Meat Science 82 (2009) 179–184

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The effects of ripening period, nitrite level and heat treatment on biogenic amine formation of "sucuk" – A Turkish dry fermented sausage

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ARTICLE INFO

Article history:
Received 6 November 2008
Received in revised form 31 December 2008
Accepted 6 January 2009

Keywords:
Sucuk
Sausage
Nitrite
Biogenic amine
Heat treatment

ABSTRACT

The effects of ripening period (1–13 days), nitrite level (45–195 ppm) and heat treatment (30–90 °C) on biogenic amine formation of sucuk were investigated using the central composite rotatable design of response surface methodology.

Increasing the ripening period of sucuk caused significant increases in putrescine, cadaverine, tyramine, histamine, spermidine and spermine levels ($P < 0.01$). During the ripening period, the biogenic amines putrescine and tyramine increased the most. Increased nitrite levels caused decreases in cadaverine and tyramine. However, the effect of heat treatment on the biogenic amines was not significant ($P > 0.05$).

1. Introduction

The production conditions of sucuk, a dry fermented Turkish sausage, may cause the formation of biogenic amines such as putrescine, cadaverine, tyramine, and histamine. Biogenic amines are organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several food products, and are mainly generated by decarboxylation of the corresponding precursors (i.e., amino acids), through substrate-specific microbial enzymes. During meat fermentation, microbial growth, acidification and proteolysis provide favourable conditions for biogenic amine formation. Some biogenic amines can have negative effects on human health by causing toxicological symptoms (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000). Some of these amines, such as tyramine, putrescine and cadaverine, increased considerably in dry fermented sausages during ripening and storage. Putrescine and cadaverine react with nitrite to form carcinogenic nitrosamines, which may also cause hemoglobinaemia (Gökalp, 1984). High amounts of biogenic amines can occur in foods due to the use of poor quality raw materials, contamination, or inappropriate conditions during processing and storage (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001a; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). Therefore, biogenic amines and nitrite levels are important factors to examine during the production of dry fermented sausages, and decreasing levels of these substances is an essential part of production (Halász et al., 1994). The use of raw materials of good microbiological quality, and changes in production and ripening conditions such as the processing and ripening period, temperature, relative humidity, and hygienic medium, can prevent the accumulation of biogenic amines.

Aside from affecting the microbiological quality, the ripening period determines the physical, chemical and sensorial characteristics of sucuk (Kurt, 2006). Kurt (2006) reported that the ripening period can be shortened from 13 to 7–8 days using a heat treatment. A shortened ripening period and heat treatment may positively affect microbiological quality of sucuk, leading to the prevention of biogenic amine formation. The effects of nitrite level, ripening period and heat treatment on biogenic amine formation were evaluated using responce surface methodology.

2. Materials and methods

2.1. Reagents and standards

Dansyl chloride from Acros (Acros Organics, Belgium), and ammonia (25%), acetone, acetonitrile (HPLC grade), ammonium acetate and perchloric acid from Merck (Darmstadt, Germany) were used in the HPLC analysis. Biogenic amine standards used were 1,7-diaminoheptane (internal standard), spermidine trihydrochloride, spermine tetrahydrochloride from Sigma (St. Louis, MO), and cadaverine dihydrochloride, histamine dihydrochloride, putrescine dihydrochloride, tryptamine hydrochloride, 2-phenylethylamine hydrochloride, tyramine hydrochloride from Acros

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doi:10.1016/j.meatsci.2009.01.008

(Acros Organics, Belgium). Nitrite was used in the form of sodium nitrite (Merck, Darmstadt, Germany).

2.2. Sucuk formulation and preparation

Sucuk was prepared as follows: 84.6% meat (beef), 9.4% lamb tail fat, 1.9% salt, 0.94% garlic, 0.66% red pepper, 0.47% black pepper, 0.85% cumin, 0.24% allspice, 0.47% sugar and 0.47% phosphate (K_2HPO_4; Merck, Darmstadt, Germany). The meat and fat pieces (~4 cm^3 in size), spices, garlic, salt, sugar, and phosphate were mixed and minced in a grinder (Cem, Turkey). Starter cultures (*Lactobacillus sake, Pediococcus pentosaceus, Staphylococcus carnosus* and *Staphylococcus xylosus*; Bactoferm™; Chr. Hansen, Denmark) were added to the sucuk dough and mixed in. Sucuk dough was divided into 18 equal parts and varying amounts of nitrite, which was dissolved in 20 ml distilled water, were added to each part as shown in Table 1. Each of resulting batches of dough was rested for 12 h at 4 °C and stuffed into collagen casings (Naturin Darm, Germany) of 35 mm diameter using a filling machine (Cem, Turkey). Each sample was washed under running water, and then a 10% potassium sorbate solution was sprayed on it. Samples were ripened at 20 ± 1 °C. For equilibration, the relative humidity was adjusted to 60% in the first 6 h of the ripening period and was then increased to 87 ± 3% and decreased every day by 1 unit. At the end of the each ripening period (Table 1), sucuk samples were heated by steam. The temperature difference between the ambient and core temperatures was adjusted to 5 ± 1 °C. Ambient and core temperatures were controlled with PID (Proportional with Integral and Derivative) temperature controllers (Emko, Turkey) equipped with probes (Pt-100, Emko, Turkey). Sucuk samples were heated from 20 ± 1 °C to the required core temperatures (Table 1) and immediately cooled to 25 ± 1 °C with a cold water spray. Samples were maintained at 20 ± 1 °C for 2.5 h for equilibration and were stored at −20 ± 1 °C until analysis.

2.3. Determination of biogenic amines

The chromatographic method of Eerola, Hinkkanene, Lindfors, and Hirvi (1993) was used for the determination of tyramine, putrescine, cadaverine, histamine, 2-phenylethylamine, tryptamine, spermidine and spermine levels. Amines were separated using high performance liquid chromatography (Agilent 1100, Agilent Technologies, Germany). The separation was carried out by gradient elution with 0.1 M ammonium acetate/acetonitrile on a reverse-phase column (Spherisorb ODS-2; 5 μm, 125 x 4 mm; Waters, Waters Corporation, U.S.A) at a flow rate of 1 ml/min using a diode array detector (G1315B DAD, Agilent Technologies, Germany) at 254 nm with 550 nm as a reference.

A 4 g sample was weighed into a test tube and 250 μl internal standard (1.7 diaminoheptane) and 10 ml 0.4 M perchloric acid solution was added and homogenised (Pro260, Pro, USA). The homogenised sample was centrifuged (Universal 32R, Hettich International, Germany) for 10 min at 2400 g and rinsed with supernatant into a 25 ml bottle through filter paper. The extraction was repeated with 10 ml 0.4 M perchloric acid solution, mixed thoroughly with a Vortex mixer (Reax top, Heidolph, Germany) and centrifuged as above. Supernatants were combined and adjusted to 25 ml with 0.4 M perchloric acid solution.

The alkalinity of a 500 μl sample extract was adjusted using 200 μl 2 M sodium hydroxide solution. A 300 μl saturated sodium bicarbonate was added as a buffer. A 1 ml dansyl chloride solution (10 mg/1 ml acetone) was added and incubated at 40 °C for 45 min. Residual dansyl chloride was removed by adding 100 μl ammonia (25%). After 30 min., dansylated extract was adjusted to 5 ml with 0.1 M ammonium acetate/acetonitrile (1/1), and filtered through a 0.45 μm syringe filter (Sartorius, Germany).

Quantities of biogenic amines in the sample were calculated as:

$C_u = 250 \times RF \times (H_A / H_i) \times C_i / W_S$.

where C_u is the mg unknown/kg sample, 250 is the dilution factor, RF is the response factor, H_A is the peak height of unknown, H_i is the peak height of internal standard, C_i is the concentration of the internal standard, W_S is the weight of sample.

2.4. Statistical analysis

The experimental design and statistical analysis were performed using Jump Software (SAS Institute Inc.). The experiments were based on a central composite rotatable design with a total of 18 combinations, including four replicates of the centre point were carried out in random order. The codified and actual levels are given in Table 1. The variables were coded according to the following equation:

$$X_i = (x_i - \bar{x}_i)/\Delta x_i$$

Table 1
Central composite rotatable design of three independent variables.

Run order	Codified levels			Actual levels		
	X_1	X_2	X_3	Ripening periods (day)	Heat levels (°C)	Nitrite levels (mg kg^{-1})
1	−1.5	0	0	1	60	120
2	−1	−1	−1	3	40	70
3	−1	−1	1	3	40	170
4	−1	1	−1	3	80	70
5	−1	1	1	3	80	170
6	0	−1.5	0	7	30	120
7	0	0	−1.5	7	60	45
8	0	0	0	7	60	120
9	0	0	0	7	60	120
10	0	0	0	7	60	120
11	0	0	0	7	60	120
12	0	0	1.5	7	60	195
13	0	1.5	0	7	90	120
14	1	−1	−1	11	40	70
15	1	−1	1	11	40	170
16	1	1	−1	11	80	70
17	1	1	1	11	80	170
18	1.5	0	0	13	60	120

X_1: Codified levels of ripening period, X_2: Codified levels of heat, X_3: codified levels of nitrite.