Histamine Levels in Repeated Thawing Beef

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Abstract
The aim of this research was to determine the histamine levels in repeated thawing beef. This research were using longissimus dorsi part (sirloin) of beef. Repeated thawing treatments of beef consisted of P1 as control without freezing, P2 as first thawing after one week freezing, P3 as second thawing without freezing two weeks and P4 with freezing three weeks. The slices of beef was storage in freezer at temperature of -20ºC every weeks for three weeks. The thawing process was done by storage in refrigerator at temperature of 6ºC for 270 minutes after being removed from the storage in freezer every weeks for three weeks. PIC (Protease Inhibiting Cocktail) methode were used to extraction of enzyme in beef. Enzyme Link Immunosorbent Assay (ELISA) were used to detected the histamine levels with 450 nm wave length. The quantitative data from parameters were analyzed by descriptive. The results of the study concluded that repeated thawing beef in refrigerator at temperature of 6ºC could decrease the histamine levels.

Keywords: histamine, beef, thawing

Introduction
Meat is one of the types of foodstuffs of animal origin, including beef which can hardly be separated from human life (Nurwantoro et al., 2012). Beef has enough role in national food context because it is one commodity with a complete nutrient (Emhar et al., 2014). Chemical composition of beef consists of 56-72 % water, 15-22 % protein, 5-34 % fat and 3.5 % protein not dissolved, include carbohydrates, organic salts, substances dissolved nitrogen, minerals and vitamins (Afiati, 2009).

Beef has a lot of benefits as nutritional value, especially protein. Furthermore it could being lost if handled inappropriately because beef is an excellent medium for the growth of microbes, especially bacteria. The existence of bacteria in beef that often cause damage and can even lead to the source of the disease in humans. Bacterial contaminated beef throughout cattle life and since slaughtered, the process of preparing carcass until the beef will be consumed (Boediono et al., 2012).

High demand for beef will increase at certain times, for example on religious holidays. The price of meat will increase certainly. It was caused by high demand but availability low for beef. People usually provide meat one day or two days before the celebration day, then stored in freezer (Maaniaan, 2009). Generally, upper and middle class of beef consumer, catering facilities or sellers of meat buy beef with cheap price and put it into freezer as soon as possible, then beef will be thawing before it was sold and cooked (Suryaningsih, 2010).

Beef storage by freezing can affect the conditions of microbial contamination. Some of bacteria contaminated beef can produce the enzyme L - Histidine Decarboxylase (HDC) which is a former precursor of the biogenic amines histamine. This compounds can produced by Raoultella terrigena, Enterobacter spp., Microbacterium testaceum, Brevibacterium mcbrellneri, Diversus Micrococcus, Staphylococcus spp (Heruwarti et al., 2008).

Histamine is a biogenic amine compound produced from decarboxylation of free histidine (α-amino-β-propionic acid inidosal) (Lehane and Olley, 1999). This compound is an important mediator of immediate type allergic reactions (immediate). Allergic reactio by histamine has different effects based on receptor binding sites. In the skin, histamine causes dilation and permeability of capillaries which cause redness, burning sensation, itching and swelling. In the heart, histamine will increase heart rate. In the respiratory system, histamine causes bronchoconstriction (increase of bronchus contraction), so it is very significant in patients with bronchial asthma. In the digestive system histamine will increase gastric acid secretion and intestinal peristaltic which causes vomiting and diarrhea, other effects caused by histamine are increase secretion gland of saliva, pancreas, bronchus and tear but generally these effect are weak and not fixed (Syarif et al., 2011).

Histamine formation process was influenced by presence of L - Histidine Decarboxylase (HDC) (Bennour et al., 1991). Human body has effective system to detoxify toxins in the digestive tract. Monoamine oxidase, diamine oxidase and histamine N - methyltransferase are digestive enzyme which
metabolize histamine into the digestive tract become non-toxic compounds. However, possibility of histamine poisoning still need to warn because histamine detoxification system only works on condition of the daily normal intake (Indriati et al., 2006). At very high intake of histamine, the system will not able to detoxify of this compounds. 15 ppm levels of histamine consumption can cause allergic symptoms and 100 ppm consumption may cause poisoning (Viciana et al., 1995). Based on the results from the previous studies, the authors would like to explain the effect of repeated thawing in refrigerator to the levels of histamine in beef.

**Materiale and methods**

**Procedure**

**Beef Freezing and Thawing**

250 grams beef were divided into 4 treatments, a treatment consisted of six repetitions with different samples and each sample weight of 10 grams, the first treatment (P1) was not done freezing (preparated and extracted directly), others 3 treatments (P2, P3, P4) were frozen in a freezer at -20°C temperature with thawing every week. Storage of beef (P2, P3 and P4) were done for one, two and three weeks, respectively. Thawing conducted at temperature of 6°C in refrigerator until the entire ice melt for 270 minutes with a permanent packaging.

**Preparation of Beef Extract**

10 grams of thawed beef was washed with cold PBS once, then it cut into small pieces and taken 10 mg to incorporated into the micro tube, protease inhibitor 500μL added and RIFA buffer (1:100), cooled for 30 minutes in the freezer, then vortex using multi voltexer for 15 minutes, and centrifuged using a centrifuge cool on speed 10,000 rpm at 4°C for 20 minutes, then the supernatant was tested.

**Histamine Assays Using ELISA Test**

Some steps were used to test the levels of histamine in beef using ELISA techniques. 50 mL sample was inserted in the ELISA plate wells, then added 50 mL Biotinylated Detection Ab, incubated for 45 minutes at 37°C temperature, then it was washed with ELISA washer for three times, then it was added 100 μL HRP Conjugate for each ELISA plate wells and incubated again for 30 minutes at 37°C temperature, then it was washed with ELISA Washer five times, then added 90 μL Substrate Reagent pitting each ELISA plate and incubated for 15 minutes at a temperature of 37°C, then added 50 μL Stop Solution and read its absorbance with ELISA reader at 450 nm wave length.

**Data Analysis**

Result datas will be analyzed by descriptive analysis.

**Results and Discussion**

The result of ELISA test of beef which repeated thawing was shown in Table 1.

<table>
<thead>
<tr>
<th>Repetitions</th>
<th>No thawing (ppm)</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; thawing (one week in freezer)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; thawing (two weeks in freezer)</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; thawing (three weeks in freezer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.108 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>4.312 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.006 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.002 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>2</td>
<td>7.642 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>9.514 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.636 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.686 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>3</td>
<td>0.856 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.456 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.026 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>4.144 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>0.004 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.204 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.054 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.046 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>0.15 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.048 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.516 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>0.292 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>4.416 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>16.66 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>7.21 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.208 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
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<tr>
<td>average</td>
<td>3.20 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>5.20 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.74 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>1.23 x10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
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</table>

The results showed that the histamine levels lowest in the second week (P3) and then third week (P4) along duration of freezing and frequent thawing treatment, there were 1.74 x10<sup>-6</sup> ppm and 1.23 x10<sup>-6</sup> ppm (Table 1 and figure 1). Histamine levels increased during the first week of thawing treatment (P1) was 5.20 x10<sup>-6</sup>. It was may be caused by freezing storage at -20°C, which the HDC enzyme (L-Histidine Decarboxylase) inactivated to produce the histamine, so it could reduced or stopped.

According to Matulessy et al., (2010), the inhibition of cellular metabolism in the body tissues of animals to produce HDC enzyme will take place at -18°C temperature. HDC enzyme activity was as precursor of histamine formation influenced by pH, temperature and low oxygen availability, the highest enzyme activity at 37°C and the optimum pH was 6.0 (Eitenmiller et al., 1982; Kusumawati and Indriati, 2008). Thawing process was done over -10°C temperature, bacterial activity will return...
normally, although some of them died. Sublethal bacteria will also die over time in freezing storage. It caused increase the level of histamine in P3. Maybe due to the presence of HDC enzyme as bacteria production. It was active again after sublethal phase for one week in freezing storage, then it decreased at P3 and P4 caused of continuous freezing treatment, then sublethal bacteria died and activity of HDC enzyme also halted because temperature was too extreme. Some of the HDC enzyme was produced by bacteria in sublethal phase at -20°C (Matulessye et al., 2010). The growth of bacteria, histamine forming and activity of HDC enzyme can be inhibited at 5°C or lower. Example Enterobacter, one of a family of Gram negative psychrotrophic.

Possibility the lowest histamine levels in the second and third week of freezing storage not causing allergy and poisoning if consumed. Thawing treatment on meat at temperature of 5-10°C and let at least 1 hour to inhibit microbial recontamination.

**Conclusion**

Storage of beef in the freezer at temperature of -20 °C and repeated thawing in refrigerator at temperature of 6 °C could decrease the level of histamine.

**References**