Comparison of Biogenic Amines Levels in Different Processed Cheese Varieties with Regulatory Specifications

Ekbal Mohamed Adel Ibrahim and Amr Abdel-Moamen Amer

Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt
Department of Food Hygiene, Faculty of Veterinary Medicine, Alexandria University, Egypt

Abstract: Cheeses are among high-protein-containing foodstuffs in which enzymatic and microbial activities cause the formation of biogenic amines from amino acids decarboxylation. Forty-five samples of different kinds of commercially processed cheeses made from Cheddar, Ras and Gouda (15 of each) were collected from local supermarkets, Egypt. The concentrations of biogenic amines (Tyramine, histamine and cadaverine) were assayed using high performance liquid chromatography (HPLC). Cheddar cheese had the highest content of tyramine and cadaverine (22.12 ± 1.35 and 0.36 ± 0.78 mg/100g, respectively). While, the highest histamine level of 17.43 ± 1.05 was found in processed cheese samples. In conclusion, comparing the obtained results with the recommended standard by Food and Drug Administration, high levels of biogenic amines in various processed cheeses consumed in Egypt exceeded the permissible value (10 mg%) which seemed to pose a threat to public health.

Keywords: Biogenic amines · Cadaverine · HPLC · Histamine · Processed cheese · Tyramine

INTRODUCTION

Biogenic amines are organic bases and biologically active compounds naturally occurring in animals, plants and microorganisms. Cheese, like other fermented foods, usually contains high levels of biogenic amines, which result from the breakdown of amino acids by the action of decarboxylase producing microorganisms [1]. The most important biogenic amines from the food hygiene point of view are primary amines which include tyramine, histamine and cadaverine [2]. Such amines were recorded as the most abundant biogenic amines in cheese [3].

The production and levels of biogenic amines in cheeses depend on their contents of free amino acids, bacterial load, pH and salt concentration as well as storage temperature [4, 5]. Thus, the amount of biogenic amines in cheese may act as a useful indicator of the hygienic quality of the product. In other words, their presence in cheese is related to its spoilage and safety [6].

Cheese is among the most commonly implicated foods associated with histamine poisoning and tyramine toxicity. Several cases have been reported in the Netherlands, United States and France and outbreaks have involved Swiss cheese that contained more than 100 mg histamine 100 g⁻¹ cheese [7]. Cases of cheese-related histamine poisoning involving individuals on drug therapy have also been reported. In Canada, a patient being treated with isoniazid reportedly became ill after consuming extremely aged Cheddar cheese that later was found to contain 40 mg histamine 100 g⁻¹ [7]. In Egypt, processed cheese is made from a blend of Ras cheese as the principal cheese (not less than 51% of the finished product) with smaller amounts of dairy products such as cream, milk, skim milk, whey or their concentrates with certain other optional ingredients, e.g. fruits, vegetable and meat. The blend is subjected to a heat treatment to give the desirable texture and flavor. Ras cheese may contain appreciable amounts of biogenic amines [8, 9]. It was reported that heat treatment had little effect on the content of biogenic amines in foods; consequently, processed cheese may contain biogenic amines [10].

Monitoring of some bacterial species; Enterobacteriaceae, Enterococci and Coliforms are often responsible for production of biogenic amines in cheese especially when their counts exceed 10⁶ cfu/g [11]. Accordingly, the action of decarboxylase activity-microorganisms on certain amino acids like tyrosine, histidine and lysine may result in the formation of

Corresponding Author: Ekbal Mohamed Adel Ibrahim, Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt. E-mail: ekbal_adel_2200@yahoo.com.
corresponding potentially toxic biogenic amines "tyramine, histamine and cadaverine" [7].

The interest in amine determination is due to their ability to have a direct or indirect effect on the human vascular and nervous systems. Indeed, a large amount of the biogenic amines can cause rash, headache, nausea, hypo- or hypertension, cardiac palpitation, intracerebral hemorrhage and anaphylactic shock, especially if alcohols or monoamine oxidase inhibitors (MAO-Is) are ingested at the same time [7,12,13]. Moreover, in the specific case of the cheese, it is possible to use the determination of biogenic amines as a parameter of hygienic quality in cheese-making [14, 15] or as an indicator of the degree of proteolysis and the typicalness of some particular cheeses [16, 17].

Although the microbiological quality of processed cheese in Egypt has been studied, there are rare available data on the content of biogenic amines in processed cheese. Therefore, this study was undertaken to determine the presence of biogenic amines in commercially available processed cheese in Egypt and match these levels with the regulatory specifications to determine their quality.

**MATERIAL AND METHODS**

**Samples:** A total of 45 random samples of different kinds of commercially processed cheeses (Cheddar, Ras and Gouda) (15 samples of each) were collected from local supermarkets, Egypt, to determine their contents of biogenic amines.

**Methods:** Tyramine, histamine and cadaverine were estimated by High Performance Liquid Chromatography (HPLC) according to the technique adopted by Moret and Conte [18].

**Amine Extraction:** Accurately, 25 g of processed cheese samples were homogenized with 125 ml of 5% trichloroacetic acid for 3 min. Using a warning blender, then filtration was achieved using filter paper Whatman No 1. Moreover, 10 ml of the extracts was transferred into a suitable culture tube with 4 g NaCl and 1 ml of 50% NaOH, then shaked and extracted 3 times by 5ml n-butanol: chloroform (1:1 v/v) stoppered and shaken vigorously for 2 min followed by centrifugation for 5 min at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable disposable pasteur pipette. To the organic extracts (upper layer), 15 ml of n-heptane was added and extracted 3 times with 1.0 ml portions of 0.2 NHCl, then NHCl layer was collected in a glass stopper tube. Solution was evaporated just to dryness using water bath at 95°C with aid of air currents.

**Derivative Formation (Dansyl Amines):** Two hundreds il of each stock standard solution (or sample extract) were transferred to a culture tube and dried under vacuum. About 0.5 ml of saturated NaHCO3 solution was added to the residue of the sample extract (or the standard). The tube was stoppered and carefully mixed to prevent loss due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The mixture was kept in a water bath at 70°C for 10 min. Then, the extraction of dansylated biogenic amines was carried out using 3 times of 5.0ml of diethyl ether and the ether layers were collected in a culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry film and dissolved in 1 ml methanol, then 10 il injected in HPLC [19].

**Interpretation of HPLC:** The most common technique for amine analysis is HPLC using derivatization before detection. Accordingly, 5-dimethylamino-1-naphthalene sulphonyl chloride was used as derivatization reagent which characterized by the reaction with both primary and secondary amine groups. The chromatographic separation was carried out to separate the three dansyl amines. Furthermore, 10,20,30,40 and 50 i of dansyl amine standard as well as 10 il of each of dansylated sample extract were used. However, the chromatogram was examined under long wave of ultraviolet (254 nm) to establish weather or not the dansyl amines of interest are present in the examined sample. The concentration of each biogenic amine in the examined samples was recorded as mg/100g according to the following formula:

\[
\text{Amine concentration (mg/100g)} = \frac{C \times V}{W \times 10}
\]

**Whereas,**

\[C = \text{Concentration of amine standard (ug/ml)}\]

\[V = \text{Final dilution of sample extract (ml)}\]

\[W = \text{Weight of the sample in the final extract (g)}\]

**Statistical Analysis:** The obtained results were statistically evaluated by using Analysis of variance (ANOVA) test according to Feldman et al. [20].
Table 1: Incidence of biogenic amines in the examined samples of different processed cheese varieties (n=15)

<table>
<thead>
<tr>
<th>Processed Cheese varieties</th>
<th>Tyramine</th>
<th></th>
<th>Histamine</th>
<th></th>
<th>Cadaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Cheddar</td>
<td>14</td>
<td>93.33</td>
<td>11</td>
<td>73.33</td>
<td>12</td>
</tr>
<tr>
<td>Ras</td>
<td>10</td>
<td>66.67</td>
<td>13</td>
<td>86.67</td>
<td>9</td>
</tr>
<tr>
<td>Gouda</td>
<td>9</td>
<td>69.00</td>
<td>8</td>
<td>53.33</td>
<td>5</td>
</tr>
<tr>
<td>Total (45)</td>
<td>33</td>
<td>73.33</td>
<td>32</td>
<td>71.11</td>
<td>26</td>
</tr>
</tbody>
</table>

Table 2. Concentrations of biogenic amines (mg/100g) in the examined samples of different processed cheese varieties (n = 15)

<table>
<thead>
<tr>
<th>Processed Cheese varieties</th>
<th>Tyramine</th>
<th></th>
<th>Histamine</th>
<th></th>
<th>Cadaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Mean± S.E</td>
<td></td>
<td>Min</td>
</tr>
<tr>
<td>Cheddar</td>
<td>6.2</td>
<td>78.5</td>
<td>22.12±1.35</td>
<td></td>
<td>5.1</td>
</tr>
<tr>
<td>Ras</td>
<td>4.9</td>
<td>38.1</td>
<td>14.95±0.89</td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>Gouda</td>
<td>4.0</td>
<td>27.6</td>
<td>9.56±0.72</td>
<td></td>
<td>3.2</td>
</tr>
</tbody>
</table>

*N* Significant differences between cheese varieties (*P*<0.05)

N.B: Mean values were calculated according to positive samples.

**RESULTS**

Results achieved in Table 1 declare that cheddar cheese samples have the highest contents of tyramine (93.33%) followed by cadaverine (80%), while processed cheese samples showed the highest histamine (86.67%), with mean values of such biogenic amines showed in Table 2. Comparing the obtained results with Food and Drug Administration value (10mg/100g), 46.67%, 40.00% and 20.00% of the examined cheddar, processed and Gouda cheeses were exceeded such value but, 26.67, 20.00 and 6.67% of such cheeses respectively were exceeded the recommended value (20mg/100g) stipulated by Egyptian legislation.

**DISCUSSION**

Biogenic amines are valuable to judge the hygienic quality of cheese; therefore they can be used as indicator of food spoilage and can cause toxicological effects to consumers at high levels. In this study 45 processed cheese samples (Cheddar, Ras and Gouda) were analyzed to evaluate the concentration of biogenic amines.

Results achieved declared that the occurrence of biogenic amines in the examined samples of Cheddar, Ras and Gouda processed cheeses were 93.33, 66.67 and 60.00% for tyramine, 73.33, 86.67 and 53.33% for histamine and 80.00, 60.00 and 33.33% for cadaverine, respectively.

Furthermore, the mean values of tyramine, histamine and cadaverine (mg/100g) were 22.12 ± 1.35, 12.98 ± 0.81 and 10.36 ± 0.78 for Cheddar processed cheese, 14.95 ± 0.89, 17.43 ± 1.05 and 8.25 ± 0.49 for processed cheese and 9.56 ± 0.27, 7.65 ± 0.38 and 4.84 ± 0.26 for Gouda processed cheese, respectively. The tyramine was the most biogenic amine formed in the processed cheese followed by histamine and cadaverine. Nearly similar results were recorded by Garcia et al. [21]. While, higher results were obtained by Jarisch [22] in Gouda cheese samples. In contrast lower concentrations of histamine and cadaverine in Ras processed cheese were reported by Darwish et al. [23].

The amines detected in processed cheese samples indicate that these amines were formed in the natural cheeses (Cheddar, Ras and Gouda) used as ingredients in processed cheeses and then passed to the final product. Ripened cheeses have been reported to contain appreciable amounts of several biogenic amines [7-9]. The low level of histamine may be attributed either to its presence below the detectable level or it was catabolized by microorganisms or enzymes [24]. It was reported that histamine is typically highest in Swiss or Gruyere cheese and is typically low in Ras and Cheddar cheese [7,8].

Cadaverine can be related to contamination of cheese with species of Enterobacteriaceae [25]. Although the manufacturing protocol for processed cheese includes high temperature treatment, biogenic amines are not
affected by this treatment and are passed from the raw materials (ripened cheese) to the final product (processed cheese). Once biogenic amines are formed, it is difficult to destroy them by pasteurization or cooking. Therefore, biogenic amines formation should be controlled by strict use of good hygiene in both raw material and manufacturing environment, with corresponding inhibition of spoiling microorganisms [26].

The production of biogenic amines in cheese has often been linked to non-starter lactic acid bacteria and Enterobacteriaceae [27].

In this respect, some proteolytic bacteria can convert the histidine into histamine, however, decarboxylation of lysine into cadaverine by Coliforms [2,7,11,28]. Many Enterococci isolates have the ability to decarboxylate tyrosine with production of tyramine [1,3].

Owing to their microbial origin, biogenic amines can be formed by microbial contamination. In this sense, Rodriguez et al. [29] reported that raw milk cheese had higher level of biogenic amines as compared with pasteurized milk cheese due to its higher microbial contamination particularly with enterobacteiraceae and enterococci. Moreover biogenic amines in food are used as indicator of the hygienic quality of raw materials employed in food manufacture as well as the hygienic quality during food processing [30,31].

It is of great concern to mention that presence of biogenic amines in cheese could be attributed to lactic acid cultures with decarboxylase activity [32]. Moreover, the high storage temperature may favor the growth of some undesirable bacteria and enhances their decarboxylation activity against the amino acids of processed cheeses resulting in formation of such serious biogenic amines [33].

Inspection of Table 3 indicated that 46.67, 33.33 and 26.67% of Cheddar cheese, 33.33, 40 and 13.33% of Ras processed cheese and 20, 20 and 6.67% of Gouda cheese samples exceeded the maximum permissible limit of tyramine, histamine and cadaverine (10 mg/100g) stipulated by FDA [34], respectively.

On the other hand, EOS [35] recommended a value of 20 mg/100g to be the safe permissible value of biogenic amines in food. Consequently, 26.67, 13.33 and 20% of Cheddar cheese and 20, 20 and 6.67% of Ras processed cheese exceeded such limit either of tyramine, histamine or cadaverine, respectively. Concerning to Gouda cheese, all the examined samples were accepted and within the permissible limit of cadaverine stated by EOS [35], however, 6.67% of such samples were unfit based on their contents of tyramine or histamine as shown in Table 4. These amines have been studied for their potential risk for human health, since they can cause "cheese syndrome" and histamine intoxication related to tyramine and histamine, respectively. It was observed that histamine at 20 mg/100g may sufficient to cause the symptoms of scombroid poisoning [36].

Table 3: Comparison of biogenic amines levels in different processed cheese with international legislation (n = 15)

<table>
<thead>
<tr>
<th>Biogenic amines</th>
<th>Permissible limit (mg/100g)**</th>
<th>Cheddar</th>
<th></th>
<th></th>
<th>Ras</th>
<th></th>
<th></th>
<th>Gouda</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td>10</td>
<td>No</td>
<td>7</td>
<td>46.67</td>
<td>No</td>
<td>5</td>
<td>33.33</td>
<td>No</td>
<td>3</td>
<td>20.00</td>
</tr>
<tr>
<td>Histamine</td>
<td>10</td>
<td>No</td>
<td>5</td>
<td>33.33</td>
<td>No</td>
<td>6</td>
<td>40.00</td>
<td>No</td>
<td>3</td>
<td>20.00</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>10</td>
<td>No</td>
<td>4</td>
<td>26.67</td>
<td>No</td>
<td>2</td>
<td>13.33</td>
<td>No</td>
<td>1</td>
<td>6.67</td>
</tr>
</tbody>
</table>

** FDA (2001)

Table 4: Comparison of biogenic amines levels in different processed cheese with Egyptian legislation (n=15)

<table>
<thead>
<tr>
<th>Biogenic amines</th>
<th>Permissible limit (mg/100g)**</th>
<th>Cheddar</th>
<th></th>
<th></th>
<th>Ras</th>
<th></th>
<th></th>
<th>Gouda</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td>20</td>
<td>No</td>
<td>4</td>
<td>26.67</td>
<td>No</td>
<td>3</td>
<td>20.00</td>
<td>No</td>
<td>1</td>
<td>6.67</td>
</tr>
<tr>
<td>Histamine</td>
<td>20</td>
<td>No</td>
<td>2</td>
<td>13.33</td>
<td>No</td>
<td>3</td>
<td>20.00</td>
<td>No</td>
<td>1</td>
<td>6.67</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>20</td>
<td>No</td>
<td>3</td>
<td>20.00</td>
<td>No</td>
<td>1</td>
<td>6.67</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Processed cheese has been documented as a food borne source of histamine poisoning and incorporation with tyramine and cadaverine may enhance its toxicity [37,38]. Approximately, 2-13% of cheese includes Gouda, Swiss, Cheddar and Gruyere have been implicated in cases of tyramine and histamine intoxication [38]. Until now, these have no official limits for the concentrations of biogenic amines in dairy products, it has been estimated that slight histamine- induced poisoning can occur at levels below 40-50 mg/kg whereas severe symptoms can occur at levels exceed 100 mg/Kg fish and fish product [38].

The main symptoms of histamine- rich food is facial flushing, urticaria, edema, diarrhea, stomach ache, cramps, hypotension, anaphylaxis, headache with respiratory disorders [22,39,40].

Based on exposure trials with healthy persons and toxicological studies with animals, it was noticed that the intake of 20-100 mg tyramine can cause toxic effects in humans, whereas, the outbreak information suggest that the levels of 100-800 mg/kg tyramine is hazardous [38].

Hypertension, nausea, severe headache or migraines, emesis, respiratory distress, rashes and increasing the cardiac palpitations are associated with tyramine intoxication [41-43]. However, cadaverine intoxication is mainly accompanied with hypotension [44].

Symptoms appeared due to ingestion of biogenic amines- rich food or conjugation with potentiating factors including; consumption of amine oxidase- inhibiting drugs or alcohols or persons suffering from gastrointestinal diseases may finally leads to heart failure or even brain haemorrhage [44 - 47].

As recommendation, the permissible level of biogenic amines stipulated by EOS should be modified to meet the more safe standard adopted by FDA and their levels can be lowered by using of good quality raw milk and maintaining hygiene standards during manufacturing and storage processes. In conclusion, presence of high concentrations of biogenic amines (tyramine, histamine and cadaverine) in Egyptian processed cheeses reflects the bad hygienic conditions under which they produced and stored. Accordingly, the levels of biogenic amines in different cheeses should be come in accordance with the safe permissible limit recommended by FDA to ensure human safety.

REFERENCES