Histamine content in various types of canned foods (fruits and syrups) stored under different temperature conditions over time - an *in vitro* study

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Abstract

Histamine (Biogenic amines) is one of the most important nitrogen containing compounds present in many vegetables, fruits and poultry food products and has biological importance in creating allergic reaction most of the times. Some toxicological descriptions of food poisoning are often associated with histamine - the most prominent biogenic amines in the category. The secondary amines involved in nitrosation and form nitrosamines which are toxic initiates illnesses to our organs. Their concentration in high level is detected in processed foods especially. In considering the food safety they have been related to food spoilage and fermentation processes. The aim of our study is to correlate the histamine in canned foods safety and analyse the presence of biogenic amines especially histamine in processed fruit syrups and pickles under different storing temperatures over time. The 5 different chosen varieties of canned fruits (mango pickle, fermented durian, canned pineapple syrup, canned rambutan syrup and canned lychee syrup) were brought into the laboratory unopened and on the day of purchase, they were opened and analysed for histamine for the first time. Further they have portioned into five, and each of the portion was stored in six different temperatures (0°C, 10°C, 20°C, 30°C, 40°C and 50°C) for 14 days and 28 days period and at the end of 14 days and 28 days they were analysed. The second part of the experiment was to fix the room temperature as the storage temperature for these canned foods (25±2°C) and analysed on 7, 14, 21 and 28 days period. It was revealed that fermented food item (canned fermented durian)
significantly has higher level of histamine over other preserved food items regardless of storage temperature and period. In general, it was found that the storing time along with the increased temperature increase the level of histamine in food items. In this study, we found that the stable room temperature (25±2°C) under the dried conditions favours the most suitable for maintaining the histamine level in food. Temperature variation revealed that has an impact over the increased concentration of histamine in food items.

Key words: Biogenic amines, histamine, temperature, food toxicity, canned fruits, storage time

1. Introduction

Histamine is one of the frequently occurring biogenic amines (BA) which are present naturally in most of the food items. The nitrogen containing amines are formed majorly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Maijala, RL. & Eerola, 1993). They are produced from histidine, tyrosine, ornithine, lysine, β-phenylalanine amino acids. They are low molecular weight bases which are synthesized by microbial, vegetable and animal metabolisms (Brink et al., 1990). These amines in food and beverages are formed by the enzymes of raw material by naturally or are generated by microbial decarboxylation of amino acids when they acted upon, but some of the aliphatic amines can also be formed by amination from equivalent aldehydes (Maijala, RL. & Eerola, 1993). These BA's are present in a wide range of food products, including fish, meat, cheese, wine, beer, vegetables, fruits and nuts. In fermented foods their presence is as a result of the fermentation process.

1.1 Importance of Histamine

Histamines are usually released by mast cells and basophils, and its biological effects are usually seen in the course of allergic and other reactions. Histamine can cause pseudo-allergic reactions meant to include the symptoms such as: urticaria, eczema, diarrhea, or spasm of bronchi etc., (Bardocz, 1993). There are 3 histamine receptors where they seen in epithelial cells of major organ system. Histamine effect is applied only when they bind to the receptors on cellular membranes in the major organ tissues. Skin rashes and itching is associated with the urticarial lesions due to sensory and motor neuron stimulation (Karovicova J & Kohajdova Z, 2005). So the symptoms exert according to the tissue type where they bind to the receptors.
For example, it causes dilatation of blood vessels, capillaries and major arteries, thus resulting in hypotension and headache. Histamine induces contraction of intestinal smooth muscles resulting in abdominal cramps, diarrhoea, and vomiting. It also induces gastric acid secretion (Rice SL et al., 1976).

The histamine toxicity can be well treated by using antihistamines drugs. Histamine is the most toxic amine detected in food products (Brink et al., 1990; Huis in’t Veld et al., 1990). The toxicological effect depends on histamine intake, presence of other different amines, amino oxidase activity and the absorbing capacity of the individual intestine. If the concentration of amines is above normal level which is usually determined by the various factors, harmful effects may occur (Christine et al., 2007)

1.2 Histamine in Food

The concentration of histamine is used as a criterion of the quality of food. BA in food can be of endogenous, which means that they were formed by metabolic conversion of the plant or animal and thus they may be present in the raw material of the food or exogenous, which means that if bacteria are the contaminant favor the histamine production and have even if they have been killed, the enzyme activities still continue to produce it (Linares DM et al., 2012). Foods rich in proteins such as fish, meat, and cheese as well as fruits and vegetables are regarded as histamine containing products since fermentation of these increases the histamine production (Landete et al., 2005).

Endogenous origin of BAs are usually present in low concentrations in unfermented foods like fruits and vegetables, meat, fish and milk (Önal A, 2007). Exogenous biogenic amines are the result of microbial decarboxylation of free amino acids and are present in higher concentrations. Most of the time the precursor amino acids were already present in the raw materials (Bodmer et al., 1999).

But there are many factors which contribute to increase the histamine concentration even if the origin is of endogenous.

The storage time and temperature is considered as among the important factors in determining BAs content. Increased temperatures and longer storage time yield higher amounts of biogenic amines. It is also explained that Klebsiella pneumoniae produced more biogenic amines at 20°C than at 10°C (Silla santos MH , 1996). Normally BA production is increased between 10°C and 37°C, and it can be inhibited by storing less than 10°C. In general, the levels of BA will be lower in refrigerated foods compared to the foods stored in increased temperature (Suzzi et al., 2003).
At relatively high temperature, during fermentation favors the production of biogenic amines, however perusal of report demonstrated that the effect of fermentation temperature is highly dependent on the starter culture used (Maijala et al., 1995).

The detoxification mechanisms exist in our body is capable of metabolizing normal dietary presence of biogenic amines in various foods which we consume daily. (Huis in’t Veld et al., 1990). The dietary exogenous source of amines are absorbed from food are readily detoxified by the action of group of enzymes called as amino oxidases or by conjugation reaction -one of the detoxification reaction. There are certain circumstances where there is a presence of monoamine oxidase (MAO) inhibitors, high level consumption and the issues in the detoxification process (defective enzymes for detoxification or genetic causes) in an individual disturbs and as a result the biogenic amines accumulate in the body. Amino oxidases are inducible enzymes in the presence of mono- or diamines (Joanna Stadnik & Zbigniew J. Dolatowski, 2010). The enzymes MAO and di amino oxidases play an important role in the detoxification process.

In this context, the study is focused to present an analysis on the histamine level in five categories of preserved fruits and syrups in different brands which normally consumed by the people as available in the major supermarkets in Malaysia (mango pickle, fermented durian, canned pineapple syrup, canned rambutan syrup and canned lychee syrup). We also took two major factors (storage temperature and storage period) which has an implications on our safety on consumption of food to check their influence over the histamine content of the selected food items. The reason for our analysis preferred on preserved fruits and syrups is that since they are the one which is commonly consumed as such and regardless of age.

2. Materials and Methods

Enzymatic methods including radio immuno assays and enzyme-linked immunosorbent assay system (ELISA) have been applied to detect histamine with the advantages of rapidity and not requiring expensive instrumentation like HPLC (Stratton et al., 1991).

Enzyme-linked immunosorbent assay (ELISA) is a detection system based on the binding of an antibody to an antigen and detection using an enzyme label. The enzyme acts on a colorless substrate to give a colored product, which is readily detectable at specific wavelength. The histamine detection kit was purchased from BioVision Company through the local supplier in Kuala Lumpur, Malaysia.
2.1 Sample Preparation

The method for amine determination involves enzymatic quantitative analysis, by using Histamine assay kit which can be used to identify the levels of histamine in various fruit and vegetable products, as well as other fermented foods. The histamine sample buffer was prepared according to the guidelines given in the kit procedure in 1:1 ratio with 100% Methanol. The rambutan, pineapple and lychee syrups were assayed directly. About 300 mg of pickled mango and fermented durian were homogenized by using 1000 µl of histamine sample buffer. The samples were boiled for 10 min at 90°C in sealed tubes in a water bath, and then cooled on ice. They were centrifuged further at 10,000 X g for 5 min. supernatant was collected and was used for the assay.

2.2 Experimental Design

The 5 different chosen varieties of canned fruits (mango pickle, fermented durian, canned pineapple syrup, canned rambutan syrup and canned lychee syrup) were bought from the super market in Kuala Lumpur; Malaysia with the expiry date mentioned on it and was served as the sample for the analysis.

They brought to the laboratory as unopened and on the day of purchase, they were opened and analysed for histamine for the first time. Further they have portioned into 5, and each of the portion was stored in six different temperatures (0°C, 10°C, 20°C, 30°C, 40°C and 50°C) for 14 days and 28 days period. At the end of 14 days and 28 days period the samples were taken out and analysed.

The second part of the experiment was to fix the room temperature as the storage temperature for these canned fruits (25±2°C) and analysed on 7, 14, 21 and 28 days period. The following formula was used to calculate the amount of histamine present in the canned fruit samples.

\[ \text{Eq.1: Concentration of histamine} = \frac{B}{V} \times D \]

Where ‘B’ is the amount of histamine in the sample well from standard curve (in nmol) ‘V’ is the sample volume added into the reaction well (in µl) and ‘D’ is the sample dilution factor if any.

2.3 Statistical Analysis

Triplicate of each samples were analysed and used for further statistical analysis. The values were expressed as mean± SD. Statistical analysis was done by using student’s t test. The results were presented for discussion.
3. Results and Discussion:

The histamine level (in triplicates) in the five canned food items we have chosen was carefully analyzed by using the kit method based on immuno assay principle. After the food sample extraction was done, the level of histamine was analysed and calculated. The level of histamine was given in table I. It was found that the level of histamine in the mango pickle and fermented durian were comparatively high among the selected canned food items and believed that the storage temperature conditions played a major role on this. While picking up the items from the supermarket, mango pickle and fermented durian were kept in the goods rack and it was at the normal room temperature. The canned pineapple, rambutan and lychee syrups were stored and picked up from the refrigerator showed the storage temperature of 18°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Level of Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango pickle (mg/Kg)</td>
<td>92.8±2.62</td>
</tr>
<tr>
<td>Fermented durian (mg/kg)</td>
<td>102.5±2.18</td>
</tr>
<tr>
<td>Canned pineapple syrup (mg/L)</td>
<td>15.4±0.2</td>
</tr>
<tr>
<td>Canned rambutan syrup (mg/L)</td>
<td>10.6±0.48</td>
</tr>
<tr>
<td>Canned lychee syrup (mg/L)</td>
<td>13.5±0.77</td>
</tr>
</tbody>
</table>

Table I: Histamine level in sample triplicates (in mg/kg or mg/l) while at the time purchased from the market. All values were expressed in mean ± SD.

The important consideration is amino acid decarboxylation and it is the major route of formation of histamine and other biogenic amines (Shalaby, 1994). This reaction occurs through either by the action of decarboxylase which is present naturally in food or by the microorganisms present in the food items (Silla, 1993; Granata, 2012). It was also proven that the biogenic amines can also produce by the addition of preservatives and other chemicals which are used extend their shelf life. These are referred as potentiators which can be classified as either food borne putrefactive amines or pharmacological agents (Stratton et al., 1991). Certain drugs like antihistamines, antimalarial agents and other medications can inhibit histamine-metabolizing enzymes may be the reason to get accumulated in our body (Brink et al., 1990; Stratton et al., 1991).
Table II: Histamine level in sample triplicates (in mg/Kg or mg/L) stored in different temperatures for 14 days period. All values were expressed in mean ± SD (ND: Not detected).

Table II showed the results of histamine level in various temperatures which we commonly preferred to store the food items (0°C – 50°C). We opened the canned food items for the first time and aliquoted it, stored in the listed temperatures for 14 days period. Upon storing at different temperature ranges, even at very low temperatures (0°C and 10°C mango pickle and fermented durian, found that have significant level of histamines. The other three items we did not find any histamine. It was found that the raising temperature ultimately increase the amount of histamine. This may be constituted either by the action of microorganisms or by the chemical preservatives or additives added to the food items oxidized and decarboxylated to produce the histamines and other biogenic amines. The increase in temperature help the microorganisms to grow on it to produce the biogenic amines thus the level of histamines was found to be increased. The level of histamine was very high at 50°C for mango pickle and fermented durian and it was found 105.0±2.44 mg/Kg, and 147.3±2.98 respectively.

Sometime it is possible that these microorganisms either cause the microbiota of the product or may be introduced before, during or after food processing (Rokka et al., 2004). Biogenic amines are present in low concentrations are not detected in fresh food normally (Granata et al, 2012).
Table III: Histamine level in sample triplicates (in mg/Kg or mg/L) stored in different temperatures for 28 days period. All values were expressed in mean ± SD (ND: Not detected).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>0°C</th>
<th>10°C</th>
<th>20°C</th>
<th>30°C</th>
<th>40°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango pickle (mg/Kg)</td>
<td>26.8±0.3</td>
<td>33.4±1.17</td>
<td>88±1.75</td>
<td>91.4±1.22</td>
<td>102.2±0.98</td>
<td>116.4±2.4</td>
</tr>
<tr>
<td>Fermented durian (mg/Kg)</td>
<td>40.5±1.56</td>
<td>46.2±1.46</td>
<td>82.3±0.92</td>
<td>110.5±2.14</td>
<td>126.7±1.9</td>
<td>179.7±1.45</td>
</tr>
<tr>
<td>Canned pineapple syrup (mg/L)</td>
<td>ND</td>
<td>8.2±0.64</td>
<td>18.6±1.4</td>
<td>22.2±0.8</td>
<td>50.4±1.55</td>
<td>66.3±1.77</td>
</tr>
<tr>
<td>Canned rambutan syrup (mg/L)</td>
<td>ND</td>
<td>5.6±0.38</td>
<td>20.9±1.14</td>
<td>31.5±1.22</td>
<td>50.0±2.5</td>
<td>62.8±2.2</td>
</tr>
<tr>
<td>Canned lychee syrup (mg/L)</td>
<td>ND</td>
<td>6.4±0.9</td>
<td>16.6±0.86</td>
<td>29.4±1.36</td>
<td>44.3±2.0</td>
<td>60.5±2.56</td>
</tr>
</tbody>
</table>

The selected canned food items stored in different temperatures for 28 days period revealed that at 0°C mango pickle and fermented durian have found 26.8±0.3 mg/Kg and 40.5±1.56 mg/Kg respectively. In the other three items, histamine was not detectable. It was found that the raising temperature along with the longer storage period influence the level of histamine even than stored for 14 days period (Table III). The gradual increase in the histamine level was observed in canned pineapple, rambutan and lychee syrup over the temperature with the longer duration of storage (28 days). This would be probably due to accumulation of biogenic amines; however the accumulation of these amines in food depends primarily on the availability of free amino acids and the presence of microorganisms. The micro organisms which have decarboxylases can easily generate the biogenic amines. (Önal, 2007). The level of amines and different types are linked to the nature of the food and type of microorganism present (Arena et al., 2001; Deng-Fwu Hwang et al., 1997). It was observed from our study that fermented durian always maintained higher level of histamine indicated that the presence of histamine producing micro organisms were high. Since the product was fermented, they can possibly induce a chemical poisoning. This was also suggested from other studies that food of animal origin such as seafood, meat and fermented foods, contains biogenic amines in high concentration (Granata et al, 2012).
Table IV: Histamine level in sample triplicates stored in room temperature (25±2°C) for 7, 14, 21 and 28 days period. All values were expressed in mean±SD.

Table IV showed that the results of histamine level in canned food items stored at room temperature in dry conditions were considerably better even though there was an increase in histamine level. However this increment was within the legal limits of histamine for food consumption. The dry conditions with the constant room temperature (25±2°C) had not giving any room to microorganisms to grow on it even though they have stored for longer duration. On a trial we also observed that if the temperature was not constant and variate typically the level of histamine was high. (Data was not shown here). This indicated that the temperature was one of the most important deciding factors to influence the histamine level. Microorganisms such as Lactobacillus, Aspergillus niger and Trichosporon spp., carry the oxidase enzymes possible induce the decarboxylation which lead to the synthesis of amines (Halhsz A. et al. 1994). Some types of yeasts were found in cheese varieties are capable of assimilating cadaverine, putrescine and histamine (Taylor SL et al., 1978).

The toxic level of amines was very difficult to establish because it depends on various factors which include principally individual characteristics and the presence of other biogenic amines like putrescine and cadaverine. Earlier studies based on food borne histamine intoxication revealed that the level of 1000 mg/Kg (amine/food) was considered as dangerous for health (Sumner S et al., 1985). Literatures reported that the values of 100-800 mg/Kg for tyramine and 30 mg/Kg for phenyl ethylamine have been considered as toxic doses in foods (Brink et al., 1990; Halhsz A et al., 1994).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mango pickle (mg/Kg)</td>
<td></td>
<td>22.3±0.3</td>
<td>55.1±1.37</td>
<td>70.3±2.62</td>
<td>88.2±1.08</td>
</tr>
<tr>
<td>Fermented durian (mg/Kg)</td>
<td></td>
<td>35.5±1.0</td>
<td>69.3±1.5</td>
<td>72.7±1.7</td>
<td>89.5±2.0</td>
</tr>
<tr>
<td>Canned pineapple syrup (mg/L)</td>
<td></td>
<td>11.4±0.74</td>
<td>18.6±1.8</td>
<td>19±0.86</td>
<td>19.7±1.66</td>
</tr>
<tr>
<td>Canned rambutan syrup (mg/L)</td>
<td></td>
<td>11.6±1.85</td>
<td>16.8±1.42</td>
<td>18.9±1.47</td>
<td>25.1±0.92</td>
</tr>
<tr>
<td>Canned lychee syrup (mg/L)</td>
<td></td>
<td>14.5±0.58</td>
<td>19.5±1.25</td>
<td>20.3±1.88</td>
<td>21.9±2.34</td>
</tr>
</tbody>
</table>
The European Community has recently proposed that the average content of histamine should not exceed 10-20 mg/100 g of fish (Joosten, HMLJ & Northolt, MD 1987). Addition of nitrite to raw meat possibly enhances the reactions with amines and amino acids present in meat (Shahidi et al., 1993). Range between 100 to 200 mg/ kg is considered as the safest level for histamine in meat products approved by the Netherlands Institute of Dairy Research and also by the Czech Republic (Bunkova et al., 2013). A maximum limit of 100 mg histamine/Kg food and 2 mg/l in alcoholic beverages have been suggested as safest level for histamine.

4. Conclusion

Histamine based food poisoning is the major concern over the consumption of canned foods specially. There are many factors which contributed to the histamine food poisoning. Most of the time it is contributed by the availability of precursor amino acids, food storage time, temperature, pH, various cooking methods, oxygen tension, availability of carbon sources, presence of vitamins, co-enzymes, concentration of free amino acids, potentiatators and other fermentable carbohydrates. If the amines and the factors responsible for increasing amines are optimal and the individual is not allergic to histamine, the consumed food would not cause any problems to the health. In this study, we found that the stable room temperature under the dried conditions favours the most suitable for maintaining the histamine level in food. Temperature variation revealed that has an impact over the increased concentration of histamine in food items. It was also revealed that fermented food item (canned fermented durian) significantly has higher level of histamine over other preserved food items. Storing time along with the increase temperature overall increase the level of histamine in food items. Thus, the main factors that influence the biosynthesis of amine compounds are storage time and conditions, temperature as well as good manufacturing practices which principally make them to keep within the safety level. A better knowledge of these factors controlling amines formation is necessary in order to improve the quality and safety of canned food items.

Conflict of interest:

Nil
References


