INVESTIGATION OF HISTAMINE LEVELS IN PASTEURIZED, HIGH HEAT-TREATED MILK AND TYPES OF CHEESE

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ABSTRACT

Histamine is the most common and toxic biogenic amine in foods. The presence of histamine in some foods is considered as an indicator of microbiological quality and hygiene. In this study, it was aimed to investigate the amount of histamine in some milk and cheese consumed in Ankara. A total of 116 samples of 20 different pasteurized milk, 20 high heat-treated milk and 76 cheese samples (tulum cheese, white cheese, fresh kashar cheese, and labne cheese) were analyzed by enzyme-linked immunosorbent assay (ELISA) technique. As a result, In 29 (72.5%) of 40 milk samples, the presence of histamine was detected in concentrations ranging between 0.10 and 0.32 mg/L. The mean levels (±S.E) of histamine of milk samples were found to be 0.18±0.01 mg/L. In 58 (76.3 %) of 76 cheese samples, the presence of histamine was detected in concentrations ranging between 2.52 and 189.79 mg/kg. Regarding the tulum cheese, white cheese, fresh kashar cheese, and labne cheese samples, mean levels (±SE) of histamine in were found to be 56.60±12.56 mg/kg; 3.68±0.29 mg/kg; 4.88±0.35 mg/kg and 4.48±0.39 mg/kg, respectively. The mean levels of histamine of cheeses were higher than mean levels of histamine of milk. The differences between mean levels of histamine in milk samples and the mean levels of histamine in cheese samples were statistically significant (P<0.001). At present, there are no established limits on Turkish Food Codex for histamine concentrations in milks and cheeses.

Keywords: Cheese, ELISA, Histamine, Milk

INTRODUCTION

Milk has special importance in the nutrition of people and especially children due to its rich nutritional content including protein and calcium (Er et al., 2010; Er Demirhan et al., 2015). Milk products should be safe in health as they are included in the diet and its consumption is required in all age groups. (Erkan et al., 2018). Pathogenic microorganisms that may arise from raw milk pose a risk in cheese production (Ducková et al., 2020). Starter cultures used in cheese production are mixed cultures of different microorganisms, and functions of starter cultures are lactic acid production, the formation of sensory active compounds and antimicrobial effect (Květoslavá et al., 2020). Food safety and quality are a major concern of consumer and health care organizations around the world (Ruiz-Capillas and Herrrer, 2019). Foodborne diseases caused by microorganisms, biotoxins and chemical pollutants pose a serious risk to human health (Sumandoulougou et al., 2015). The occurrence of toxic compounds has a negative impact on the safety and nutritional value of foods (Er Demirhan et al., 2015).

Histamine is the causative agent of scombroid poisoning, is biogenic amine and is produced by microorganisms (Er et al., 2014). Histamine is formed by microbial decarboxylation of histidine amino acid by the histidine decarboxylase enzyme. Generally, fresh foods do not contain histamine. (Stratton et al., 1991; Ede, 2017). During the food processing steps, the microbial activity could cause the production of histamine (Er et al., 2014).

Histamine could cause foodborne intoxication. Alcoholic beverages such as red wine and beer, and various foods such as milk, fish, cheese, fruits, vegetables, sausages, and salama are considered as histamine-rich foods (Worm et al., 2009; Švare-Gajic and Stojanovic, 2011; Er et al., 2014; Diaz et al., 2015; Diaz et al., 2018). The histamine content increases in the improper food processing of milk and milk products. Normally, fresh milk contains very low levels of histamine, while commercially sold pasteurized or ultra-high temperature (UHT) milk is reported to have a higher histamine content. A significant increase in histamine content is observed with the fermentation of milk (Botmer et al., 1999). In particular, cheese is one of the most ideal mediums in the formation of biogenic amine (Vale and Glória, 1998; Budak et al., 2008; Spizirri et al., 2013). Many factors are effective in the formation of histamine in cheese. These factors could be specified as starter culture used in cheese making, pH of cheese, salt concentration, contamination during production, storage temperature and ripening time. The highest histamine production occurs during the ripening of the cheeses. This is explained by the degradation of proteins into free amino acids (Madejška et al., 2018).

Although histamine has important physiological functions in humans, consuming food or beverages containing high amounts of histamine can lead to histamine poisoning, which causes symptoms such as headache, diarrhea, asthma, hypotension, pruritus, urticaria, and dizziness (Joosten and Nunez, 1996; Leszczyńska et al., 2004; Er et al., 2014; Diaz et al., 2018). Generally, in case of a low level of histamine exposure, histamine could not be absorbed by the gastrointestinal (GI) tract. Otherwise, histamine is absorbed by the GI tract and histamine intoxication occurs due to the high level of histamine intake and the increase of the blood histamine levels (Taylor, 1986; Er et al., 2014). Healthy people are able to rapidly detoxify dietary histamine by amine oxidases, but histamine detoxification capacity can be exceeded by the accumulation of histamine, thereby histamine toxicity could occur (Maintz and Novak, 2007; Chong et al., 2011; Akan and Demirağ, 2018). In this case, people with low diamine oxidase (DAO) enzyme activity are at risk for histamine toxicity (Maintz and Novak, 2007). However, toxicological reactions may also occur after food intake containing relatively high amounts of histamine. Histamine intolerance is due to the accumulation of histamine in the human system (Rai et al., 2014). Consumption of histamine-rich food or drugs that histamine release or block the DAO can cause diarrhea, headache, rhinoconjunctival symptoms, asthma, hypotension, arrhythmia, urticaria, pruritus, redness and other disorders in patients with histamine intolerance. In this instance, symptoms can be reduced with a histamine-free diet or eliminated by antihistamines (Maintz and Novak, 2007). The presence of histamine has no positive effect on foodstuffs and reduces the quality of food. Thus, the determination of histamine levels in food is necessary to indicate deterioration and quality. Fresh and suitably stored or processed foods and beverages have low histamine content. However, products with lower histamine content in the same type of food are of better quality (Botmer et al., 1999).
The occurrence of biogenic amines in foods is considered as a chemical indicator of food spoilage. Consequently, monitoring of biogenic amine levels in foods has remarkable importance (Zarei et al., 2011; Er et al., 2014). Many cases of poisoning related to cheese have been reported and it is indicated that the cause of the majority of these poisoning is histamine (Joosten and Nunez, 1996). In some countries, food regulations specify the highest levels of residues where histamine can be found in some foods. However, the type of food is generally limited to fish. In the Turkish Food Codex (TFC), the levels of histamine are regulated with fish and fishery products (Turkish Food Codex, 2011). There is no legal regulation on milk and milk products. The objective of our study was to determine the presence and levels of histamine in some milk and cheeses consumed in Ankara.

**MATERIAL AND METHODS**

**Samples**

A total of 40 pasteurized and UHT milk of different brands (A, B, C, and D), and 76 Tulum cheese (E, F), White cheese (G, H), Kashar cheese (I, J) and Labne cheese (K, L) samples of different brands, were collected from supermarkets in Ankara, Turkey. Milk and cheese samples had different serial numbers. These samples were collected before their expiration date and were kept at +4°C (except UHT milk) until they were opened.

**Analysis of Histamine**

The levels of histamine were determined with an ELISA, using the Ridascreen histamine/ELISA kit (r-Biopharm AG, Darmstadt, Germany) (Anonymous, 2018). Samples were prepared according to the instructions of the Ridascreen kit procedure.

**Preparation of Milk Samples**

A 4 mL of each of the pasteurized or UHT cow milk samples were centrifuged at room temperature (20-25 °C) at 3000 g for 10 minutes (Jouan mr 1822, France), then lipid layer was removed. 20 µL of this skimmed milk transferred into other sterile plastic centrifuge tubes, 9 mL distilled water was added and mixed well by vortexing (Firlabo Sa, France).

**Preparation of Cheese Samples**

A 10 g of the sample was weighed and homogenized. A 1 g of the homogenized sample was transferred to sterile plastic centrifuge tubes, 9 mL distilled water was added and mixed well by vortexing. Subsequently, these were centrifuged at 2500 g for 5 min at room temperature (20-25 °C) and after the lipid layer was removed. A 1 mL of supernatant was transferred into other sterile plastic centrifuge tube, 9 mL distilled water was added and mixed well by vortexing. Then, 200 µL of this solution was diluted with 9.8 mL distilled water.

**Application of ELISA**

A 100 µL of the standard solutions and prepared samples and controls were added to 96 wells in the acylation plate, respectively. 25 µL of the acylation reagent and 200 µL of the acylation buffer were added to each acylation well and mixed before incubation for 15 min at room temperature (20-25 °C). 25 µL of acylated standard solutions, controls, and prepared samples were used for the ELISA procedure. The absorbance was measured at 450 nm in ELISA plate reader (Versamax Tunable Molecular Devices LLC, Sunnyvale, CA). Concentrations of histamines were calculated through the guideline of the Ridascreen kit (Anonymous, 2018). Ridasoft Win PC Software for the evaluation of data was used. The detection limits of milk and cheese were reported as 100 ppb and 2.5 ppm, respectively in the test kit.

**Statistical Methods**

The data were analyzed by one-way ANOVA and t-test. Significant differences in means were determined by the Duncan test (Daniel, 1991).

**RESULTS AND DISCUSSION**

Levels of histamine were detected in the commercial pasteurized milk, UHT milk and cheese samples using ELISA. In the applied method, detection limits were 100 ppb for milk and 2.5 ppm for cheese. The levels of histamine for milk samples are shown in Table 1. The mean histamine levels of cheese samples were higher than the mean histamine levels of pasteurized milk and UHT milk in all of the samples.

**Table 1** The levels of histamine in positive samples of cheese and milk

<table>
<thead>
<tr>
<th>Samples</th>
<th>The sample tested, n</th>
<th>Positive sample (%)</th>
<th>The concentration of positive samples (mean ± SE)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td>76</td>
<td>58 (76.3 %)</td>
<td>21.53 ± 5.18 mg/kg</td>
<td>2.52 mg/kg</td>
<td>189.79 mg/kg</td>
</tr>
<tr>
<td>Milk</td>
<td>40</td>
<td>29 (72.5 %)</td>
<td>0.18 ± 0.01 mg/L</td>
<td>0.10 mg/L</td>
<td>0.32 mg/L</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>87 (75 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Means within a column with different superscripts differ (P<0.001)

Our data revealed that the presence of histamine was detected in 29 (72.5%) of 40 milk samples. In commercial pasteurized and UHT milk samples, mean levels (± SE) of histamine were found to be 0.20±0.01 mg/L and 0.16±0.02 mg/L, respectively. Minimum and maximum histamine levels of pasteurized and UHT milk were determined as not detected-0.32 mg/L. In 58 (76.3%) of 76 cheese samples, the presence of histamine was detected in concentrations ranging between 2.52-189.79 mg/kg. Regarding the commercial Tulum, White, Kashar and Labne cheese samples, mean levels of histamine were found to be 56.60±12.56 mg/kg, 3.68±0.29 mg/kg, 4.88±0.35 mg/kg and 4.48±0.39 mg/kg, respectively. Minimum and maximum histamine levels of Tulum, White, Kashar and Labne cheese samples were determined as 6.72-189.79 mg/kg, 2.52-6.26 mg/kg, 2.73-8.76 mg/kg, and 3.00-5.69 mg/kg, respectively. According to the statistical analysis for histamine, the difference between brands was insignificant in pasteurized and UHT milk (P>0.05). The histamine values of the pasteurized milk and UHT milk were compared and no statistically significant difference was found (P>0.05). The differences between cheese types were compared, the mean histamine levels of Tulum cheese brand (E) were higher than the others and this was found to be statistically significant (P<0.001) (Table 2). When the histamine values of milk and cheese products were compared, the difference between milk and cheese was statistically significant (P<0.001). This difference is due to the high histamine content of long-ripened cheese samples. Some cheese samples had higher histamine contents compared with others, likely because of the expiration dates of samples. These higher histamine values were maintained throughout the imminent expiration date of these samples.

**Table 2** Statistical analyses for histamine in positive samples of cheese

<table>
<thead>
<tr>
<th>Cheese Types</th>
<th>The sample tested, n</th>
<th>The concentration of positive samples, mg/kg (mean ± SE)</th>
<th>Minimum (mg/kg)</th>
<th>Maximum (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulum</td>
<td>19</td>
<td>56.60 ± 12.56*</td>
<td>6.72</td>
<td>189.79</td>
</tr>
<tr>
<td>White</td>
<td>12</td>
<td>3.68 ± 0.29*</td>
<td>2.52</td>
<td>6.26</td>
</tr>
<tr>
<td>Fresh Kashar</td>
<td>20</td>
<td>4.88 ± 0.35*</td>
<td>2.73</td>
<td>8.76</td>
</tr>
<tr>
<td>Labne</td>
<td>7</td>
<td>4.48 ± 0.39*</td>
<td>3.00</td>
<td>5.69</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>21.53 ± 5.18*</td>
<td>2.52</td>
<td>189.79</td>
</tr>
</tbody>
</table>

*Means within a column with different superscripts differ (P<0.001)

The high histamine content in food is influenced not only by microbial contamination but also by free amino acids, water activity, pH, salt concentration, humidity, and storage temperature in the environment (Bodmer et al., 1999, Rohani et al., 2013, Mahmoudi and Mardani, 2015). During the ripening of cheese, caseins is broken down with proteolytic enzymes and the amount of free amino acid increases (Büyük and Marangoz, 2018). The ELISA is a rapid and precise method for the determination of the histamine in many food samples (Leszczynska et al., 2004).

Histamine contents of some foods have been investigated in several studies. To our knowledge, the presence of histamine in pasteurized and UHT cow milk samples has not been investigated in Turkey. Therefore, this is the first study that related the investigation of histamine content in pasteurized and UHT cow milk in Turkey. In 87 (75%) of 116 milk and cheese samples, the levels of histamine...
were detected in concentrations ranging between not detected-0.32 mg/L for milk and not detected-189.79 mg/kg for cheese. The present study indicated that the mean levels of histamine were 0.18±0.01 mg/L in 72.5% of 40 milk samples and 21.53±5.18 mg/kg in 76.3% of 76 cheese samples. Nizamoğlu, (1999) reported that substantially higher amounts of histamine as 54.5 mg/100g and 23.3 mg/100g in commercial Kashar and Tulum cheeses, respectively. Durlu-Özka ya et al., (1999) found 6.35 mg/100g of histamine maximum level in White cheeses. Numanoğlu et al., (2008) found 91.5 mg/kg of histamine maximum value in Tulum cheeses. These histamine values in Tulum cheese samples were lower compared with the value reported in the present study. Şimşek and Tunec, (2018) reported levels of histamine as 3.55-18.9 mg/kg and 5.80-44.6 mg/kg in fresh and old akcaatik cheese (similar labane cheese). These results in cheese samples were found to be higher than our research (3.00-5.69 mg/kg).

The literature available on the occurrence of histamine in different types of cheeses indicates contamination. Similar or even higher levels of these amines have been found by other authors in other cheeses from different countries. Zarkower et al. (1965) analyzed histamine in 47 cow’s milk using fluorometric analysis and reported that to be 0.272 µg/mL. The studies about dairy products have been focused on cheeses. Higher levels of histamine were detected in different cheeses by Chang et al., (1985) and in North Serbia by Švare-Gajie and Stojanovic, (2011). On the contrary, Bunkova et al. (2013) reported that biogenic amines levels in a total of 112 cheese and fermented dairy products examined in the Czech Republic. Researchers reported to histamine levels as not detected level to 24.2 mg/kg in cheese. Rohani et al., (2013) determined that biogenic amines in some cheese samples by HPLC and reported mean levels of histamine in koopshe, lighwan, red salmas as 70.80 mg/kg, 27.59 mg/kg and 105.21 mg/kg, respectively.

The cheese should be stored at low temperature after ripening in order to reduce histamine deposition. Lactic acid bacteria carrying the histidine decarboxylase gene can be held responsible for the formation of histamine in fermented milk products. These bacteria may be present in milk and may be part of starter cultures (Diaz et al., 2018). Therefore, bacteria must be selected carefully in the selection of starter cultures. Starter cultures that produce little or no histamine should be used (Rai et al., 2014).

CONCLUSION

In this study, the histamine content was investigated in 116 milk and cheese samples and histamine was found in 72.5% of 40 milk samples and 76.3% of 76 cheese samples. Histamine levels in pasteurized and UHT milk samples are quite different. Changes in different types and brands may be due to production technology, lack of hygiene, and inappropriate storage conditions. Quality manufacturing is required to prevent histamine formation in cheese and thus the possible health hazards to the consumer. Therefore, it is important to monitor histamine levels and to determine legal values in ripened cheeses.

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REFERENCES


