

Short Communication

Determination of histamine in Iranian cheese using enzyme-linked immunosorbent assay (ELISA) method

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Histamine is a simple chemical substance created during processing of the amine acid histidine. Histamine is also an agent in inflammation and the increased presence of histamine causes allergic reaction. Histamine may play a role in the increased prevalence of food intolerances. The objective of this study was to determine histamine contents. Forty four (44) samples of traditional and commercial cheese were analyzed by enzyme-linked immunosorbent assay (ELISA) method in Iran. In the two cheese samples of the 44 samples (4.5%), the presence of histamine was 26 and 46.7 mg/100 g. Histamine in any of the cheese samples was not higher than the tolerance limit of histamine contents (50 mg histamine/100 g) accepted by European countries. The values were comparable and in the range of the literature values. The results of this study indicate that the produced cheese and marketed cheese in Iran have concentrations below 50 mg histamine/100 g. Further studies should be done to investigate the presence of this toxin in different foodstuffs.

Key words: Histamine, cheese, enzyme-linked immunosorbent assay (ELISA), Iran.

INTRODUCTION

Histamine is a biogenic amines foodborne chemical toxin gotten by eating some spoiled or bacterially contaminated food especially fish and cheese as products of enzymatic activity of certain microbial agent (Standra et al., 2002). Cheese is an ideal substrate for the production of biogenic amines by microbial decarboxylation of the corresponding amino acids. Histamine content of cheese is affected by many factors including the presence of substrate and microbial enzymes, temperature, pH, salt and water content and presence of enhancing substances in catabolism of amines (Standra et al.,

2002). The histamine poisoning disease is complicated but is generally associated with high levels of histamine (50 mg/100 g) in the spoiled fish (Lehane, 2000; Lehane and Olley, 2000). Symptoms usually subside within a few hours spontaneously. The most commonly encountered symptoms are tingling and burning sensations around the mouth, gastrointestinal complaint and a rash with itching (Mahendradatta, 2003). Nausea, vomiting, headache and other symptoms are induced by consumption (ingestion) of foods containing high levels of histamine and also the same symptoms can occur after the consumption of red wine, especially in individuals with naturally reduced ability of histamine decomposition (Smajlovic et al., 2008; Mitchell, 1993). Consequently, human histamine poisoning is mostly and rightly associated with canned fish containing high levels of this amine. Currently, there is limited information regarding the contaminant levels of histamine in cheese in Iran.

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Table 1. Presence of histamine in different samples ($P \leq 0.01$).

Parameter	Value												
Industrial	0.114	0.099	0.104	0.102	0.103	0.108	0.101	0.100	0.101				
Traditional 1	0.114	0.102	0.106	0.104	0.103	0.104	0.111	0.110	0.110	0.105	0.106	0.102	
Traditional 2	0.106	0.103	0.106	0.102	0.104	0.104	0.113	0.112	0.117	0.103			
Traditional 3	0.115	0.110	0.107	0.104	0.080	0.117	0.114	0.105	0.105	0.102	0.115	0.104	

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The objective of this study was to determine histamine contents in 44 samples of traditional and commercial cheese by enzyme-linked immunosorbent assay (ELISA) method in Iran as one of the food borne toxin.

MATERIALS AND METHODS

Collection of samples

From April to September 2011, a total of 44 cheese samples including 36 traditional and eight commercial cheese samples were randomly selected in Esfahan and Chaharmahal va Bakhyari, Iran. The samples were immediately transported to the laboratory in a cooler with ice packs and stored at -20°C until the time of analysis.

Method of analysis

To measure histamine in cheese samples, a competitive ELISA was employed using RIDASCREEN® histamine kit (R-Biopharm AG, Germany). The assay was performed according to the manufacturer's recommendation. The limit to the detection of this test for cheese, fresh fish and canned fish is 2.5 ppm. After the sample preparation, histamine was quantitatively converted to N-acetylhistamine, using an acylation reagent. After washing, the secondary peroxidase-conjugated antibodies (enzyme conjugate) are added. These antibodies bind to the antibody-histamine complex; then, unbound antigen is removed by washing. Substrate (urea peroxide) and chromogen (tetramethyl-benzidine) are added into wells of the micro-titration plate and then are incubated. During incubation, the bound enzyme conjugation converts a colorless chromogen into blue product, and blue color changes into yellow after addition of stop solution. After the substrate reaction, the optical density was measured at 450 nm on the ELISA plate reader (Stat Fax 2100, England). The amount of bound complexes to the plate and the optical density are inversely in proportion to the histamine concentration of the samples.

Statistical analysis

All statistical analyses were performed by using SPSS software, version 16 (SPSS Chicago, IL, USA) and the data were expressed as mean \pm standard deviation (SD). Chi-square test and Fisher's exact two-tailed test analysis were performed and differences were considered significant at values of $P < 0.05$.

RESULTS AND DISCUSSION

In the two cheese 44 samples (4.5%), the presence of

histamine was found as 26 and 46.7 mg/100 g (Table 1). Results indicate significant differences between histamine contents among the traditional and commercial cheese samples ($P < 0.05$). Out of the 36 total traditional cheese samples, 5.6% (two samples) showed histamine contamination. The values were comparable and in the range of the literature values. Histamine in the cheese samples was not higher than the tolerance limit of histamine contents (50 mg histamine/100 g) accepted by European countries. Symptoms of clinical illness have been associated with the consumption of a minimum of 100 to 180 mg histamine; it seems unlikely that any of the analyzed cheese in their study could cause intoxication, unless has been consumed in very large quantities (Innocente et al., 2002). Standara et al. (2000) determined the biogenic and showed the histamine concentration between 12.2 to 15.8 mg/100 g which were lower than the level of histamine in cheese samples in our study. Numanoglu et al. (2008) in Turkey noted that 94% of traditional cheese samples obtained from different Turkish markets contained histamine at levels of 65.9 and 91.5 mg/100 kg. Erzincan Tulum cheese contained the highest level of histamine (91.46 mg/kg) among all the types of cheese samples, followed by Izmir Tulum cheese (65.89 mg/kg). Since Tulum cheese is a ripened cheese, it can be concluded that ripened cheese contain more histamine than fresh cheese (Numanoglu et al., 2008). This can be explained by the formation of free amino acids due to hydrolysis of casein during ripening. Results of the researches are similar to the level of histamine in traditional cheese in our study. There are also reports of extremely high levels of histamine in some fresh and fermented cheese in Asia (Durlu-Ozkaya, 2002).

A novel screening immunoassay for histamine was used for the detection of histamine in different foodstuffs. The detection limit of this assay was $20 \mu\text{g kg}^{-1}$. The concentration of histamine varied between 182 and 982 $\mu\text{g kg}^{-1}$ in sauerkraut, cheese and fish samples and 26 and 18433 $\mu\text{g l}^{-1}$ in milk, sparkling wine and wines. The applied competitive enzyme immunoassay (ELISA) seemed a reliable technique for simple and rapid determination of histamine in food (Sarkadi et al., 2003). A study in Taiwan reported that 54.8 and 15.4% of the natural and processed cheese samples, respectively, had

histamine in cheese samples at a level of 7.9 mg/100 g as mean (Durlu-Ozkaya, 2002). The difference in histamine content in these cheese products can attribute to the type of cheese, the storage temperature of the cheese, and the hygienic condition of the environment for cheese processing and handling. The situation regarding a toxic dose is unclear (at least because the responsible chemical is not known). Approximately 100 mg/100 g histamine is considered to be toxic but a number of incidents have involved foods containing less than 5 mg/100 g histamine. A limit used is commonly 30 mg/100 g, although the Food and Drug Administration (FDA) have a limit of 50 mg/100 g. Another scheme states that <5 mg/100 g is safe to eat, 5 to 20 mg/100 g is possibly toxic, 20 to 100 mg/100 g is probably toxic and >100 mg/100 g is toxic and unsafe for human consumption (Lehane and Olley, 2000). Histamine production is known to be associated with the growth of bacteria that possess the enzyme histidine decarboxylase. In cheese, several histamine-producing bacteria have been implicated as primary contributors to histamine formation (Kim et al., 2001; Cemaek et al., a). Permanent control of the histamine presence in food rich in proteins and in wine should be introduced because the possibility of histamine development in such foodstuffs is detrimental to human health. Since the screening method for quantitative determination of histamine is easy to perform, the control of histamine presence should legally regulate for the protection of human health.

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