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 trypamine, from 0 to 65.625 mg/kg for 2-phenylethylamine, from 0 to 253.625 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, tryp- tamine, 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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Sancck 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 $3.3 \times 10^{6}$ /gr., $5.2 \times 10^{5}$ /gr., $5.1 \times 10^{5}$ /gr., $6.7 \times 10^{5}$ /gr., $1.9 \times 10^{6}$ /gr., $1.7 \times 10^{6}$ /gr. and $7.3 \times 10^{5}$ /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 ($A_w$) 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 content were found in the following $44.26\%$ and $3.79\%$ respectively. The maximum values of pH and water activity ($A_w$) 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 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 N, 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).

2.4. 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 NaHCO₃ (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 NH₄OH 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 N, 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...
### Table 1

Results of biogenic amine levels found in fermented sausage samples (mean ± Standard error, mg/kg wet weight).

| BA      | Mean ± SE | N   | J    | I    | H    | G    | F    | E    | D    | C    | B    | A    | Mean with different letter are statistically significant P < 0.05. |
|---------|-----------|-----|------|------|------|------|------|------|------|------|------|------|-----------------------|
| TRY     | 20.00 ± 11.08 a,b | 121 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | |
| PHE     | 2.47 ± 1.20 b | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 2.47 ± 1.20 b |
| PUT     | 24.60 ± 5.71 a | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 24.60 ± 5.71 a |
| HIS     | 99.88 ± 36.83 b | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | |
| TYR     | 160.67 ± 45.15 b | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | |
| SPD     | 34.41 ± 10.62 b | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | |
| SPM     | 7.55 ± 1.33 b | 12 | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | 12   | |

*Means with the same letter are not statistically different (P > 0.05). *a Different letter are statistically significant P < 0.05.

No standards or guidelines exist for the allowable concentrations of biogenic amines in fermented sausages. Determination of the exact toxicity threshold of biogenic amines in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual. Although the toxicity of biogenic amines to man is a controversial subject, ingestion of from 70 to 1000 mg histamine will usually cause clinical symptoms intoxication [14]. The FDA has established a hazard action concentration for histamine in tuna fish of 50 mg of histamine/100 g [27]. Moreover, it must be noted that smaller amounts of biogenic amines may cause poisoning particularly if the person is vulnerable, because of the inhibition of the biogenic amines detoxification mechanism in the body due to reasons such as personal predisposition, gastrointestinal diseases, the use of certain medicines and alcohol intake, and the existence of other amines [26,28].
Shalaby [9] suggested amounts of BAs (tyramine 100–800 mg/kg, histamine 50–100 mg/kg, phenylethylamine < 30 mg/kg) as parameters for evaluation of good manufacture practices (GMPs). It is expected that 2–10 mg histamine for every day per individual is perfect with people. A toxicological sum differs from 70 to 1000 mg, as indicated by the powerlessness of a person to this substance. It is further expected that an admission of 100 mg histamine will normally bring out harming side effects. Little is thought about the toxicological measurements of other biogenic amines. About 8.33% (10 samples) of all fermented sausage samples had higher phenylethylamine concentration than 30 mg/kg.

The histamine contents of the different groups were significantly different (P < 0.05) from each other. Histamine concentrations ranged from 0 to 469.375 mg/kg, in fermented sausage samples in this study. Whereas tyramine is the amine in higher concentration (372 mg/kg) followed by histamine (165 mg/kg) in “Salamini italiani alla cacciatora PDO” was detected by Coisson et al. [44].

Histamine is the amine most contemplated as to its toxicological impacts. An intake of 5–10 mg of histamine can be considered as abandoning to some delicate individuals, 10 mg is considered as a middle of as far as possible, 100 mg affect a medium poisonous quality and 1000 mg is very lethal [48,49]. Some different scientists Eerola et al. [50] and Gardini et al. [51] recommended taking after intoxicated points of confinement of histamine, 8–40 mg causes slight intoxication, 40-100 mg middle of the road injuring and more than 100 mg can bring about concentrated poisoning.

Nout [52] called attention to the allowable greatest of histamine and tyramine substance for foods from 50 to 100 mg/kg 100 to 800 mg/kg, respectively. About 26.66% (32 tests) and 22.5% (27 tests) of fermented sausages had higher histamine fixation than 100 and 200 mg/kg, respectively (Table 1). Variation in histamine concentrations of fermented sausages could be because of the clean nature of crude materials, producing practices, and the presence of the particular microorganisms.

Tyramine and putrescine were found from (0 to 438.125 and 0–255.625 mg/kg), respectively. The results obtained in this study are close to the lower mentioned limits, 100-800 mg of TYR [53]. Tyramine is the most abundant BA in fermented sausage as reported by Bozkurt and Erkmen [15], Bover-Cid et al. [6] and Latorre-Moratala et al. [46]. Tyramine levels was found from not detected to a maximum of 411 mg/kg which is the most abundant BA in fermented sausage followed by putrescine as testified by Latorre-Moratala et al. [46]. In another study related to fermented sausage, essential amine were putrescine (more than 400 mg/kg), followed by tyramine (250 mg/kg) [41]. While cadaverine was the predominant biogenic amine 1387 mg/kg in Egyptian fermented sausage [47]. Cadaverine generally found in higher concentrations which are ranged in the mean of (23.8 to 681.2 mg/kg) in different plants [5].

Putrescine and spermidine and spermine amounts in this work had a variable value compared to other BAs in this study. However, handling of raw materials and production technology for fermented sausages is relatively primitive in Turkey. For this reason, must be developed methods for preventing formation of biogenic amines that aim at eliminating the decarboxylating microbes in fermented sausages, the use of high-quality raw materials, amine negative starter cultures and processing conditions which favour growth of the starter strains.

4. Conclusion

It is concluded that the histamine levels obtained from the study were not important from a public health viewpoint but these levels may be a potential risk according to susceptible individuals. However, handling of raw materials and production technology for fermented sausages is relatively primitive in Turkey. For this reason, must be developed methods for preventing formation of biogenic amines that aim at eliminating the decarboxylating microbes in fermented sausages, the use of high-quality raw materials, amine negative starter cultures and processing conditions which favour growth of the starter strains.

Transparency document

The Transparency document associated with this article can be found in the online version.

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