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**DETERMINATION OF HISTAMINE IN TRADITIONAL CHESES OFFERED IN
THE MARKET MARAND BY HPLC**

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ABSTRACT

Biogenic amines are low molecular weight organic bases with biological activity that are produced primarily by microorganisms via enzymatic decarboxylation of amino acids. Histamine, tyramine, putrescine, tryptamine, Kadaorin, spermine, and 2-phenyl ethyl amine, are biogenic amines. Histamine is a good indicator for evaluating the quality of food and is used as an index of the freshness degree of food. Histamine toxicity is a chemical poisoning resulted of food and is usually a mild toxicity associated. With symptoms such as headache, skin rashes, pruritus, urticaria, localized inflammation, irritation of the mouth and gastrointestinal symptoms. As a most-consumed dairy product, Cheese is a suitable environment to create histamine. This study was conducted using Boston HPLC station (Silice for powerful LC sepuration) (CEC 150 / 4.6nucleodur C18 grovity 0.5 µm). The study was conducted in the summer of 1393 in which 50 samples were randomly selected from traditional cheeses of Marand. Based on the results, in 10 samples (20%) the amount of histamine was more than 100 ppm, in 10 samples (20%) was about 50 to 100 ppm and in 30 samples (60%) was less than 50 ppm. High levels of histamine in traditional white cheese kept in maximum brine were 170ppm maximum, with an average of 53.45 ppm in understudied samples indicates the presence of predisposing conditions of histamine production production, processing and storage of cheese. If the conditions are unfavorable for the growth of histamine-producing bacteria the amount of histamine reduces. Pasteurization or cooking doesn't destroy the biogenic amines. So, the production of biogenic amines must be done using hygienic raw materials and production environment as well as a

storage, temperature control in order to inhibition of spoilage microorganisms. The results of this study show that the identification of effective actions to control the production of biogenic amines, such as histamine, in the production, processing and storage of traditional cheese are necessary.

Keywords: Histamine, Traditional Chesses, HPLC, Marand

INTRODUCTION

Biogenic amines are organic bases of low molecular weight and are formed in foods mainly by the microbial decarboxylation of certain amino acids [1, 2]. One of the most studied amines, histamine (HIS) arises in foods by the growth of microorganisms that possess histidine decarboxylase and thus, foods rich in free histidine can contain high level of HIS. Although fish of the families *Scombridae* and *Scomberesocidae* are commonly implicated in incidents of HIS poisoning, non-scombroid fish, cheese and other foods have also been attributed in cases of such poisoning [3].

Besides fish, cheese is the second most significant source of biogenic amines and many organisms may be the cause of amine formation in cheese [3, 4]. Several studies have been performed for the identification of lactic acid bacteria capable of producing HIS, and several species have been recovered from cheese, including *Lactobacillus buchneri*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Streptococcus faecium* and *Streptococcus*

lactis. Since some of these bacteria are utilized as starter cultures and reach high populations during ripening, their identification as HIS producers is important to the dairy industry [2, 3].

The presence of biogenic amines in food constitutes a potential public health concern because of their physiological and toxicological effects [5]. HIS is a powerful biologically active chemical that can directly stimulate the heart, cause extra vascular smooth muscle to contract or relax, stimulate both sensory and motor neurons, and control gastric secretion. Therefore, a wide variety of symptoms can be attributed to this type of poisoning [3].

The predominant biogenic amines found in several types of cheese are tyramine, putrescine, cadaverine and histamine [1, 3, and 6]. Amine contents may vary among different types of cheese, and ripened cheeses usually contain higher concentrations of amines than unripened cheeses. This difference can be related to casein proteolysis during ripening [6, 7]. Moreover, the duration

and conditions of the ripening period and starter culture types used are important factors for the production of amines [6]. Also, bacterial quality of milk, heat treatments applied, pH, salt concentration, and temperature are the other parameters leading to differences among a variety of cheese [3, 6].

Due to the impact of biogenic amines on human health and food safety, monitoring their levels in foodstuffs is still gaining importance [6]. Quantitative instrumental analyses developed so far for the analysis of biogenic amines in food samples include thin-layer chromatography, gas chromatography, liquid chromatography, capillary electrophoresis (CE) and biochemical assays. Among these, high performance liquid chromatography (HPLC) by pre- or post-column derivatization is most frequently reported [6, 8], and only a few analyses do not require time consuming derivatization procedures, such as enzymic sensor array method detection, HPLC with conductometric detection and some CE methods [6]. This study was conducted using Boston HPLC station (Silice for powerful LC separation) (CEC 150 / 4.6nucleodur C18 gravity 0.5 μm).

MATERIALS AND METHODS

The study was conducted in the summer of 1393 in which 50 samples were randomly selected from traditional cheeses of Marand.

Histamine hydrochloride and Dansyl chloride were purchased from Sigma Co. Acetonitrile, distilled water with a degree Hplc, butanol and hydrochloric acid were purchased from Merck.

Chromatography systems include:

- Column: euro sphere 150/4.6 nucleodur c18 gravity 0.5 μm (silica for powerful lc separation)
- Well chrom HPLC pump , k-1001 (KNAUER Germany)
- Dynamic mixing chamber
- Degasser (KNAUER)
- Uv – detector K-2501 (KNAUER Germany)

Preparation of the samples according to the method defined by acid extraction and derivatization took place. 10 g of each sample was weighed directly into centrifuge tubes. 20 ml of 1.0 M hydrochloric acid containing the internal standard (1 and 7 Di-amino heptanes) was added. The sample tubes for 2 minutes with a standard homogenizer and was homogenized heidolph diax 9000. Resulting in the suspension was centrifuged in 12000 for 20 min at 4 ° C. The supernatant was collected from each tube and deposit the remaining supernatant by centrifuge and again

with the same conditions as the previous mixed with 1.0 M HCl in a volume of 50 ml. To derive the samples, 1 mL organic extract with 2 drops of hydrochloric acid 1M and mixed under vacuum dried. Then 1 ml of 1.0 M hydrochloric acid, saturated solution of sodium bicarbonate and 1 ml of 500 microlitres chloride solution Dansyl 5 (mg / ml) added and incubated for 1 h at 40 C. Finally, after drying under vacuum 2 mL acetonitrile was added, the solution is filtered (Varian, Bond Elut C18) was injected into the HPLC column. Histamine also took steps to standardize. To measure histamine HPLC column (Silica for powerful TLC separation)

(EC 150 / 4.6nucleodur C18 gravity 0.5 μ m) was used. The combination mobile phase contains of water and acetonitrile, peak production of 254 nm devices were identified and assessed. The concentration gradient of the flow velocity (flow - rate) 8/0 ml per minute has been shown in Table 1. Then extracted with an organic solvent (butanol) was done, This means that in a 5 ml test tube with 5 ml of butanol extraction and mixing for 2 minutes and 3 times the vortex. Organic extracts with saturated NaCl and NaOH brought to pH= 5.11.

Table 1: Biogenic amines used to analyze the concentration gradient

Water (%)	Acetonitrile (%)	Time (min)
50	50	0
50	50	1
35	65	5
25	75	6
20	80	10
10	90	15

RESULTS

Central and distributive indices including mean, standard deviation, minimum and maximum amount of histamine in the 50 samples is shown in Table 2. The results obtained that the lowest and highest amount in samples were 7.5 ppm and 170 ppm and

the average amount of histamine in the sample was 53.45 ± 7.88 ppm.

Based on these findings in 10 samples (20%) the amount of histamine over 100 ppm, in 10 cases (20%) the amount of histamine approximately was 50 ppm to 100 ppm and in 30 samples (60%) of histamine was less than 50 ppm.

Table 2: Descriptive characteristics of histamine (ppm) in each test sample.

50	Number of samples
53.45	Mean
7.88	Standard deviation
7.5	Minimum
170	Maximum

DISCUSSION

Cheese is an ideal substrate for biogenic amine formation because of the possible presence of decarboxylase- positive microorganisms, the convenience of environmental conditions for the growth of these microorganisms, and the presence of some cofactors [1]. The U.S. and the European Community (EC) have set a maximum limit of 100 ppm for HIS in fish, but not in cheese. However, a maximum limit of 900 mg/kg was suggested for tyramine, HIS, putrescine and cadaverine combined [7]. The acceptable level of HIS in cheese was reported as 100 mg/kg by Durlu-Özkaya [9] and Durlu-Özkaya et al. [10]. Although the concentration of HIS in all cheese samples analyzed in this study was lower than the acceptable limit, the Erzincan Tulum cheese content was found to be fairly close to the limit. On the other hand, since symptoms of clinical illness have been associated with the consumption of a minimum of 100 to 180 mg of HIS, it seems unlikely that any of the cheeses analyzed in this study could cause intoxication, unless consumed in very large quantities [2].

Based on the results, in 10 samples (20%) the amount of histamine was more than 100 ppm, in 10 samples (20%) was about 50 to 100 ppm and in 30 samples (60%) was less than 50

ppm. High levels of histamine in traditional white cheese kept in maximum brine were 170ppm maximum, with an average of 53.45 ppm in understudied samples indicates the presence of predisposing conditions of histamine production production, processing and storage of cheese. In a study by Razavi Rouhani and his colleagues in 2012 was taken on the kind of traditional cheese, It was found that the amount of histamine in traditional cheese was 2.43 ppm and the highest dose observed in this type of cheese was 1102.24 ppm. Another study by Mehdi Zarei and Amir Hesam Jafarnejad was taken in 2000; the results showed that the amount of histamine in traditional cheese in the range of zero to 8.12 mg ppm per kg and the average of it is 5.37mg per kg. Although the standard is not defined in the amount of histamine in cheese, But considering the standard of canned tuna can be said that the composition of the compound in this kind of cheese is acceptable and safe. In another study by Zohreh Meshak and her colleagues in 2001; on the rainbow trout were purchased from the market in Tehran that Histamine levels in samples between 40 ppm to 280 ppm with an average 180.14 ppm. Of these, 12.5% of samples were above the limit of international. In addition to this direct relationship between histamine and the number of bacteria in fish samples there.

The results of this study show that the identification of effective actions to control the production of biogenic amines, such as histamine, in the production, processing and storage of traditional cheese are necessary.

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