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DETERMINATION OF BIOGENIC AMINES IN GEORGIAN TRADITIONAL CHEESE

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ABSTRACT

This article discusses the formation of toxic compounds such as biogenic amines in cheese. The issue of biogenic amine contamination of dairy products remains relevant today. Biogenic amines ingested with food are rapidly detoxified by amine oxidases. If the detoxification process is impaired or the concentration of biogenic amines is too high, they become toxic metabolites and pose a serious threat to human health. Adaptation of dairy production practices continues to evolve as consumers demand stricter rules on ingredients and transparency to ensure their protection. With increasing speculation about production standards, the dairy industry needs to be able to verify current operations to meet these requirements, which can also serve as quality indicators for consumers. One of the main topics of discussion over the past decade has been concerns about a class of nitrogenous compounds known as biogenic amines.

KEYWORDS: Biogenic amines, cheese, histamine, HPLC.

INTRODUCTION

Biogenic amines (BA) are nitrogenous compounds that have biological activity and are derived from the microbial decarboxylation of amino acids during the ripening process. These compounds are found in various types of cheese and are produced by the activity of microorganisms, fungi and bacteria, both in the raw materials used for cheese production and at any stage of production and storage/ripening.

The content of biogenic amines in food products is of great interest, as it is an indicator of food safety and quality assessment. Biogenic amines are organic bases that play several physiological roles in the human body; they act as local hormones and neurotransmitters. When consumed in normal quantities, biogenic amines do not have a proven toxic effect on human health. However, improper implementation of technological processes, as well as the influence of other factors, leads to their significant accumulation in lactic acid products, which cause serious effects on the human body with the development of various diseases. Therefore, the maximum permissible concentration of biogenic amines in dairy products is strictly regulated. In general, cheese is an ideal environment for the production of biogenic amines, although their concentration varies and depends on factors such as the type of cheese, storage temperature, ripening duration, pH, salt concentration and the number of microorganisms with high amino acid decarboxylase activity. One of the conditions for the presence and subsequent accumulation of BA in fermented products, including cheese, is the presence of microorganisms capable of synthesizing these compounds themselves. Among the identified microorganisms were both cheese microbiota and BA-producing yeasts, gram-positive and gram-negative bacteria. However, all of the above do not affect the concentration of BA in the final product.

The aim of this work was to assess the biogenic amine content of Georgian traditional cheeses in Georgia and also to discuss the factors that influence the formation of biogenic amines. For this purpose, 20 samples of Georgian cheeses were obtained from local production. After sample preparation, the biogenic amine content was assessed by liquid chromatography HPLC method. The presence of histamine, tyramine, cadaverine and tyrosine was observed in the tested cheeses. The total amount of biogenic amines was highest after 60 days at 1379.04 ppm. The average content was 977.54 after 30 days and the lowest amount was 537.49 ppm after 20 days. The levels of biogenic amines formed vary depending on the ripening period and the function of the microflora.

MATERIALS AND METHODS

The main components of a liquid chromatography (HPLC) system are: a solvent reservoir, a high-pressure pump, a column, an injector, and a detector. A liquid chromatography system (HPLC) is a column chromatography that samples a mixture or analyte in a solvent at high pressure through a column containing a chromatographic packing material (stationary phase). In this method, a moving stream of helium or nitrogen gas is carried through the sample.

High-performance liquid chromatography techniques can separate and identify compounds present in any sample. In this method, a liquid carrier stream, called the mobile phase, carries the injected sample to a separation column and detector. In the separation column, individual components are separated according to physicochemical interactions. The separated components are detected by a detector based on changes in light absorption or refractive index, electrochemical or conductometric changes, or simply the size distribution of the separated molecules.

Sample preparation Determination of BA was carried out by acid extraction and derivatization using the methods of Eerola, Hinnkkalnenn, Linfors, & Hirvi (1993) and Moret & Conte (1996). For acid extraction, 20 mL of 0.1 M HCl was added to 10 g of each sample. (1,7-diamino-heptane, 10 mg l- 1) The homogenized suspension was centrifuged at 12000 g for 20 min at 4 ˚C and the supernatant was collected. 5 mL of acid extract vortexes with three portions of 5 mL butanol. The organic extracts were saturated with NaCl and pH was adjusted to 11.5 with NaOH. For derivatization, 1 mL of organic extract was mixed with 2 drops of 1 M HCl and dried under vacuum. Then, 1 mL of 0.1 M HCl, 500 µL of saturated solution of NaHCO3 and 1 mL of dansyl chloride solution (5 mg mL-1) were added. The reaction vessel was transferred to an incubator and kept at 40 ˚C under agitation for 1 hour. then, the solution was dried under vacuum and 2 mL of acetonitrile was added. The solution was filtered (Varian Bond Elut C18; Varian, Inc., Palo Alto, CA, USA) and injected onto the chromatographic column.

RESULTS AND DISCUSSION

The results obtained showed the maximum and minimum limits of biogenic amines. The maximum permissible limits of BAs (histamine, tyramine, cadaverine and tyrosine) were tested in traditional cheese and the results are given in Table 1. The total BA content was the highest at 1379.04 ppm, the average at 977.54 and the lowest at 537.49 ppm.

Table 1: Biogenic amine levels (ppm) after 20 days of ripening.

Cheese type	Histamine	Tiramine	Cadaverine	Tyrosine	Total
Georgian cheese					
Average	160.7	288.35	78.25	10.82	537.49
Max	2752.15	3452.24	1306.12	2192	
Min	3.56	2.11	2.05	1.94	

Table 2: Levels of biogenic amines (ppm) after 30 days of ripening.

Cheese type	Histamine	Tiramine	Cadaverine	Tyrosine	Total
Georgian cheese					
Average	264.51	347.28	40.61	325.14	977.54
Max	762.16	1195.62	82.08	675.52	
Min	41.64	23.82	7.62	95.12	

Table 3: Levels of biogenic amines (ppm) after 60 days of ripening.

Cheese type	Histamine	Tiramine	Cadaverine	Tyrosine	Total
Georgian cheese					
Average	441.11	668.07	102.81	167.05	1379.04
Max	784.72	1011.75	256.62	428.21	
Min	68.35	321.42	22.15	9.58	

CONCLUSION

Cheese is an ideal environment for amine production, but amine concentration is very different and depends on factors such as cheese type, age, and microflora.

The enzymatic activity of proteases derived from microorganisms is important for the production of BA in cheese, which in turn directly affects the taste and aroma of the product through peptides and amino acids. The presence of amino acids, in turn, affects the quantity and quality of BA forms. It seems that the most abundant biogenic amines are histamine and tyramine, which are present at all stages of ripening. Cadaverine and tyrosine have also been detected in large quantities in ripened cheese. However, a wide variation in their concentrations has been observed, which is mainly related to milk hygiene.

Overall, on average, tyramine had the highest levels and cadaverine the lowest, compared to the other amines. The toxicity of tyramine appears to be enhanced by the presence of other amines, such as cadaverine, histamine and tyrosine, which act as potentiators of its toxicity.

In addition, some BAs, such as tyramine and cadaverine, when exposed to heat, can lead to the formation of secondary amines and in the presence of nitrites, they can form nitrosamines, chemical agents that are considered to have major carcinogenic properties.

In conclusion, cheese is an ideal environment for amine production, especially during the final stages of ripening, where the ability to produce amino acids is increased, which also contributes to the high concentration of BA. The concentration of amines varies greatly and depends on factors such as the type of cheese, age and microflora. It is also influenced by

several factors such as physicochemical properties, pH, water activity, salt, humidity, temperature and the presence of microorganisms.

Spoilage microorganisms in milk and cheese, mainly represented by Pseudomonas, Enterobacteriaceae and Micrococci, possess active decarboxylases and lactic acid bacteria (LAB) are less active in decarboxylating amino acids but become more active during cheese ripening.

Decarboxylase-positive microorganisms contribute to the accumulation of BA by acting on free amino acids. The presence of specific precursor amino acids affects the quantity and quality of BA formation. Biogenic amine formation can be controlled by inhibiting microbial growth or by inhibiting microbial decarboxylase activity (Wendakoon and Sakaguchi 1995).

Control of BA accumulation in cheese

Therefore, the prevention of biogenic amine formation in food is based on various factors. In addition to good manufacturing practices, the inhibition of BA-causing microorganisms through pasteurization should be carried out. reducing the amount of proteolytic activity to limit the availability of precursor amino acids, the use of starter cultures, reducing the duration of maturation, constant temperature control and the use of high-quality raw materials.

Among food poisoning cases, poisoning caused by BA is quite common, although in many cases it remains unnoticed. Control of this toxin in dairy products will contribute to the reduction of food poisoning.

To achieve this goal, new approaches to safe food need to be adopted. This approach includes knowledge of both the routes of contamination and their causes, as well as the toxic effects that can be caused to the consumer.

The results obtained highlighted the need to control hygienic conditions. which are responsible for the production of large amounts of BA. It is also recommended to use competitive auxiliary starter cultures for fermentation, (Dapkevicius et al. 2000) which will also reduce the production of biogenic amines in the final product.

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