Rapid detection of histamine in foods

Kikkoman Biochemifa Company

1 Introduction

Histamine poisoning is one of the most common forms of food intoxication and is caused by the ingestion of foods containing high level of histamine. Histamine poisoning is characterized by a variety of symptoms similar to allergic reactions and include hypotension, flushing, headache, puffy eyes, and skin rash (Taylor, 1986). In Japan, instances of histamine poisoning occur every year (Table 1).

Histamine is a biogenic amine produced through the decarboxylation of free histidine by histamine-forming bacteria (Ladero et al., 2010). This ability has been described in different genera, species, and strains of bacteria. Histamine is rarely found in fresh fish but its level increases with the progress of decomposition. Freezing, cooking, or canning can inhibit the histamine-forming reactions, but histamine formation cannot be eliminated because histamine is heat stable. In addition to fish, histamine can also be found in aged or fermented foods such as soy sauce, fish sauce, miso, wine and cheese, in which histamine-forming bacteria can be found (Okamoto et al., 1997). Histamine poisoning caused by Japanese seasonings such as soy sauce and miso have rarely occurred and most cases of poisoning are caused by raw and processed seafood. It is likely that the small intake of seasonings does not contain sufficient amounts of histamine causing poisoning (Guidi and Gloria, 2012).

2 Regulations in histamine

Fish handling practices are critical with regard to histamine production. For the purposes of consumer protection, fish importing countries have regulations and varying limits for histamine in fish and fishery products. For example, according to Commission Regulation EC No 2073/2005 (Regulation 2073/2005/EC) maximum levels for histamine were established in fishery products from fish species associated with high histidine amounts, value raising from 100 to 200 mg/kg, while the products which have undergone enzyme maturation treatment in brine, the aforementioned limits raised to 200 and 400 mg/kg. The last Regulation amended Annex I to Regulation EC No 2073/2005 added a maximum value for fish sauce produced by fermentation of fishery products, equal to 400 mg/kg. According to the FDA guidelines, the toxicity and defect action levels of histamine established for fish is equal to or greater than 50 ppm. (FDA 2011).

3 Analytical methods for histamine detection

A number and variety of methods exist for determination of histamine levels in fish, fishery products, and fermented foods including the well-accepted Association of Official Analytical Chemists (AOAC) fluorometric method (AOAC 977.13), enzyme-linked immunosorbent assay (ELISA) methods (Pessatti et al., 2004, Kose et al., 2011), the colorimetric enzyme test (Sato et al., 2005) and high-performance liquid chromatography (HPLC) methods that can measure multiple biogenic amines (Malle et al., 1996; Brillantes and Samosorn, 2001). While each method has strengths and limitations, and they vary in terms of related cost, operator expertise, time to obtain a result, portability, etc. (Table 2), most methods provide good agreement and are capable of reliably measuring histamine in seafood at levels of interest.

Table 1: Histamine poisoning outbreaks in Japan

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>7</td>
<td>206</td>
</tr>
<tr>
<td>2012</td>
<td>9</td>
<td>113</td>
</tr>
<tr>
<td>2013</td>
<td>7</td>
<td>190</td>
</tr>
<tr>
<td>2014</td>
<td>7</td>
<td>61</td>
</tr>
<tr>
<td>2015</td>
<td>13</td>
<td>405</td>
</tr>
<tr>
<td>2016</td>
<td>15</td>
<td>283</td>
</tr>
<tr>
<td>2017</td>
<td>8</td>
<td>74</td>
</tr>
</tbody>
</table>
Fluorescent assay (AOAC 977.13)

Fluorescent measurement (AOAC 977.13) has been established and is recognized as the most suitable method for the determination of histamine contained in fish and fermented foods. In this method, product is extracted with 75% (v/v) methanol. Extract is passed through ion exchange column. o-Phthalaldehyde as a fluorescent reagent is added to elute to form fluorescent histamine derivatives. The intensity of the fluorophore is measured using photofluorometer and histamine is quantified using external standards. In this method, the procedure is not simple and the samples need to be extracted with hot methanol. In order to obtain derivatives from this fluorophore and histamine, impurities in the sample must be removed by column. Thus, well-trained laboratory labor must be employed and time required to carry out “cleanup” procedures are unavoidable.

High-performance liquid chromatography (HPLC)

HPLC is suitable method of histamine assay. In addition to histamine, biogenic amines such as putrescine and cadaverine can be simultaneously and quantitatively determined by HPLC. In this method, however HPLC analysis is not run simultaneously, so sequential analysis of multiple samples can take time. The HPLC equipment needed in this assay is also expensive.

Enzyme-linked Immune Sorbent Assay (ELISA)

ELISA assay kits are commercially available. ELISA kits provide good reproducibility, however, the kits are expensive for routine testing of numerous samples on a quality-assurance or preventive testing basis.

### Rapid Enzymatic assay

Kikkoman Histamine Test (code 61341)

Histamine test kits using rapid enzymatic method with easy extraction are commercially available (Kikkoman Histamine Test Code 61341). This product has been issued the AOAC Performance Tested Methods (PTM) certificate (License number 041802).

#### 4.1 Assay principal

A colorimetric enzyme assay for quantitative analysis of histamine in foods has been developed using histamine dehydrogenase (Sato et al., 2005). Histamine dehydrogenase specifically catalyzes the oxidation of histamine. In the test kit of Kikkoman Biochemifa Company, the principle of this photometric assay is as follows;

Histamine dehydrogenase catalyzes oxidative deamination of histamine in the presence of 1-methoxy PMS (electron carrier), which

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<table>
<thead>
<tr>
<th>Table 2: Comparison of the test methods for determination of histamine levels (modified from the FAO Meeting report, 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Product</td>
</tr>
<tr>
<td>Testing time</td>
</tr>
<tr>
<td>Equipment</td>
</tr>
<tr>
<td>Limit of quantification</td>
</tr>
<tr>
<td>Procedure</td>
</tr>
<tr>
<td>General Advantages</td>
</tr>
</tbody>
</table>

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![Figure 1: Determination of histamine using enzyme](image-url)
converts WST-8 (tetrazolium salt) to a formazan (Figure 1). Thus, one molecule of formazan is formed by one molecule of histamine. This product is measured in the visible range at 460-470 nm.

The correlation between histamine level and absorbance is excellent when assay is performed using histamine standard in terms of linearity passing the origin of coordinate axes (Figure 2). Therefore, the histamine in a tested sample can be determined with good precision from absorbance using only one point of histamine concentration as a standard. In addition, the cost of this enzymatic method is very reasonable as compared to other method such as Fluorescent assay, HPLC, and ELISA method.

4.2 Analytical protocol of raw and canned tuna
Histamine level in raw and canned tuna can be determined as below (Figure 3);

[Preparation of sample solution]
1. Approximately 10 g of the sample was weighed and homogenized.
2. Weigh out precisely 1 g of homogenized sample and transfer into a heat resistant plastic tube with cap.
3. Precisely 24 ml of 0.1 M EDTA•2Na solution (pH8.0) is added and mixed vigorously. In this case, the sample is diluted 25-fold.
4. The sample tube is boiled for 20 min and then cooled on ice. In a case of heated sample such as canned tuna, this boiling process is not needed.
5. The sample is mixed vigorously and the supernatant is collected by filtering through folded filter paper or centrifugation (10,000g for 5 min).

[Determination of histamine amount]
1. To set the absorbance of the spectrophotometer to zero, distilled water should be used as reference according to its instruction manual.
2. To assay N samples, prepare (2N + 2) plastic tubes for duplicated assay.
3. To carry out sample assay, add 0.5 ml of the extracted sample solution. Then add 0.5 ml each of the colorimetric reagent and incubate for 15 minutes.
4. Measure the absorbance at 470nm.

Figure 3: Determination of histamine in raw fish using enzymatic method

Figure 2: Determination of histamine standard

\[
y = 0.3403x + 0.0127 \\
R^2 = 0.99989
\]
the enzyme solution. Mix well and incubate at 37°C for 15 min. The sample should be protected from light, if possible, especially irradiate strong light and, in particular sunlight, during the operations. Measure the absorbance at 470 nm.

4. To carry out sample blank assay, add 0.5 ml of the buffer solution instead of the enzyme solution. Carry on the same operation as in (3). Measure the absorbance at 470 nm.

5. To carry out histamine standard assay, use 0.5 ml of histamine standard solution instead of extracted sample solution. Carry on the same operations as in (3). Measure the absorbance at 470 nm.

6. To carry out reagent blank assay, add 0.5 ml of distilled water instead of the extracted sample solution and add 0.5 ml of buffer instead of enzyme solution. Carry on the same operation as in (3). Measure the absorbance at 470 nm.

The histamine concentration in the sample can be calculated by the ratio of the absorbance of the sample and standard solution with the subtractions of each blank. In this method, no harmful reagents are used and each operation is not difficult. All procedures including extraction can be completed within 1 hour.

**Table 3** shows recovery of spiked sample in raw and canned tuna. Good recovery was obtained at the concentration from 10 ppm through 75 ppm. When commercial raw tuna is stored at 20°C for 2 days, histamine levels can be increased. As shown in **Table 4**, high concentration of histamine is detected, and the result of this method showed good correlation with HPLC, AOAC fluorescent assay, and EIA methods. Histamine concentration in a commercial canned tuna sample, in which a high concentration of histamine was present, was also measured by four different methods. The result using this enzymatic method was consistent with the other methods. In conclusion, this simple and rapid enzymatic histamine test is as reliable as the other conventional methods tested.

### 4.3 Interference by oxidation and reduction substrates in some foods

In this method, the tetrazolium salt develops color due to the electron transfer, which is caused by histamine decomposition by enzyme. If any oxidation or reduction substrate is present in the reaction, interference or enhancement of color development is possible with the result that histamine level is not determined properly. Such substrates are known to be present in fermented foods such as miso, soy sauce, and fish sauce. Sample dilution can eliminate these interferences when significant.

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**Table 3**: Spike and recovery test for raw and canned tuna

<table>
<thead>
<tr>
<th>Spiked histamine (ppm)</th>
<th>Recovered histamine (ppm)</th>
<th>Raw tuna</th>
<th>Canned tuna in oil</th>
<th>Canned tuna in water</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>11.3</td>
<td>10.8</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>21.6</td>
<td>21.2</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>51.0</td>
<td>49.5</td>
<td>50.9</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>72.0</td>
<td>73.5</td>
<td>77.6</td>
<td></td>
</tr>
<tr>
<td>Recovery rate</td>
<td>96-113%</td>
<td>98-108%</td>
<td>100-106%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4**: Comparison of results from four histamine test methods in raw and canned tuna (unit: ppm)

<table>
<thead>
<tr>
<th>Method</th>
<th>Raw tuna (stored at 20°C for 2 days)</th>
<th>Canned tuna</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Enzymatic test</td>
<td>2,886</td>
<td>537</td>
</tr>
<tr>
<td>HPLC</td>
<td>2,545</td>
<td>566</td>
</tr>
<tr>
<td>AOAC 977.13</td>
<td>2,646</td>
<td>530</td>
</tr>
<tr>
<td>EIA method</td>
<td>2,728</td>
<td>574</td>
</tr>
</tbody>
</table>

**Table 5**: Comparison of results from two histamine test methods in fish sauce produced in Thailand (unit: ppm)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colorimetric enzymatic method</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brand A</td>
<td>42</td>
<td>47</td>
</tr>
<tr>
<td>Brand B</td>
<td>148</td>
<td>148</td>
</tr>
<tr>
<td>Brand C</td>
<td>290</td>
<td>288</td>
</tr>
<tr>
<td>Brand D</td>
<td>290</td>
<td>309</td>
</tr>
</tbody>
</table>

**Figure 4**: Interference by oxidation and reduction substrates
Significant concentrations of histamine are present in food. In fact, we found that the histamine could be determined in fish sauce when sample is diluted at least 200 times. Four commercial fish sauces were tested in two methods - 1) the rapid enzymatic test as described above and 2) HPLC. As shown in Table 5, the rapid enzymatic test produced results in very close agreement to the results in HPLC method. Thus, the rapid enzymatic method could produce in accurate and consistent results for fish sauce. In the case of soy sauce, because histamine levels are not typically as high as in fish sauce, dilution is not applicable. In this case, pre-treatment of sample with column could be applied (Figure 5). The procedure of column-treatment in soy sauce is shown below.

1. 0.1 ml of soy sauce is diluted in 10 ml of 20 mM phosphate buffer (pH 6.0).
2. This solution is applied to column (Sep-Pak Plus Accell CM, Waters).
3. Column is washed by 10 mL of 20 mM phosphate buffer (pH 6.0).
4. Histamine is eluted by 10 ml of 175 mM NaCl in 20 mM phosphate buffer (pH 7.0). The eluent was applied to histamine test.

As shown in Figure 6, various concentration of histamine in soy sauce were determined by enzymatic method and HPLC method. Both results were consistent and their correlation coefficient was 0.998.

**Conclusion**

The rapid enzymatic method for the measurement of histamine in food has been developed and Histamine Test (AOAC-PTM 041802) is commercially available. A high correlation was observed between the results obtained by this method and those obtained by conventional methods. This method has a lot of advantages; extraction procedure is simple, calibration curve of histamine can be easily drawn, and the histamine amount can be rapidly measured. In the FAO Meeting report (2012), this method was cited as reliable analysis as same as AOAC fluorescent assay, HPLC, and ELISA methods. Thus, this method would be a useful tool for assessing food spoilage and preventing histamine poisoning.


