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Histamine, Nitrate and Nitrite Content of Meat Products Marketed in Western Anatolia, Turkey

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Abstract: Biogenic amines (such as histamine) and nitric oxide (such as nitrate and nitrite) are basic nitrogenous compounds occurring in many foods. The histamine, nitrate and nitrite are key signaling molecules in numerous physiological and pathological events. The purpose of this study was to determine the histamine, nitrate and nitrite contents on meat products (53 Turkish styles fermented sausages, 19 salami, 16 fresh sausages and 12 pastırma) of different brands obtained from retail stores in the Afyonkarahisar area of Turkey. The maximum histamine in pastırma samples in the range 30.20-51.82 mg kg^{-1} (mean 42.11 mg kg^{-1}) and minimum histamine in fresh sausage in the range 7.73-30.68 mg kg^{-1} (mean 19.67 mg kg^{-1}) were found. Nevertheless maximum nitrate and nitrite in fermented sausage (mean 149.31 mg kg^{-1}) and salami (mean 45.33 mg kg^{-1}) samples, minimum nitrate and nitrite fresh sausage (mean 97.84 mg kg^{-1}) and fermented sausage (mean 23.49 mg kg^{-1}) were found. According to the results of the present study, meat products samples showed a comparable good quality from a toxicological and public health point of view.

Key words: Turkish styles fermented sausages, salami, fresh sausages, pastırma, histamine, nitrate and nitrite

INTRODUCTION

The meat products such as Turkish style fermented sausage, salami, fresh sausage and pastırma (Turkish dry meat product) are very popular in Turkey and similar products are known in most Middle Eastern Countries and Europe. Many authors have discussed the toxicology of histamine, nitrate, nitrite and their occurrence and formation in foods, with special emphasis on meat products. Histamine can be formed in a variety of foods and beverages, especially in protein rich and fermented foods. Several biogenic amines as histamine play important roles in many human and animal physiological functions[1]. They are formed mainly by decarboxylation of amino acids. Despite their important physiological functions (they promote growth, metabolic activity and immunological system of gut, are active in the nervous system and in the control of blood pressure, some are free-radical scavengers), biogenic amines may have toxicological effects in humans when consumed in food in high amounts: Dilatation of peripheral blood vessels, resulting in hypotension and headache, or contraction of intestinal smooth muscle, causing abdominal cramps, diarrhoea and vomiting[2,3]. Nitrate and nitrite are commonly monitored for food control. Nitrate, as well as nitrite, has been added intentionally during the curing process of certain meat products, due to their ability to inhibit the growth of spores of *Clostridium botulinum* and to impart characteristic color and flavor to this kind of foodstuffs[4]. Although useful as a curing agent, residual nitrite in the meat poses a health risk to humans. The National Institute of Occupational Safety and Health reported that nitrite is a primary irritant, carcinogen and mutagen and causes undesirable reproductive effects. If too much is added, there is a risk for illness, even death of consumers[5, 6]. The aim of this study was to determine the histamine, nitrate and nitrite content of meat products marketed in Afyonkarahisar, Turkey.

MATERIALS AND METHODS

Investigation material constituted a total of 100 meat product samples. Including 53 Turkish style fermented sausage, 19 salami, 16 fresh sausage and 12 pastırma (Turkish dry meat product). All samples were obtained from various markets in the Afyonkarahisar area of Turkey and analyzed for histamine, nitrate and nitrite content. Samples were transported under cold conditions in

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ice-boxes from their place of collection to the laboratory. Analyses were started without any delay. The histamine, nitrate and nitrite levels in meat product samples were determined by colorimetric modifed methods according to Patange *et al.*[7] and Miranda *et al.*[8], respectively.

Histamine analysis: Histamine was extracted from meat product samples. The meat sample (5 g) was homogenized with 20 mL of 0.85% NaCl solution for 2 min using a high-speed blender and centrifuged at 12000 g for 10 min. The supernatant was made up to 25 mL with saline. The extract was used immediately for further analysis. The extract (1 mL) was diluted to 2 mL with saline and 0.5 g of salt mixture containing 6.25 g of anhydrous sodium sulfate to 1 g trisodium phosphate monohydrate was added. The tubes were stoppered and shaken thoroughly. 2 mL of n-butanol was then added and the tubes shaken vigorously for 1 min and allowed to stand for 2 min and then shaken briefly to break the protein gel. The tubes were further shaken vigorously for few seconds and then centrifuged at 3100 g for 10 min. The upper butanol layer (only 1 mL) was transferred into a clean and dry test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water and then reacted with the reagent. The absorbance of the color produced was measured immediately after 5 min at 496 nm[7].

Nitrate and nitrite analysis: Nitrite and nitrate calibration standards were prepared by diluting sodium nitrite and sodium nitrate in pure water. After loading the plate with samples (100 μL), addition of vanadium (III) chloride (100 μL) to each well was rapidly followed by addition of the Griess reagents, sulfanilamide (50 μL) and N-(1-Naphthyl) ethylenediamine dihydrochloride (50 μL). The Griess solutions may also be premixed immediately prior to application to the plate. Nitrite mixed with Griess reagents forms a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines, such as N-1-(naphthyl) ethylenediamine. Blank sample values were obtained by substituting diluting medium for Griess reagent. Nitrite was measured in a similar manner except that samples and nitrite standards were only exposed to Griess reagents. The absorbance at 540 nm was read to assess the total level of nitrite and nitrate in all samples[8].

RESULTS AND DISCUSSION

Histamine, nitrate and nitrite levels in meat products of different brands obtained from Afyonkarahisar retail markets are shown in Table 1 and 2. As seen from the tables, the lowest average histamine and nitrate levels in fresh sausage and also nitrite level in fermented sausage were obtained and the highest average values were obtained for pasturma, fermented sausage and salami, respectively.

Table 1: Histamine levels of meat products from different brands

		Histamine (mg kg^{-1})		
Meat product	n	Minimum	Maximum	Mean±SD
Fermented sausage	53	16.18	80.75	38.41±17.1
Salami	19	14.41	40.95	26.94±9.7
Fresh sausage	16	7.73	30.68	19.67±6.8
Pasturma	12	30.20	51.82	42.11±4.1

Histamine level in stored feedstuff is a product of complex enzymatic activity involving microbial histidine decarboxylase, microbial and endogenous proteolytic enzymes and microbial diamine oxidases. Enzyme activity depends on amino acid content and microbial contamination in the feedstuff and storage conditions, such as temperature, pH and oxygen availability. In common storage conditions, significant activity of histidine-increasing proteolytic enzymes and histamine-degrading diamine oxidase cannot be expected due to high temperature and oxygen level and neutral or alkaline pH needed for their activity. High histidine content in stored feedstuff and presence of bacteria with high histidine decarboxylase activity (mainly *Enterobacteriaceae*) are the main factors affecting histamine levels. Acid pH and anaerobic conditions, often present during storage, facilitate decarboxylase activity. A limit of 100 mg histamine/kg food was suggested [9,10].

Concentrations of harmful nitrogen-containing compounds, such as nitrate and nitrite, have increased in our foods. Although nitrate ions (NO_3-) are not directly toxic, they are transformed to harmful nitrite ions (NO_2-) by bacterial nitrate reductase. In addition, nitrite can also interact with haemoglobin forming methaemoglobin by oxidation of ferrous iron (Fe^{2+}) to ferric state (Fe^{3+}) preventing or reducing the ability of blood to transport oxygen, a condition described as methaemoglobinaemia that is harmful especially in infants (so-called blue-baby syndrom). Nitrites however can have several adverse effects upon human health. For example, the in vivo reaction between nitrite and secondary or tertiary amines produces N-nitrosamines, which are potential carcinogens, mutagens and/or teratogens[11-15]. The maximum levels of residual nitrate and nitrite levels according to Turkish Food Regulations[16] are 250 mg kg^{-1} and 100 mg kg^{-1}, respectively. Therefore, widespread and frequent monitoring surveys should be carried out.

A recent study[17] conducted on Turkish style dry-sausages from 46 different brands, the mean histamine

Table 2: Nitrate and Nitrite levels of meat products from different brands

		Nitrate (mg kg^{-1})			Nitrite (mg kg^{-1})		
Meat product	n	Min	Max	Mean±SD	Min	Max	Mean±SD
Fermented sausage	53	59.91	341.66	149.31±66.2	8.2	152.1	23.49±22.5
Salami	19	76.45	298.31	124.47±62.8	20.4	104.6	45.33±25.8
Fresh sausage	16	68.57	159.60	97.84±22.8	14.40	64.02	38.80±13.6
Pastırma	12	72.91	185.61	126.73±29.4	29.33	57.33	44.55±8.10

levels in meat product were 32.13±16.5 mg kg^{-1}. In another research of Senoz *et al.*[18], the histamin concentrations were between 6.72 and 362.22 mg kg^{-1} in fermented sausages. Bozkurt and Erkmen[19] reported histamine concentrations between 0.85 and 378.29 mg kg^{-1} in Turkish dry fermented sausages. Kose[20] found the histamine content in Turkish dry fermented sausages from local markets, had a mean of 0.195 mg 100 g^{-1}. Taylor *et al.*[21] reported histamine levels between 0.50 and 0.83 mg 100 gr^{-1} in sausages. These results are parallel with our study findings. Pamukçu[22] also reported that the nitrite concentrations in pastırma, fresh sausage, fermented sausage and salami samples were 114, 288, 214,8 and 229,8 mg kg^{-1}, respectively. Furthermore, in an investigation carried by Şanlı and Kaya[23] the nitrate and nitrite concentrations in pastırma; 79.79 mg kg^{-1} and 28.62 mg kg^{-1}, salami; 214.19 mg kg^{-1} and 64.45, fresh sausage; 143.63 and 94.25 mg kg^{-1}, fermented sausage; 155.87 and 30.93 mg kg^{-1} were found.

According to the results above, Turkish style fermented sausage, salami, fresh sausage and pastırma of the different brands analyzed comply with the Regulations currently in force for histamine, nitrate and nitrite concentrations. Nevertheless, meat products samples showed a comparable good quality from a toxicological and public health point of view.

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