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The determination of some biogenic amines in Turkish fermented sausages consumed in Van☆

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ABSTRACT

Biogenic amines, has been implicated as the causative agent in several outbreaks of food poisoning. Fermented food such as Turkish style fermented sausages can also contain biogenic amines, microorganisms possessing the enzymes decarboxylase, which converts amino acids to biogenic amines, are responsible for the formation of biogenic amines in fermented sausages. The purpose of this study was to determine the amount of biogenic amines in Turkish fermented sausage consumed in Van in Turkey, and to evaluate their concentrations in term of public health risks. High performance liquid chromatography method was used to the determination of 8 biogenic amines in 120 sausage samples collected from 10 different brands sold in the local markets of Van. The detection levels of biogenic amines in the samples was ranged from 0 to 129.375 mg/kg for tryptamine, from 0 to 65.625 mg/kg for 2-phenylethylamine, from 0 to 255.625 mg/kg for putrescine, from 0 to 1148.75 mg/kg for cadaverine, from 0 to 469.375 mg/kg for histamine, from 0 to 438.125 mg/kg for tyramine, from 0 to 554.375 mg/kg for spermidine, and from 0 to 614.375 mg/kg for spermine.

1. Introduction

Biogenic amines (BAs) describe as a group of low molecular weight, heat stable, non-volatile, basic nitrogenous compounds with biological activity [1,2]. The term biogenic amine defines decarboxylation products such as histamine, serotonin, tyramine, phenylethylamine, tryptamine, and also aliphatic polyamines [3]. Biogenic amines chiefly created by microbial decarboxylation of amino acid in foodstuffs, or by amination and transamination of aldehydes and ketones by amino acid transaminases [2,4]. Knowing the levels of histamine in foods is necessary to assess the health hazard arising from consumption of these products [5] and can be used as indicators for food quality markers [6,7]. They have been related to several outbreaks of food-borne intoxication in the world and are very important in public health concern due to their possible poisonous properties [2,8]. Biogenic amines investigated as a possible mutagenic precursor, since some amines may be nitrosated by react with nitrite and generate volatile nitrosamines which are carcinogenic and pose a potential health threat to humans [1,9]. Some strains of bacteria, for example, *Escherichia, Enterobacter, Pseudomonas, Salmonella, Shigella, Clostridium perfringens*, *Streptococcus, Lactobacillus*, and *Leuconostoc* are identified to be able of histamine creation [8,10] Much research has shown that biogenic amines in fermented foods has been implicated as the causative agent in several outbreaks of food poisoning. Canned tuna [11], tuna burger [12], and fermented meat products [13] contain excessive amounts of histamine. Henry [14] suggested that dry sausages should contain only 1–2 μg histamine/g, based on physiological muscle levels but sausages contaminated by undesirable microorganisms may easily reach histamine concentrations of 100 μg histamine/g or greater during the early stages of fermentation.

Turkish fermented sausage (sucuk) is an old-style dry fermented sausage which is the most prevalent meat product in Turkey and is known in almost all Middle Eastern Countries and Europe [15–17]. Turkish style fermented dry sausage is very popular in Turkey. It is a traditional fermented dry sausage, mostly produced by traditional methods in small-scale enterprises by air drying. It is made from sheep or beef meat. Meat is mixed with tail fat, salt, sugar, dry garlic, spices and vegetable oil [7,15,17]. In manufacture lean meat and fat are ground through a 3 mm plate and the mixture stuffed into cattle small intestines. Traditionally they are hung for fermentation and dried at ambient temperature for 25 days [18].

The microbiological, physico-chemical characteristics of Turkish fermented sausages were studied by Sancak et al. [19], Gokmen et al. [20] and Sezer et al. [21]. In the microbiological analyses reported by

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Sancak et al. [1996] in Turkish fermented sausages consumed in Van showed the average value of the total colony, coliform, E. coli, fecal streptococci, staphylococci, coagulase positive staphylococci, *Clostridium perfringens* and yeast-mould were found to be 3.3 × 10 /gr., 5.2xl0 /gr., 6 × 10 /gr., 5.1 × 10 /gr., 6.7 × 10 /gr., *l*.9xl0 /gr., 1.7 × 10 /gr. and 7.3xl0 /gr. respectively. In the chemical and physical analyses, the average values of moisture, fat, salt, ash, and protein contents were found to be 38.57%, 36.13%, 4.12%, 3.99%, 16.43% respectively. The average values of pH and water activity (Aw) were found to be 5.50 and 0.91 respectively.

Sezer et al. [21] reported the presence of *Escherichia coli* in fermented sausages, neither *Clostridium perfringens* nor *E. coli* O157:H7 were identified in any of the fermented sausage samples. Also *Listeria monocytogenes* and *Salmonella* species was isolated in the fermented sausage samples. In the chemical and physical analyses, the maximum values of fat, ash, and salt contents were found to be 58%, 5.42%, and 3.15% respectively. The maximum value of pH was found in the range of 6.97. Gokmen et al. [20] reported the isolation and identification of *Staphylococcaceae* and *Enterobacteriaceae* species in Turkish sausage. In the chemical and physical analyses, the maximum values of humidity and salt contents were found in the following 44.26% and 3.79% respectively. The maximum values of pH and water activity (Aw) were found to be 5.20 and 0.78 respectively.

In many studies, the total number of aerobic microorganisms in fermented sausages is reported to be between 10^6 and 10^7 cfu/ g [22]. According to Turkish Standards Institute, TS-1070, pH value of Turkish sausage is between 4.7–5.4% and moisture content maximum is 40%, while the fat content can be up to 30% for the first grade, and up to 40% for the second grade. Protein concentration can be up to 20% for the first grade and up to 18% for the second grade [23].

This study was undertaken to determine the amount of biogenic amines in Turkish style fermented sausages since biogenic amines are important with regard to toxicological effects.

2. Materials and methods

2.1. Sample origin

In the present paper, 120 Turkish style dry fermented sausages belonging to ten different brands [Brands A, B, C, D, E, F, G, H, I, J] were obtained from retail stores in the Van area of Turkey, and analyzed for biogenic amines content. Twelve samples were collected from each brand. Samples were kept chilled until analysis. Assays were done on duplicate samples with the results being averaged.

2.2. Biogenic amines analysis

Sample preparation and biogenic amines analysis were done by using HPLC method as described by Eerola et al. [24].

2.3. Sample preparation and homogenization procedure

The samples of fermented sausages, whole sausage samples were sliced with a clean stainless steel knife. Some parts of the sausage samples (2 g of the sample) were randomly chosen and transferred into falcon plastic tubes then homogenized with a metallic staff homogenizer tools (T-25 digital Ultra-Turrax from IKA®-Works, Inc. Wilmington, NC 28405 USA) for about 2 min. The homogenization was done by adding 125 µl from internal standard (1.7-diaminoheptane) with 10 ml of 0.4 M perchloric acid (Merck Germany). In the next step the homogenate samples were centrifuged (3000 rpm for 10 min under 4 °C) by High-Speed Refrigerated Centrifuge (Hitachi Koki Co., Ltd. Japan) then the extraction solvents were transferred and filtered with filter paper (Schleicher and Schuell 589 Black ribbon Ø 70 mm) into a volumetric flask. The remain (supernatant) part again centrifuged with 10 ml perchloric acid and filtered into the same volumetric flask then complete to 25 ml with 0.4 M perchloric acid. An aliquot of 1 ml of the final extract was then used for analysis after derivatization while the remaining volume was stored at 4 °C for no more than one week.

2.4. Derivatization of standards and sample extracts

The dansylated derivatives of the amines were formed by adding 1 ml of extract or standard solution mixed with 200 µl of 2 N NaOH (Merck, Germany) and 300 µl of saturated $NaHCO_3$ (Merck, Germany) solution and vortexed (Heidolph D-91126 Schwabach, Reax top, Germany), and 2 ml of dansyl-chloride solution 2 mg dansyl-chloride per ml in acetone (Sigma Chemical Co., USA) were added and solution and again vortexed. Fresh dansyl-chloride solutions were prepared each time just before use. After shaking, samples were left in the incubator at 40 °C for 45 min. After reaction time has passed, the residual dansyl-chloride was removed by addition of 100 µl of NH_4OH 25% (v/v) then vortexed and wait 30 min at room temperature. The derivatization was completed upon addition ammonium acetate and acetonitrile mixture (1:1; v/v) and adjusted to 5 ml. Finally, the mixture was centrifuged (Hettich Zentrifugen, Werk Nr, Germany) at 3000 rpm for 5 min under 4 °C and the supernatant was filtered through 0.45 µm-pore-size filters (Millipore Co., USA).

2.5. Chromatographic conditions

Two solvent reservoirs containing (A) ammonium acetate and (B) acetonitrile were used to separate all the amines with an HPLC elution program. The gradient–elution system was 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The gradient–elution program was started at 50% solvent B and ended at 90% solvent B in 25 min. The system was equilibrated for 10 min before next analysis. The flow rate 1.0 ml/min and the column temperature was 40 °C. A 20 µl sample was injected onto the column. The quantitative determinations were carried out by an internal standard (1.7-diaminoheptane) method, using peak heights.

2.6. Statistical analysis of data

In the statistical analysis of the data obtained in this study, SAS 9.4, a software package was used. In order to assess significant differences between brands means, we performed ANOVA based on the Duncan multiple comparison tests. A p-value of 0.05 or less ($P < 0.05$) was considered statistically significant [25]. The results of statistical analysis are shown as mean values ± standard error, mg/kg wet weight in (Table 1).

3. Results and discussion

Table 1 shows the biogenic amines contents of the Turkish style dry-sausages of different brands obtained from Van retail markets. As seen from the Table 1 there are statistically significant differences ($P < 0.05$) in the contents of biogenic amines in fermented sausage samples. Differentiation in the biogenic amines concentrations of sausages could be due to the hygienic quality of raw material, manufacturing practices, the specific bacteria, ripening period and the type of culture. The biogenic amines concentration may be used as a quality index for this kind of meat product. Handling of raw materials and production technology for fermented sausages are relatively primitive in Turkey. These results indicate that the natural fermentation process used for dry sausages can result in the accumulation of high biogenic amines levels. The brand specific variation suggests that different environmental conditions have some effect on histamine content.

Although the natural fermentation process used in the preparation of these sausages probably did not involve growth of any major biogenic amines - producing bacteria, the lack of quality control in their production [26] and the use of natural fermentation makes selection of