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EFFECTS OF POPPY OIL ON BIOGENIC AMINE IN FERMENTED TURKISH SAUSAGE

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ABSTRACT

Biogenic amines are generally created by the decarboxylation of amino acids via microbial enzymes and these compounds may have a toxic effect on humans and animals. The ripening and storage conditions of meat and meat products directly affect the quality of such products in terms of biogenic amines. The aim of this study was to investigate the effects of poppy oil, which is produced and consumed in large quantities in Afyonkarahisar province, on the formation of biogenic amines in fermented sausages. In the study, 2 groups of sausages were produced including control and poppy oil groups. 300 mg / kg poppy oil was added to the sausage that would be produced in the poppy oil group. The sausages were ripened for 15 days in the surrounding where relative humidity (90-60%) and the temperature (25-18 ° C) could be adjusted. During this period (0, 2, 4, 6, 8, 10, 13 and 15 days), physico-chemical, sensory, microbiological properties and biogenic amine (histamine, putresin, tryptamine, phenyl ethylamine and tyramine) formations were determined in the samples. As a result, it is recommended that the poppy oil used does not cause a significant difference in the formation of biogenic amines, does not adversely affect the general characteristics of the sausage and that the amount of animal fat is reduced and alternatively, the use of poppy oil in the production of fermented sausage is recommended.

Keywords: Poppy oil, Biogenic amine, Fermented sausage

INTRODUCTION

Sausage is one of the most popular traditional fermented meat products produced in Turkey (**Genis and Tuncer, 2018**). Ripening process of sausage depends on many complex reactions as well as the interaction of these reactions. Chemical, biochemical and microbiological changes occur during production; furthermore these changes create the characteristic flavor of the product (**Coskuner et al., 2010**).

Biological amines formed as a result of microbial activity in food products have lower molecular weight. Almost all fermented foods contain free amino acids or proteins. Biogenic amine formation is an expected result when microbial or biochemical activity conditions are met properly (**Ten Bring et al., 1990**). Biogenic amines in foods are important for two reasons. First, the amount of biogenic amine is considered to be a quality indicator whereas the other factor is bound to be the presence of toxic effects on health. Since decarboxylase activity is increased during microbial spoilage of foods, the presence of biogenic amines is of importance because it is the indicator of food spoilage (**Bardocz, 1995**).

Major biogenic amines formed in nutrients are putresine, cadaverine, histamine, tyramine, tryptamine, 2-phenylethylamine, spermine, spermidine, methylamine, agmatine, ethylamine and ethanolamine (**Shalaby, 1996; Toy, 2010**). Biogenic amines have important biological functions in the body. They form the first step in the synthesis of protein, hormone and nucleic acid. They can also affect the body temperature balance and increase or decrease the blood pressure. Polyamines are essential for the development of all organs in the body, for the regeneration and metabolism of the cells, and for the strengthening of the immune system. However, consumption of foods containing high concentrations of these compounds may cause toxic reactions. It is very difficult to give precise limits on the toxicity of biogenic amines. Because, the type of food consumed, the amount and the presence of inhibitors with the content of the amine make it difficult to determine the limits related to the toxicity of biogenic amines (**Santos, 1996**). Typical symptoms of biogenic amine poisoning are listed as diarrhea, nausea, headache, hyper- or hypotension (**Yerlikaya and Gokoglu, 2002**).

Poppy is a single-year cultivated plant as *Papaver somniferum L* type of the Papaveraceae family in the Rhoedales family (**Anonymous, 2017**). Poppy oil has colors ranging from light yellow to dark yellow. Poppy oil is also semi-drying; it has a pleasant smell and taste (**Koc et al., 2006**). Poppy oil consists of an average of 11.0% palmitic, 0.4% palmitoleic, 1.9% stearic, 15.0% oleic, 71.3% linoleic and 0.6% linolenic fatty acids (**Atakisi, 1991**). Poppy oil is rich in Omega-6 and Omega-9 fatty acids. This oil contains high levels of vitamin E. It has antioxidant effect. It forms important building blocks of tissue cells in our body. It strengthens the immune system. Regulates blood circulation. Helps skin look younger, looks younger and regulates the functions of all skin cells (**Anonymous, 2012**). On the other hand, calorie taken with animal fat is the same as calories from other fatty acids; causes fat accumulation and weight gain in the body (**Altunkaynak et al., 2006**). It prevents the removal of low density lipoprotein (LDL, bad cholesterol) in the blood. As a result, it can cause atherosclerosis by forming deposits in the vessels (**Baysal, 2002**). Therefore, in order to avoid health problems, poppy oil may be preferred instead of animal fat.

In similar studies, the effects of various oils have been documented on of biogenic amine formation in different foods such as zahter oil (a blend of powdered thyme) (**Bozkurt, 2006**), yoghurt (**Somer, 2013**), olive (**Ozdestan et al., 2011**), fish and fish products (**Olgunoglu et al., 2009**). In this study, the effect of poppy oil on the formation of biogenic amines in fermented sausage was investigated accordingly.

MATERIAL AND METHODS

Sausage Preparation

Sausage paste was prepared by mixing 80% red meat and 20% oil with 2% salt, 0.6% sucrose sugar, 1% dried garlic, 0.7% red pepper, 0.5% powdered black pepper, 0.9% cumin, % 0,25 kg allspice (**Gokalp et al., 1999**).

The prepared sausage paste was divided into two parts: as control and poppy group. Some (additive-free) part formed one group. The other group was prepared as two different types of sausage samples by adding 300 mg / kg of poppy oil. The prepared sausages were ripened for 15 days in the relative humidity (90-60%) and the temperature (25-18 ° C).

Sampling

During the ripening phase, samples were taken from the sausage samples on the days 0, 2, 4, 6, 8, 10, 13 and 15, then their physico-chemical, sensory and microbiological properties and formation of biogenic amine (histamine, putresin, tryptamine, phenyl ethylamine and tyramine) were determined.

Microbiological Analyses

For microbiological evaluation of the samples, 90 ml of peptoned water was added to the 10g sample and the mixture homogenized. It was then diluted to 107-8 with serial dilutions. From the prepared dilutions, planting was made for total mesophilic aerobic bacteria count (TMAB) (**ISO 4833, 2003**), lactic acid bacteria count (LAB) (**Lopez-Diaz et al., 2000**) and yeast-mold count (**Pichhardt, 1993**).

Physico-Chemical Analyses

The humidity determination of the sausage samples was carried out according to **ISO 1442** (**1997**), while pH values were measured using WTW brand (Microprocessor pH meter, Germany) pH meter **ISO 2917** (**1999**), and titratable acidity was determined according to **Gokalp (1993**).

Color Measurement

Color analysis of sausage samples was realized with (B * (Brightness), r * (redness and y * (yellowness) using Minolta (CR-A70, Japan) color measurement device.

Sensory Evaluation

Sensory evaluation of the samples was performed by panelists (n=10). The panelists were trained on the quality characteristics of sausages before going into consideration. The sensory evaluation of the samples was carried out at the same time in all repeats under the fluorescent light. The panelists evaluated the color and appearance, taste and aroma, texture, and taste of sausages in terms of general taste. The panelists were given water and bread to avoid interactions between the samples. They made the evaluations using hedonic scale via points 1 - 3 (very bad - unacceptable), 4-5 (moderate), 6-7 (good), 8-9 (very good) (**Altug, 1993**).

Biogenic Amine Analysis

The levels of biogenic amines (histamine, putresine, tryptamine, phenyl ethylamine and tyramine) in the sausage samples were determined using the HPLC tool. Biogenic amine analysis in sausage samples were carried out at Mehmet Akif Ersoy University, Scientific and Technological Application and Research Center. Prepared samples were centrifuged according to the HPLC method via Shimadzu Prominence Brand adding 10 mL of 0.6 N perchloric acid to 10 g of sample and at 4000 rpm for 4 minutes at 20° C. The supernatant liquid was incubated with 100 µl 2 N NaOH, 150 µl of saturated sodium bicarbonate and 1mL of densyl chloride and then incubated at 40 ° C for 45 minutes. After keeping at room temperature for 10 minutes, 50 µl of 25% NH 3 was added and the mixture was again kept at room temperature for a further 30 minutes. 5 mL of ammonium acetate was added to it and filtered through a 0.45 µm filter, and then injected into the HPLC system.

RESULTS AND DISCUSSION

In the study putresin, histamine, tyramine, tryptamine, PEA (phenyl ethyl amine) levels determined in the sausage samples during fermentation are shown in Table 1.

Table 1 Levels of Putrescine, Histamine, Tiramine, Triptamine, PEA (phenyl ethyl amine) in the sausage samples during fermentation period

Time (day)	Putrescine (%-µg/g)		Histamine (%-µg/g)		Tyramine (%-µg/g)		Tryptamine (%-µg/g)		PEA (%-µg/g)	
	K	H	K	H	K	H	K	H	K	H
0	0.29	0.23	0.16	0.12	0.11	0.09	0.03	0.03	39.59	42.61
2	0.63	0.67	0.23	0.13	0.21	0.11	0.05	0.11	62.69	48.47
4	0.76	0.68	0.11	0.11	0.21	0.14	0.21	0.25	59.73	51.50
6	0.83	0.85	0.11	0.16	0.18	0.18	0.27	0.25	63.01	58.12
8	0.83	0.80	0.10	0.15	0.20	0.16	0.37	0.32	61.51	54.18
10	0.80	1.19	0.40	0.22	0.17	0.20	0.34	0.40	65.14	57.07
13	0.84	0.67	0.22	0.28	0.23	0.18	0.43	0.37	67.72	55.62
15	0.58	0.64	0.36	0.25	0.16	0.21	0.42	0.45	64.31	63.63

K: Control, **H:** Poppy

Putrescine levels were revealed to have increased during the fermentation in both groups, while no significant difference was observed between the groups in terms of putrescine levels. Similar studies were conducted by **Erkmen and Bozkurt** (**2004); Ten Brink et al.** (**1990**). They found the amount of putrescine as 0.0-918.9 mg / kg and 1-190 mg / kg. Regarding the effect of the use of starter on the amount of putresine, many researchers reported that the addition of starter reduced the putresin formation, but did not prevent the formation (**Roig-Sogues and Eerola, 1997; Hernandez-Jover et al., 1997; Ayhan et al., 1999; Bover-Cid et al., 2000**).

At the end of the analysis in the histamine levels; the poppy group was detected to be at a lower level than the control group. In their study, **Ten Brink et al.** (**1990**), determined the amount of histamine in fermented sausages as between 1-63 mg / kg. On the other hand, **Buncic et al.** (**1993**), revealed 0.3% GDL, starter culture (*Lactobacillus plantarum*) and control group of sausage histamine concentration as 19, 41, 17, 20 and 18.64 mg / kg respectively. In their studies on the samples of histamine **Ekici et al.** (**2004**); **Erkmen and Bozkurt** (**2004**); **Bozkurt and Erkmen** (**2004**), reported the histamine levels to be 19.64-87.47, 1.5-478.2 and 0.0-242.2 mg / kg.

For tyramine levels; no significant changes were observed in the control group; the tyramine level was found to have increased during the fermentation in the poppy group. Similar studies were performed by **Senoz et al.** (**2000**); **Erkmen and Bozkurt** (**2004**), and they found that the amount of tyramine in sausage samples ranged between 125–1173.28 and 1.2- 316.3 mg / kg, respectively. In the study of **Ten Brink et al.** (**1990**), the amount of tyramine was found to be 40-310 mg / kg in fermented sausages. **Coisson et al.** (**2004**), detected that the most important biogenic amine in fermented dry sausage was tyramine (372 mg / kg). According to the results of the current study, tyramine was found to be the most important biogenic amine formed in the sausage, but other researchers found the amount to be lower (**Ayhan et al., 1999; Senoz et al., 2000; Bozkurt and Erkmen, 2002; Colak and Ugur, 2002; Erkmen and Bozkurt, 2004**).

Triptamine levels of sausage samples in both groups were determined to have increased during the fermentation; no significant difference was observed between the two groups in terms of tryptamine levels. **Eitenmiller et al.** (**1978**), revealed that tryptamine in starter cultured samples was formed in lower amounts than the control group. **Bover-Cid et al.** (**2000, 2001a**), regarding with amine negative *Lactobacillus sakei and Staphylococcus spp*. detected that tyramine, cadaverine and putresine formation in fermented soes decreased and other amines did not form histamine, phenyl ethylamine and tryptamine. **Bozkurt and Erkmen** (**2004**), pointed out that tryptamine was not available in the samples at the beginning of ripening, and that the highest amount was found in the control group, whereas tryptamine formed in lower amounts in the starter cultured samples when compared to the control group.

PEA levels of the samples were detected to have increased during the fermentation in both groups. In terms of PEA levels, the poppy group was found to be lower when compared to the control group. Similar studies conducted by **Ayhan et al.** (**1999**), did not detect phenyl ethylamine in any of the sausage samples in their study. **Bozkurt and Erkmen** (**2002**), examined the effects of starter culture besides the use of different amounts of chemicals on biogenic amine formation and reported that phenyl ethylamine was not available in the samples. On their research of fermented sausages produced by adding sulfide, **Bover-Cid et al.** (**2001b**), determined that the tryptamine and phenyl ethylamine were not formed during the ripening period. **Bover-Cid et al.** (**2001c**), also found that phenyl ethylamine was created only in the sausages that were produced in the natural flora. **Bover-Cid et al.** (**2000, 2001a**), reported that the amine-negative *Lactobacillus sakei and Lactobacillus curvatus* did not produce phenyl ethylamine. **Ansorena et al.** (**2002**), stated that *Staphylococcus carnosus* produced phenyl ethylamine. **Ten Brink et al.** (**1990**), determined the amount of phenyl ethylamine in fermented sausages ranging between 5-45 mg / kg. **Bozkurt and Erkmen** (**2004**), expressed that in many cases phenyl ethylamine was absent while in the determined samples, the amount was less than 30 mg / kg.

In this study, total aerob mesophilic bacteria (TAMB), lactic acid bacteria and yeast / mold results determined in the sausage samples during fermentation are shown in Table 2. Bacterial numbers of TAMB and Lactic acid increased during the fermentation period. The yeast / mold levels of the poppy samples were found to be approximately 1 log lower than the control group levels.

Table 2 Microbiological Analysis Findings

Time (day)	Groups	TAMB	LAB	Yeast/Mold
0	K	6.45	4.76	3.25
	H	6.05	4.69	3.24
2	K	6.75	5.27	4.30
	H	6.33	5.21	3.50
4	K	7.31	5.63	4.45
	H	6.45	5.69	3.40
6	K	7.50	6.49	4.38
	H	7.06	6.78	3.60
8	K	7.54	6.73	4.68
	H	7.51	6.80	4.10
10	K	7.60	7.25	5.08
	H	7.63	7.45	4.20
13	K	7.68	7.30	6.78
	H	7.60	7.42	4.12
15	K	7.56	7.65	4.50
	H	7.60	7.24	3.90

TAMB: Total Aerobic Mesophilic Bacteria, LAB: Lactic Acid Bacteria, K: Control, H: Poppy

The pH, dry matter and acidity (% LA) results of sausage samples during the fermentation period are shown in Table 3. In the course of the whole ripening, the pH generally did not fall below 5.0 and there was no significant difference between the two groups. In terms of dry matter and acidity values, an increase was detected during fermentation, however no significant difference was found between the two groups.

Table 3 Chemical Analysis Findings

Time (day)	Groups	Ph	Dyr Matter (%)	Acidity (%LA)
0	K	5.64	41.21	0.71
	H	5.65	41.21	0.71
2	K	5.31	44.20	0.82
	H	5.31	44.22	0.83
4	K	5.17	48.11	0.87
	H	5.11	48.16	0.87
6	K	5.17	52.51	0.93
	H	5.17	53.15	0.96
8	K	5.17	53.63	1.35
	H	5.11	54.15	1.36
10	K	5.07	57.31	2.10
	H	4.97	58.20	2.10
13	K	5.00	58.23	2.15
	H	4.90	58.35	2.15
15	K	5.00	60.49	2.19
	H	4.95	61.91	2.18

K: Control, H: Poppy, LA: Lactic Acid

In the study, during the fermentation period, no significant change was observed in the r-redness and y-yellowness values in the color measurement findings in the sausage samples; on the other hand, b-brightness was found to be higher in the poppy group compared to the control group.

Table 4 Color Analysis Findings

Groups	L	a	b
K	43.76	25.10	18.16
H	44.19	25.15	18.55

K: Control, H: Poppy, L: lightness, a: redness, b: yellowness

The sensory results of the study detected in the sausage samples during fermentation are shown in Table 5. In terms of the sensory values, the control group received higher evaluation scores compared to the poppy group in terms of appearance, bad smell and general taste, while there was no significant difference between the two groups in color and texture values.

Table 5 Sensory Analysis Findings

Groups	Apperance	Bad Smell	Taste	Colour	Texture	General
K	8.17	8.33	7.50	7.83	7.50	8.17
H	7.50	8.17	7.17	7.83	7.50	7.50

K: Control, H: Poppy

CONCLUSION

As a result, the poppy oil used in the ratio specified in the fermented sausage production did not adversely affect the general characteristics of the sausage. Poppy oil use was also revealed to have reduced histamine formation; microbiologically reduced the number of yeast molds by 1 log via suppressing the proliferation. The amount of animal oil should be reduced, while the use of poppy oil is recommended alternatively in fermented sausage productions.

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