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THE EFFECTS OF MICROENCAPSULATED *L. RHAMNOSUS* AND STORAGE ON BIOGENIC AMINE AMOUNT OF SUCUK

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ABSTRACT
In this study, the effect of microencapsulated *L. rhamnosus* was investigated on biogenic amine amounts in sucuk during the 6 months storage. Traditional and probiotic sucuk production were occurred with *L. plantarum* and *L. rhamnosus*. Probiotic sucuk was produced with free or microencapsulated *L. rhamnosus*. Microencapsulation protected survival of *L. rhamnosus* in sucuk. It was observed that biogenic amine amounts of sucuk increased with storage. Putrescine, cadaverine, histamine, tyramine and tryptamine were detected as the most abundant biogenic amines in sucuk. It was also determined that addition of *L. rhamnosus* plus *L. plantarum* were reduced effectively biogenic amine amounts in sucuk samples. Total biogenic amine amounts in sucuk without probiotic *L. rhamnosus* was found higher than in sucuk with probiotic *L. rhamnosus*. In conclusion, short storage period and the use of probiotic culture are recommended for the production of sucuk with low biogenic amine level.
Keywords: Biogenic amines, microencapsulation, probiotic, sucuk

SUCUĞUN BİYOJEN AMİN MİKTARI ÜZERİNE DEPOLAMANIN VE MİKROENKAPSÜLE *L. RHAMNOSUS*'UN ETKİSİ

ÖZ
Bu çalışmada 6 aylık depolama periyodu sırasında sucuktaki biyojen amin miktarı üzerinde depolamanın ve mikroenkapsüle *L. rhamnosus*'un etkisi araştırılmıştır. Geleneksel ve probiyotik sucuk üretimi *L. plantarum* ve *L. rhamnosus* ile gerçekleştirilmiştir. Probiyotik sucuk, serbest ve mikroenkapsüle *L. rhamnosus* ile üretilmiştir. Mikroenkapsülasyon işlemi sucuktaki *L. rhamnosus*'un canlılığını korumuştur. Sucuktaki biyojen amin miktarının depolamayla birlikte arttığı gözlenmiştir. Putresin, kadaverin, histamin, tiramin ve triptamin'in sucukta en bol bulunan biyojen aminler olduğu ortaya çıkarılmıştır. Ayrıca *L. plantarum*'a ilave olarak *L. rhamnosus* ilavesinin sucuk örneklerindeki biyojen amin miktarlarını etkin bir şekilde azalttığı belirlenmiştir. Probiyotik *L. rhamnosus* bulunmayan geleneksel sucuklardaki toplam biyojen amin miktarı *L. rhamnosus*'lu probiyotik sucuklardan daha yüksek bulunmuştur. Sonuç olarak, kısa depolama süresi ve probiyotik kültür kullanımı düşük biyojen amin seviyeli sucuk üretimi için tavsiye edilmektedir.
Anahtar sözcükler: Biyojen aminler, mikroenkapsülasyon, probiyotik, sucuk

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INTRODUCTION

Fermented meat products could be suitable vehicle for probiotics. Especially, fermented dry sausages containing probiotic are attracted to consumers because of both their functional properties and dietary composition with high protein content (Rubio et al., 2013; Cavalheiro et al., 2015; Wang et al., 2015). In this regard, a number of studies were conducted on probiotic fermented sausages. *Lactobacillus* strains were mostly used as probiotic cultures in fermented sausages. However these strains suffer from adverse conditions such as low pH and water activity, curing agents, competitive microorganisms, spices and other ingredients in fermented sausages (De Prisco and Mauriello, 2016). Microencapsulation technique has been emerged and developed to protect probiotics against these stress factors in various food products (Champagne and Fustier, 2007; Kalkan et al., 2018). However, there are very few studies about application of microencapsulation in fermented meat products (Cavalheiro et al., 2015).

Sucuk known as a typical Turkish dry-fermented sausage is the most popular and widely consumed meat product in Turkey (Özdemir, 1999; Genccelep et al., 2008). The safety of fermented meat products is a major concern for industry and consumer (Wang et al., 2015). For example some biogenic amines can have undesirable effect on human health due to their toxicity properties (Kongkiattikajorn, 2015; Lu et al., 2015). The microorganisms from fermentation process may contribute to biogenic amines accumulation and their activities vary according to species and strain (Jairath et al., 2015; Kongkiattikajorn, 2015). The choice of suitable starter culture (amine negative starter culture) prevents the formation of high levels of biogenic amine in fermented sausages (Xie et al., 2015). Lactic acid bacteria are widely used in fermented sausages as starter or probiotic culture and inhibit undesired amine positive microorganisms to improve the quality and safety of final product because of their rapid acidification ability (Lu et al., 2015). In Turkey, recently there have been a few studies about probiotic sucuk, while no reports are available on the study of sucuk production with encapsulated probiotic strains. For example, sucuk manufacture with the use of free probiotic cultures such as *L. plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* was achieved by some researchers in Turkey (Kaya and Aksu, 2005; Ergönül and Kundakçı, 2011; Bağdatlı and Kundakçı, 2016; Özer et al., 2016). On the other hand, in other countries, works based on fermented sausage with microencapsulated or free probiotic strains were conducted by some of previous researchers (Muthukumarasamy and Holley, 2006; De Vuyst et al., 2008; Espinoza and Navarro, 2010; Sidira et al., 2014).

In this study, sucuk samples obtained by microencapsulated and free *L. rhamnosus* were compared in terms of their biogenic amine contents.

MATERIALS AND METHODS

Starter culture and probiotic culture used in the present study were *L. plantarum* (Blessing-Biotech GmbH-Stuttgart/Germany) and *L. rhamnosus* (Danisco USA INC.), respectively. Prebiotic (fructooligosaccharide), growth promoter (peptide), sodium alginate, gellan gum and gelatin were coating materials applied in microencapsulation.

Microencapsulation of Probiotic Strain

Incorporation of probiotic strain *(L. rhamnosus)* was carried out with 1.89% sodium alginate, 0.96% gellan gum, 0.15% gelatine, 1% peptide and 1.45% fructooligosaccharides by extrusion method based on rate of optimal coating material combination in terms of probiotic survival found by Unal (2014). Also, microencapsulation technique practiced by Chen et al. (2007) was performed in this study.

Probiotic Sucuk Production

For the manufacturing of probiotic sucuk, the method of previous researchers was followed with a bit modification (Kaban and Kaya, 2009). Sucuk productions were occurred with 3 varied culture combinations (A: Control sample containing free *L. plantarum*, B: Sucuk sample containing free *L. rhamnosus* and free *L. plantarum*, C: Sucuk sample containing microencapsulated *L. rhamnosus* and free *L. plantarum*). Detailed information related to probiotic sucuk production with microencapsulated and free cells was given in previous paper of the present author (Ünal Turhan et al., 2017). The sucuk samples were vacuum packaged and stored at 10-15°C for 6 months.

Sampling and Sample Preparation

From three replications, sucuk samples were taken on the 0, 2, 4 and 6 months of storage. Biogenic amine amounts, *L. plantarum* and *L. rhamnosus* counts were analyzed. All analyses were carried out in duplicates.

Biogenic Amine Analysis

a) Reagents: The water used in the study was purified by a Millipore-Q system (Millipore Corp., Saint-Quentin, France). 1.7 diaminoheptan, perchloric acid, NaOH, $NaHCO_3$, dansyl chloride, ammonium acetate, acetonitrile and amine standard solutions including histamine, tryptamine, tyramine, putrescine, 2-phenylethylamine, cadaverine, spermidine and spermine were purchased from Sigma Chemical Co. (USA).

b) Extraction and Quantification: Biogenic amine levels of the samples were determined according to the method of Eerola et al. (1993) and Gençcelep et al. (2008) with slight modification. Amine standard solutions (1 mg/mL) were prepared in 0.4 M perchloric acid. Working solutions (0.0, 2.0, 5.0, 10 and 20 mg/mL) of each amine standard solution were prepared in 0.4 M perchloric acid and stored at 4°C. For the extraction of biogenic amines 2.0 g samples were mixed with 0.4 M perchloric acid and the detection of biogenic was carried out as their dansyl derivatives by HPLC. Elution solvents were 0.1 M ammonium acetate (solvent A) and acetonitrile (solvent B). The gradient elution was programmed as starting at 50% solvent B and ending at 90% solvent B in 25 min. The equilibration of the system was last for 10 min before following analysis. 20 µL of derivatized biogenic amine solution was injected onto the column in the HPLC apparatus. The column was termostated at 30°C and flux was set at 1.0 mL/min.

The detection of peaks were carried out at 254 nm using the HPLC system with a column nucleodur 100-5-C18 (12.5 X 4 mm) and a gradient pump. This instrumental system consists HPLC (Shimadzu LC-20AT, Japon), quaternary pomp (G1311A), vacum degasser (G1322A), auto sampler (G1313A), Diode Array Detector (DAD, SPD-M20A), variable wavelength (G1314A) and a computer with the Agilent package program. The quantitative determinations were performed according to internal standard (1.7-diaminoheptane) method, utilizing peak heights. Biogenic amine amounts were given in mg/kg.

Statistical Analysis

Data obtained from the present study were represented as averages of triplicate data with their standard deviations. Statistical analysis was performed according to one-way ANOVA. Duncan's multiple test also was applied to detect the significance or insignificance of differences among averages ($P < 0.05$) based on "Windows SPSS 15 Software" (Bek and Efe, 1988).

RESULT AND DISCUSSION

Table 1 represented biogenic amine amounts found in sucuk samples during storage. Total biogenic amine amounts changed from 360.68 mg/kg to 1378.35 mg/kg.

The maximum threshold of total biogenic amines that has been considered dangerous from human health was reported as 1000 mg/kg by Dominguez et al. (2016). Total biogenic amine amounts of A, B and C samples in 6 months of storage and of A and C samples in 4 month of storage exceeded this dangerous limit. As seen from results, there were significant differences in biogenic amines amounts of sucuk. In accordance with our study, previous researchers stated that the main biogenic amines in fermented sausages and sucuk were putrescine, cadaverine, histamine, tyramine and tryptamine (Gençcelep et al., 2007; Gençcelep et al., 2008; Kurt and Zorba, 2009; Lu et al., 2015; Xie et al., 2015). Putrescine was biogenic amine with the highest amount in sucuk samples whereas phenylethylamine and spermidine amounts in sucuk samples were found at low levels. Similar to the present results, in the paper of Ergönül and Kundakçı (2011) putrescine content (ranged from 3.94 to 35.48 ppm) in probiotic sucuk samples was found higher than other biogenic amines such as cadeverine, histamine, tyramine (between 3.90 and 18.50 ppm).

Table 1. Biogenic amine amounts in sucuk samples during the storage (mg/kg)

	A-0	B-0	C-0	A-2	B-2	C-2	A-4	B-4	C-4	A-6	B-6	C-6
Tryptamine	27.98^{a}	8.99^{c}	10.06^{b}	43.55^{ab}	28.87^{b}	15.57^{b}	57.39^{a}	37.7^{ab}	53.19^{a}	61.66^{a}	52.24^{a}	56.09^{a}
Phenyl-ethylamine	10.28^{c}	1.47^{b}	1.03^{bc}	14.66^{bc}	3.02^{ab}	0.74^{c}	18.89^{b}	4.99^{a}	3.83^{ab}	25.14^{a}	4.99^{a}	5.40^{a}
Putrescine	260.57^{d}	177.92^{c}	165.07^{d}	404.41^{c}	314.48^{b}	334.21^{c}	447.49^{b}	338.38^{b}	399.89^{b}	535.13^{a}	522.57^{a}	510.16^{a}
Cadaverine	60.59^{c}	16.29^{c}	8.96^{c}	63.98^{c}	21.67^{c}	12.83^{c}	156.30^{a}	93.29^{b}	91.17^{b}	121.96^{b}	169.27^{a}	166.84^{a}
Histamine	115.95^{d}	84.01^{d}	54.62^{d}	145.72^{c}	110.76^{c}	113.31^{c}	270.34^{a}	207.77^{b}	283.92^{b}	232.31^{b}	401.90^{a}	358.78^{a}
Tyramine	121.02^{d}	78.88^{c}	82.40^{c}	211.69^{c}	158.25^{b}	180.75^{b}	259.69^{b}	164.58^{b}	192.95^{b}	312.16^{a}	236.27^{a}	238.12^{a}
Spermidine	5.42^{b}	4.75^{c}	5.77^{b}	4.75^{b}	6.85^{a}	7.97^{a}	6.54^{b}	5.94^{ab}	5.31^{b}	10.06^{a}	5.38^{c}	5.99^{b}
Spermine	33.83^{a}	28.63^{c}	32.77^{b}	28.23^{a}	30.57^{c}	27.90^{c}	24.20^{a}	37.71^{a}	29.99^{b}	28.61^{a}	32.54^{b}	36.97^{a}
Total Amounts	635.64	400.94	360.68	916.99	674.47	693.28	1240.84	890.36	1060.25	1327.03	1425.16	1378.35

a, b, c: values with different letters are significantly different ($P < 0.05$), A: Control sample containing free *L. plantarum*, B: Sucuk sample containing free *L. rhamnosus* and free *L. plantarum*, C: Sucuk sample containing microencapsulated *L. rhamnosus* and free *L. plantarum*, 0: The beginning of storage, 2: 2nd month of storage, 4: 4th month of storage, 6: 6th month of storage

In another study, Gençcelep et al. (2008) reported that the most important biogenic amines were tyramine (maximum 676 mg/kg) and putrescine (maximum 364 mg/kg). Spermidine amounts and spermine in sucuk samples ranged from 4.75 to 10.06 mg/kg and from 24.20 to 36.97 mg/kg within the acceptable level. Spermine and spermidine in fermented sausages are the main biogenic amines coming from raw material and their amounts rarely change during the fermentation and storage (Lu et al., 2015). Similarly spermine and spermidine values in sucuk samples did not undergo large changes during the whole storage period.

Quality characteristics of foods could change with storage period (Loizzo et al., 2016). To this regard, we followed the evolution over 6 months of biogenic amine amounts (Table 1). Storage period affected significantly ($P < 0.05$) biogenic amine amounts in sucuk samples (A, B, C), with the exception of spermine in A sample. Results of this study showed that total biogenic amine concentrations at the beginning of storage were low (635.64 mg/kg for A sample, 400.94 mg/kg for B sample and 360.68 mg/kg for C sample) and after 6 months of storage these values reached 1327.03 mg/kg, 1425.16 mg/kg and 1378.35 mg/kg, respectively. Similar to the present results, Xie et al. (2015) reported that total biogenic amine amounts in fermented sausages with *L. plantarum* as starter culture were approximately 1000 mg/kg. Biogenic amine amounts of sucuk samples mostly increased with the storage, and especially a significant increase was seen in level of putrescine, cadaverine, histamine and tyramine in accordance. These results are also in agreement with Loizzo et al. (2016) who reported that biogenic amine concentrations increased with storage time and tyramine, putrescine and cadaverine were the most abundant. Similarly, Bozkurt and Erkmen (2004) stated that storage time significantly affected biogenic amine amount. Additionally total biogenic amine amounts in A sucuk sample were found higher than B and C sample in 0, 2 and 4 months of storage except for 6 month of storage. In 6 month of storage total biogenic amine amounts in A sample showed a drop due to increase of cadaverine and histamine amount in B and C sample. During the first 4 month of storage, presence of probiotic culture in B and C sample showed a good effect in reducing the accumulation of biogenic amine. In the study of Ergönül and Kundakçı (2011) while the highest biogenic amine accumulation in probiotic sucuk samples was observed in the 4 months of storage period, there was no significant change in biogenic amine amounts of sucuk samples from 4 to 8 months of storage period.

Starter cultures are used to control the fermentation process and to provide standardized sucuk production having properties of favorable quality. During the formation of biogenic amine in sucuk, fermentative cultures influence the decarboxylase activity of microorganisms producing biogenic amine (Gücükoğlu and Küplülü, 2010; Song et al., 2018). Similarly, in the present study, culture combination had significantly effect on biogenic amine amounts in sucuk samples. Especially, a high correlation among biogenic amine amount and probiotic content was observed. As seen from results, formation of biogenic amine in probiotic sucuk samples (B and C) were found lower than A samples. This situation was considered that prevalent of probiotic culture in sucuk prevented formation of biogenic amine. Similarly, some researchers reported that probiotics caused to drop in formation of biogenic amine (Erkkila et al., 2001). For instance, in the study of Ergönül and Kundakçı (2011), putrescine amount (13.38 ppm) in sucuk containing probiotic *L. casei* was found lower than with *L. acidophilus* (35.48 ppm). The present study revealed that the type and counts of microbial cultures (probiotic and starter) affected biogenic amine amounts in sucuk samples as highlighted in the paper of Genccelep et al. (2007). As a matter of fact B and C samples containing both starter and probiotic culture had lower biogenic amine amounts than A sample and this results confirmed the literature. In summary the production of biogenic amine was limited by probiotic culture. Many researchers reported that fermentation with appropriate starter cultures or mixed cultures is effective approach to reduce biogenic amine formation during fermented sausages production (Kongkiattikajorn, 2015; Wang et al., 2015; Xie et al., 2015; Dominguez et al., 2016; Kantachote et al., 2016; Sun et al., 2016). Sun et al. (2016) reported that the lowest amounts of biogenic amines were observed in sausages inoculated with the bacterial mixture including *Staphylococcus xylosus* and *Lactobacillus plantarum*. Similarly, addition of *L. rhamnosus* as well as *L. plantarum* in the production of sausages reduced biogenic amine formation.

As regards to effect of microencapsulation on biogenic amine amounts, in general it was observed that there were no significantly differences among sucuk productions with microencapsulated *L. rhamnosus* or free *L. rhamnosus*. Thus obvious relation between microencapsulation and biogenic amine amount could not be found in B and C samples. Similarly, Song et al. (2018) reported that there were no statistical differences between biogenic amine amounts in probiotic sausages containing microencapsulated or free *B. longum*.

CONCLUSION

Culture combination and storage period affected some biogenic amine amounts. Biogenic amine amounts in sucuk samples mostly increased with storage. Sucuk samples containing probiotic culture (B and C sample) had lower amounts of biogenic amine than A sample. These results confirmed that probiotic cultures in sucuk prevented biogenic amine formation and biogenic amine amounts were negatively correlated with probiotic counts. Probiotic *L. rhamnosus* could be considered as candidates for a mixed starter culture in sucuk production to inhibit biogenic amine formation. Probiotic sucuk in the present research could contribute to the variety of probiotic functional products and safely be consumed because of positive effect of probiotic on health and protective properties of probiotic. Probiotic sucuk will possibly contribute to probiotic product diversity.

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