WALAILAK JOURNAL
http://wjst.wu.ac.th Short Communication

Histamine Levels in 3 Types of Iranian Cheese by Ion-Exchange Chromatography

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Received: 4 March 2012, Revised: 5 April 2012, Accepted: 12 July 2012

Abstract

Three types of Iranian cheese including Feta, Lighvan and Kope were investigated on the basis of histamine content as a biogenic amine. The separation and determination of this compound were performed by ion-exchange chromatography. Results indicated significant differences in histamine contents among the three types of cheeses, with the highest mean content of 42.6 mg/100g for Kope cheese, and the lowest mean level of 4.99 mg/100g in Feta cheese. In microbiological studies, Kope cheese samples had the highest total yeast (24 %) and bacterial contents (36 %). None of the isolates were found to be of major pathogenic importance.

Keywords: Biogenic amine, cheese, histamine, ion-exchange chromatography

Introduction

Biogenic amines are low molecular weight organic bases that possess biological activity. Biogenic amines are present in many biological materials, occurring as natural components of animal and plant tissues and microbial cells, or as products of enzymatic activity of certain microbial agents [1]. These compounds, such as tyramine, histamine, putrescine, cadaverine, tryptamine and 2-phenyl ethylamine have been found in several types of cheese [2,3]. Their presence can be regarded as a sign of ongoing decomposition of foods [1]. Cheese is an ideal substrate for the production of biogenic amines by microbial decarboxylation of the corresponding amino acids. Histamine content of the cheese is affected by many factors. The formation and presence of such amines depend on a variety of factors including the presence of substrate and microbial enzymes, temperature, pH, salt and water content, presence of enhancing substances in catabolism of amines [1]. The presence of biogenic amines can cause several problems for susceptible consumers, such as nausea, respiratory disorder, hot flushes, sweating, heart palpation, headache, bright red rash, oral burning [4].

It is therefore necessary to recognize the profile of ingested amines and to discover the ways of preventing their occurrence in food. Several methods have been developed for the determination of amino acids and biogenic amines, e.g. high-performance liquid chromatography, chromatography on an amino acid analyser and overpressured-layer chromatography. In comparison, ion-exchange chromatography (IEC) offers the advantages of a simple preparation of samples and the determination of a larger number of amines in food samples with sufficient sensitivity and accuracy [5].

The aim of this study was to determinate histamine levels by ion-exchange chromatography in 3 types of Iranian cheese, Feta, Lighvan and

Kope as most representative of the Azerbaijan and Kurdistan regions.

Materials and methods

Samples

One hundred-fifty samples of traditional cheese (50 for each cheese type) from different regions of West Azerbaijan and Kurdistan were obtained randomly from local markets. The samples were transferred to the laboratory and evaluated immediately.

Standard histamine preparation

Histamine (HI) dihydrochloride was obtained from Merck, Darmstadt, Germany. Stock solutions contained 1 mg of the respective amine per 1 ml. The calibration mixtures were prepared from equal volumes of the stock solutions and were diluted with dosage buffer to obtain the concentration of 50 µg of amine per 1 ml. The solution was kept cold and was protected from light by an opaque foil.

Instruments

Single-column automatic amino acid analyzer 334T (Mikrotechna, Prague, Czech Republic) with the TZ 4100 recorder (Ingos Ltd., Raha, Czech Republic) and the IC-26 integrator (Analitik, Mikhailovgrad, Bulgaria) were used.

Conditions of chromatography

The separation of histamine was carried out on a column with the OSTION LG ANB ion exchanger (Spolek pro chemickou a hutni vyrobu, Ustı nad Labem, Czech Republic). The temperature of the column was maintained at 60 ^{o}C and the flow of buffers and ninhydrin reagent at 14 ml/h and 12 ml/h, respectively. No pre-column for the removal of ammonia from buffers was used in the flow-through system of the analyzer. The amines were detected spectrophotometrically at 520 nm and the 100 mV signal of the recorder was integrated for quantitative determination. The dosage was 200 µl per column.

Sample processing and ion exchange chromatography

Preparation of 3 types of cheese samples and performance of ion exchange chromatography method were conducted according to Standara *et al.* [1].

Microbiological evaluations

All samples were evaluated for total yeast and bacterial contents. After 1/10 w/v homogenization of sample in sterile PBS solution, cultures were performed on routine media for isolation and detection of yeasts and bacteria. Isolates were differentially characterized to the species level by routine culture and biochemical microbiological methods [6].

Statistical analysis

All experiments were performed in triplicate, HI level and mmicrobiological analysis was conducted using variance analysis (ANOVA). All statistical analyses were performed using software 17 SPSS. Significant results were considered at $P < 0.05$.

Results

The mean and standard deviation for histamine content of each type of cheese are shown in **Table 1**. Results indicate significant differences of HI contents among the 3 types of cheese ($P < 0.05$). The lowest content of HI in Feta cheese was below the limit of detection (LOD) of the method while the highest was 5.64 mg/100g. Lighvan and Kope cheese showed values of HI in the ranges 4.62 - 10.32 and 7.62 - 42.6 mg/100g respectively. In comparison, Kope cheese has high levels of histamine ($P < 0.05$). As far as microbiological studies are concerned, Kope cheese samples had the highest total yeast and bacterial contents, 12 samples (24 %) and 18 samples (36 %), respectively. A total of 5 bacterial isolates belonged to family *Enterobacteriaceae*. None of the isolates found was of major pathogenic importance. Feta cheese samples had the lowest total yeast and bacterial contents, 2 samples (4 %) and 5 samples (10 %), respectively. No enterobacterial contamination was observed. Lighvan cheese samples had moderate total yeast and bacterial contents, 7 samples (14 %) and 11 samples (22 %), respectively. A total of 2 bacterial isolates belonged to family *Enterobcteriaceae*. None of isolates was found to be of major pathogenic importance.

Table 1 Means contents (± SD) of histamine in 3 cheese types.

Cheese type	Histamine (mg/100g) (Mean ± SD)
Feta (n = 50)	$4/99^{a} \pm 0.35$
Lighvan (n = 50)	$8/16^{b} \pm 0.73$
Kope (n = 50)	$26/76^{c} \pm 0.54$

Superscript letters represent significant value ($P < 0.05$)

Discussion

The presence of biogenic amines in food constitutes a potential public health concern because of their physiological and toxicological effects [7]. HI 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]. Cheese is an ideal substrate for biogenic amine formation because of the possible presence of decarboxylase positive micro-organisms, the convenience of environmental conditions for the growth of these microorganisms, and the presence of some cofactors [8].

Due to the impact of biogenic amines on human health and food safety, monitoring their levels in foodstuffs is still gaining importance [7]. Ion-exchange chromatography with amino acid analyzer seems to be one of the most specific and selective methods for the determination of amino acids and biogenic amines. This method has several advantages such as simple sample preparation and low cost [6]. The formation and accumulation of histamine is influenced directly by bacterial activity, pH, salt concentration and indirectly by water availability, storage temperature and ripening time [9,10]. Differences in histamine contents among the 3 types of cheese in the present research could be dependent on the different ripening time, storage periods, and preparation and distribution conditions for each cheese.

The results of the determination of biogenic amines in cheese by ion-exchange chromatography showed the highest concentration being recorded for putrescine (164 - 191 mg/kg) and cadaverine (85 - 89 mg/kg), HI (12.2 -15.8 mg/kg)[1].

The U.S. and the European Community (EC) have set a maximum limit of 100 ppm for histamine in fish, but not in cheese. However a maximum limit of 900 mg/kg was suggested for tyramine, histamine, putrescine and cadaverine in cheese [11]. The histamine mean contents obtained for all 3 cheese types in the present study are significantly lower than this limiting value.

The acceptable level of histamine in cheese was reported as 100 mg/kg [12,13]. Although the concentration of histamine in all cheese samples analyzed in their 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 histamine, it seems unlikely that any of the cheeses analyzed in their study could cause intoxication, unless consumed in very large quantities [14]. Investigation of the histamine contents of some cheese samples from Taiwan showed that the histamine contents were higher than the 5 mg/100 g limit set by the U.S. [15]. Food and Drug Administration for scombroid fish and/or product, in 54.8 and 15.4 % of the natural and processed cheese samples, respectively. The average histamine content of natural cheeses was found to be 7.9 mg/100 g.

Determination of biogenic amine content of Beyaz cheese has demonstrated a histamine content of 29.2 mg/kg [12]. These results are similar to the level of histamine found in Kope (26.76 mg/kg) in our study.

The results of the analysis of histamine in 16 traditional cheese samples obtained from different Turkish markets showed that in 15 samples (94 %) with a level greater than LOD[1], and in 2 (13 %) samples greater than LOQ[2], with values of 91.5 and 65.9 mg/kg [16]. Erzincan Tulum cheese contained the highest level of histamine (91.46 mg/kg) among all types of cheese samples, followed by İzmir Tulum cheese (65.89 mg/kg). Since Tulum cheese is a ripened cheese, it can be concluded that ripened cheeses contain more histamine than fresh cheeses such as Denizli Lor, Bolu Dil, Diyarbakır Orgu and White brined

[1]Limit of detection (LOD), 20 mg/kg for histamine
[2]Limit of quantification (LOQ), 61 mg/kg for histamine

cheese. This can be explained by the formation of free amino acids due to hydrolysis of casein during ripening. These results are also in agreement with the results of Durlu-Ozkaya [12], who screened histamine contents of some Turkish cheeses by HPLC, and reported that matured cheeses contained more biogenic amines than fresh cheeses. Authors determined histamine contents of Civil and Mihalıç cheese as 947.6 and 126.4 mg/kg, respectively. On the other hand, histamine levels of some other ripened hard cheese varieties such as Urfa, Balıkesir Mihalıç and Trakya Eski Kaşar were found to be lower than the quantitation limit [12]. Results of the levels of histamine in all 3 types' cheese in our study are similar to the results of these researchers. Furthermore, results of the present research are in line with those reported by Pinho and Ferreire [17]. They showed that high temperature (25 ± 3 °C) could significantly increase the contents of biogenic amines like histamine. Histamine content of Kope cheese was higher than two other cheese types, as this kind of cheese is prepared and commercially distributed in such a temperature range. Furthermore, the ripening time of this cheese is far longer than the 2 other cheese types and also has a higher risk of microbial contamination. Thus, Kope cheese when compared to other cheeses is more likely to produce histamine.

Feta and Lighvan cheese types have more hygienic preparation (According to the results of microbial counts and low presence of bacteria and yeasts), are mostly prepared in factories and their storage periods (including transportation from the cheese factory, commercial distribution, purchase and eventual consumption) of these cheese types are shorter.

Conclusion

The results presented here appear to support the need for the implementation of a well-designed refrigeration scheme throughout transportation, commercial distribution, purchase and storage by the consumer in order to guarantee low levels of histamine in cheese and therefore, less potential for health hazards by the time of consumption.

Acknowledgements

The authors express their sincere appreciation and thanks for the financial support received from the Faculty of Veterinary Medicine, Urmia University and Faculty of Veterinary Medicine, University of Tabriz.

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