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Effects of *in situ* exopolysaccharide production and fermentation conditions on physicochemical, microbiological, textural and microstructural properties of Turkish-type fermented sausage (sucuk)

Enes Dertli [a], Mustafa Tahsin Yilmaz [b,*], Nevruz Berna Tatlisu [c], Omer Said Toker [a], Hasan Cankurt [d], Osman Sagdic [a]

[a] Bayburt University, Engineering Faculty, Food Engineering Department, 69000 Bayburt, Turkey
[b] Yıldız Technical University, Chemical and Metallurgical Engineering Faculty, Food Engineering Department, 34210, İstanbul, Turkey
[c] İstanbul Gelişim University, Vocational School, Food Technology Division, 34310•Istanbul, Turkey
[d] Erciyes University, Safiye Cikrikcioglu Vocational School, Food Science Division, Kayseri, Turkey

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ABSTRACT

In this work, the role of *in situ* exopolysaccharide (EPS) production under different fermentation conditions on physicochemical, microbiological, textural and microstructural properties of sucuk was determined. For this purpose, the effect of EPS producing strains (control, strain 1, strain 2 and mixture) and fermentation conditions (fermentation temperature; 14, 16 and 18 °C and time; 8, 12 and 16 days) on physicochemical, microbiological, textural and microstructural properties were investigated using response surface methodology. *In situ* EPS production was observed to remarkably affect these properties while fermentation conditions were also seen to dominantly influence the physicochemical properties of sucuk, revealing that the ripening temperature appeared to be more determinant factor. EPS producing cultures enhanced the textural properties of sucuk which became harder, less adhesive and tougher. The microstructural analysis revealed the formation of web-like structure by *in situ* EPS production in sucuk mix during fermentation process. This study revealed the importance of *in situ* EPS production on final technological properties of sucuk.

1. Introduction

Meat fermentation results in significant alterations in taste, aroma, sensorial characteristics and shelf life of raw meat due to the biochemical and physical reactions mainly conducted by Lactic Acid Bacteria (LAB) (Rantsiou & Cocolin, 2008). Fermented meat products are popular in all over the world and sucuk as the Turkish style fermented sausage is the most consumed fermented meat product in Turkey (Bozkurt & Erkmen, 2002a). Although traditionally sucuk was produced from lamb or beef with the addition of tail fat, salt and spices without the addition of chemical additives such as nitrate, nitrite, ascorbic acid and importantly starter cultures under uncontrolled conditions, recently this traditional way was modernized in most plants and a good proportion of sucuk is now produced with the preparation of sucuk mix with lamb and beef with tail fat including antimicrobials, antioxidants, starter culture mixture and species under controlled atmospheric conditions (temperature and % RH) (Bozkurt & Bayram, 2006). Following the preparation of sucuk mix, sucuk dough is filled into artificial casing and then fermentation occurs under controlled temperature for a certain period (Bozkurt & Erkmen, 2002a). The color, texture, flavor and odor are among the most important technological properties of sucuk. These properties are directly influenced by several factors such as fermentation conditions (*e.g.* fermentation temperature and duration) and use of starter cultures due to their antimicrobial effects and exopolysaccharide (EPS) production characteristics (Bozkurt & Erkmen, 2002a; Rantsiou & Cocolin, 2008).

LAB are capable of producing EPS that may be attached to the bacterial cell wall or directly secreted to the environment (Dertli et al., 2013). EPS have unique characteristics because of the differences in the sugar subunits and glycosidic linkages present in their repeating units, which explain the great diversity among bacterial EPS and novel EPS structures (De Vuyst & Degeest, 1999; Dertli et al., 2013). EPS have crucial roles in physicochemical and textural properties of fermented food products especially dairy products as natural bio-thickening agents and *in situ* produced stabilizers (Duboc & Mollet, 2001). In addition to their structural properties the level of EPS produced by LAB plays crucial roles in technological functions of these natural polymers. Several intrinsic and extrinsic factors can affect the EPS production levels and fermentation temperature and time are important extrinsic factors determining the level of EPS production in LAB (De Vuyst & Degeest, 1999). For

* Corresponding author.
E-mail address: mtyilmaz@yildiz.edu.tr (M.T. Yilmaz).

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this reason understanding the EPS production characteristics of LAB strains under fermentation conditions is crucial to obtain the expected benefits from these polymers. Although an important number of studies were conducted to understand the functional characteristics of EPS produced by LAB strains in fermented dairy products, to the best of our knowledge, no study uncovering the functional role of EPS production in fermented meat products such as sucuk has appeared so far.

In our study, we aim to develop an EPS based fermented sucuk by fermentation at the specified fermentation temperatures and times in strain-specific conditions. Therefore, the aims of this research were to investigate the role of *in situ* exopolysaccharide (EPS) production by EPS$^+$ *Lactobacillus plantarum* and *Leuconostoc mesenteroides* strains under certain fermentation conditions (incubation temperatures, 14–18 °C and times; 8–16 h) on *in situ* EPS production levels and textural properties of sucuk by employing response surface methodology and to determine the functions of *in situ* EPS production on physicochemical, microbiological, textural and microstructural properties of sucuk.

2. Materials and methods

2.1. Bacterial strains

For preparation of sucuk samples, EPS$^+$ strains were used. In this study, the sucuk samples were produced and investigated as four different treatment groups, and will be referred as following throughout the manuscript:

(a) **Control group**: Control sucuk samples produced without LAB addition, with natural flora.
(b) **Strain 1 group**: Sucuk samples produced by using Strain 1 (EPS$^+$ *Lactobacillus plantarum* 162 R strain),
(c) **Strain 2 group**: Sucuk samples produced by using Strain 2 (EPS$^+$ *Leuconostoc mesenteroides* N6),
(d) **Mixture group**: Sucuk samples produced by using mixture of Strain 1 and Strain 2.

All strains were incubated in 10% reconstituted skim milk and stored at −70 °C until further use. The stock cultures were activated in MRS medium at 37 °C for 24 h and following another propagation in MRS medium all strains were inoculated to the sucuk mix at 1% concentration for the fermentation process.

2.2. Sausage manufacturing

Fresh, boneless beef cuts (from middle-aged cows) with approximately 14% fat and sheep tail fats were obtained from a retail market (İstanbul, Turkey). Controlled fermentation process was achieved by using the aforementioned bacterial strains. A fermentation cabinet in which the desired temperature, relative humidity and air circulation could be achieved was used in this study in order to provide the controllable fermentation conditions during the ripening period of the sausages.

The sucuk samples were produced according to a general method used in Turkish sausage manufacturing plants. The sausage formulation and spice mixture used as outlined (Gokalp et al., 1997) was comprised of 90% beef, 10% tail fat, 2% salt, 1% garlic, 0.7% red pepper, 0.5% powdered black pepper, 0.9% cumin, 0.25% allspice (Bağdat Baharat, Turkey). In sucuk production process, beef meat and tail fat were cut in small pieces, mixed with the respective spice mixture in the mixer and the mix was kept in a refrigerator at 4(±1) °C for 24 h. Table 1 shows the physicochemical and microbiological properties of beef meat and tail fat as well as final numbers of bacterial strains in the sucuk mix. The mix was separated into four groups each of which was inoculated with the respective bacterial culture mentioned above at 1% level and then each mix group was further kept for 30 h. Each mix group (control, strain 1, strain 2 and mixture groups) was separately ground through a grinder machine. Then, each batch was immediately stuffed into 36-mm-diameter collagen casings (Yıldızsa Co., Istanbul, Turkey) by the grinder machine (6.5 kg capacity, Cem Brand, Istanbul, Turkey). The prepared sausage batons were rinsed with water and then air bubbles emerged on the surface of batons were removed using a pin. Then, sausage batons were separated into 10 experimental batches and fermented according to experimental runs (R1–R10) each of which represents different processing conditions; namely, different fermentation conditions (fermentation temperature and time levels presented in Table 2). The limits of fermentation conditions were chosen, considering those applied for Turkish type fermented sucuk. For this purpose, the batons were placed in the fermentation cabinet and ripened at respective temperature levels and for respective days. At the first 2 days, the relative humidity (RH) and air circulation velocity was 90% and 0.5 m/s, respectively. At the third, fourth and fifth days, these values were 85% and 0.5 m/s, respectively. The following days, the RH was decreased to 80% RH. Treatments were repeated two times at the batch level.

Table 1
Physicochemical and microbiological properties of formulation components of sucuk samples.

	Physicochemical and microbiological properties			
Formulation components	Dry matter (%)	Protein (%)	Fat (%)	MRS (log CFU/g)
Meat	22.35 ± 0.86	20.06 ± 0.41	0.96 ± 0.02	1.4 ± 0.02
Tail fat	85.96 ± 2.3	2.35 ± 0.01	83.1 ± 2.7	–
Strain 1	–	–	–	7.5 ± 0.3
Strain 2	–	–	–	7.8 ± 0.4

2.3. Physicochemical and microbiological analysis

The physicochemical analyses were conducted by following the instructions, as outlined (Gokalp et al., 1997). The pH values of the samples were measured with a pH meter (WTW, 315İ, Germany). Dry matter (g dry matter/100 g sample) was determined by drying a 10-g sample at 105 °C to a constant weight. Fat content (g fat/100 g sample) was determined by using a Soxhelet fat extraction apparatus. Protein (g protein/100 g sample) was analyzed according to the Kjeldahl method. Factor 6.25 was used for the conversion of nitrogen to crude protein.

For the microbiological analysis of sucuk samples, serial dilutions were prepared, plated onto MRS (Merck, Germany) agar plates and incubated at 37 °C for 48–72 h for enumeration of *L. plantarum* and *L. mesenteroides* counts (De Man, Rogosa, & Sharpe, 1960; Terzaghi &

Table 2
The design matrix indicating the levels of coded and actual values for the experimental factors.

	Coded levels of factors		Actual levels of factors	
Runs	Fermentation temperature (°C)	Fermentation time (day)	Fermentation temperature (°C)	Fermentation time (day)
R1	−1.0	−1.0	14	8
R2	−1.0	0.0	14	12
R3	−1.0	1.0	14	16
R4	0.0	−1.0	16	8
R5	0.0	0.0	16	12
R6	0.0	0.0	16	12
R7	0.0	1.0	16	16
R8	1.0	−1.0	18	8
R9	1.0	0.0	18	12
R10	1.0	1.0	18	16