and 3rd and the 4th weeks. Which indicates that phenol compounds as antioxidants can play a role in inhibiting fat oxidation during storage [17]. Previous studies in which liquid smoke was added to the block supplement feed and given to cattle during fattening showed that liquid smoke levels increased the rate of fat oxidation and more or less the same antioxidant activity [13]. Giving in the form of smoke flour which was sprinkled directly on the meat in this study was more effective than feeding in the feed. The level of addition of smoke flour has not been able to maintain or reduce the level of fat oxidation.

3.2. Acetic acid content
Changes in acetic acid contents based on the type of flour and types of meat products during aging can be seen in table 1.

Analysis of variance showed that meat products had a highly significant effect (P <0.01), the type of flour and aging time had no significant effect on the contents of acetic acid of Balinese beef meatballs and fresh Bali beef.

Addition of smoked flour level 2% in this study caused fresh Bali beef to produce acetic acid contents 109.02% higher than Bali beef meatballs. The high contents of acetic acid in fresh Bali beef explain the more significant occurrence of acetic acid evaporation during the grinding process, mixing materials, boiling and storing meatballs compared to during the storage of fresh meat In previous studies, Bali beef added with 10% liquid smoke with a concentration of 20% decreased acetic acid contents of 29.82% [17]. It could be explained that the addition of smoked flour was far more effective in maintaining acetic acid contents during the process of making and storing meat products compared to giving it in liquid form. Acetic acid is one of the smoke compounds that play a role in inhibiting the bacterial development or as an antimicrobial. Thus in the future, the application of liquid smoke in fresh meat and processed meat products should be given in the form of smoke flour.

Even though the types of flour provided approximately the same contents of acetic acid in Balinese beef meatballs and fresh Bali beef, there was a tendency for the freeze-dried smoke to provided
slightly higher contents of acetic acid compared to the other two types of smoke flour. It can be considered for the use of freeze-dried smoke as an antimicrobial regarding acetic acid in future studies.

Acetic acid contents during aging were relatively constant even though there was a tendency to decrease slightly at 14 days of aging. Stability of acetic acid contents during aging time led to stability in meat products which were added to smoke level 2% regarding quality, especially the total stability of bacterial colonies.

3.3. Carbonyl content

Changes in carbonyl contents based on the type of flour and types of meat products during aging can be seen in table 1.

Analysis of variance showed that meat products had a highly significant effect (P <0.01), the type of flour had a significant effect (P <0.05) and aging time had no significant effect on the contents of carbonyl of Bali beef meatballs and fresh Bali beef.

The addition of smoke level 2% on Balinese beef meatballs produced higher carbonyl content of 58.31% than fresh Balinese beef. The carbonyl as one of the smoke compounds that characterize the aroma of smoke as a result of carbon degradation could be maintained better in processed meat products in the form of meatballs than in fresh meat. Product meatballs characterized by the boiling process (heating) triggers the formation of carbonyls that are more intense than without the heating process, in this case, the addition of smoked flour to fresh meat is not intense to produce carbonyl compounds. Previous studies have shown that the decreased carbonyl content in the fresh meat of Bali beef at a concentration of liquid smoke10% reached 15.22% lower than the concentration of liquid smoke 5% [17].

At the level, 2% freeze-dried smoke produced a significant carbonyl content (P <0.05) 14.23% higher than oven dried smoke flour and more or less the same as spray dried smoke flour. The drying and flouring of liquid smoke through freeze-drying techniques were able to maintain a better and more effective content of smoke carbonyl than both other drying techniques can explain this.

Even though the aging time gave more or less the same content of carbonyl, there was a tendency carbonyl content decreased slightly during aging. The decreasing in insignificant and relatively constant carbonyl content during aging explains the ability of level 2% smoke to maintain the aroma of smoke in processed meat products and fresh meat for 14 days aging. The aroma of smoke felt by the panelists on beef meatballs which added liquid smoke to the three types of muscles and different rigor-mortis phases resulted in more or less the same sensory quality of meatballs [7]. The comparison of the results of this study can state that giving smoke in the form of flour or liquid produces the same sensory quality.

3.4. Water content

The changes in water content based on the type of flour and types of meat products during aging can be seen in table 1.

Analysis of variance showed that meat products had a highly significant effect (P <0.01), the type of flour and aging time had no significant effect on the water content of Balinese beef meatballs and fresh Bali beef.

The addition of smoke level 2% produced freshwater content of fresh Bali beef was 5.26% higher than Bali beef meatballs. The process of grinding, mixing, boiling and cold storage at 2-5°C in making meatballs resulted in a more significant loss of water content compared to fresh meat, could explain why the freshwater content was higher than of meatballs.

Even though the level of 2% flour provided the same water content of Balinese beef meatball meat and fresh Bali beef, there was a tendency for the water content to be slightly lower in freeze-dried smoke and spray dry. That can be explained that the freeze and dry spray technique are slightly better at reducing water content compared to dry ovens. The freezing and vacuum temperatures used in drying and flouring liquid smoke can explain it. The treatment of drying and sowing techniques that
were not significantly different from the water content of meatballs and fresh meat of Bali cattle can be considered for the selection of one of these drying techniques to be used in subsequent studies.

The aging time gave more or less the same water content; there was a tendency to decrease the water content of meatballs and fresh meat of Bali beef during aging. The water content stability during aging was 70.82 - 70.85% explained the ability of 2% smoke level to maintain the water content of meatballs and fresh meat of Bali beef during aging which had implications for product durability.

4. Conclusion
The addition 2% of smoke flour produced higher content of phenol, acetic acid, and water in fresh Balinese beef while carbonyl levels are higher in Bali beef meatballs. Frozen dry flour produced higher carbonyl content reaching 0.46%. The aging time up to 14 days produced content of phenol, acetic acid, and carbonyl which were approximately the same as without and seven days aging. It could be concluded that 2% freeze-dried flour can be added to fresh meat and processed meat products as natural and environmentally friendly preservatives.

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