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Design of probiotic dry fermented sausage (sucuk) production with microencapsulated and free cells of *Lactobacillus rhamnosus*

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Abstract: The present study was performed to design sucuk production with microencapsulated and free cells of *Lactobacillus rhamnosus* and to investigate the effect of this production method on probiotic viability and quality characteristics on the final product. *L. rhamnosus* was microencapsulated with an optimal coating material combination by using response surface methodology and subsequently used in sucuk production as a probiotic culture. Microencapsulation protected *L. rhamnosus* against gastric and other stress conditions in sucuk composition. Sucuk production with microencapsulated *L. rhamnosus* was found to be similar to traditional sucuk in terms of textural, physicochemical, and sensorial properties. Novel sucuk production was developed with the use of free or microencapsulated probiotics. The addition of probiotics to the sucuk promoted the health benefits associated with lactic acid bacteria and contributed to an increase in the consumption of such products. In summary, the results of this study can provide data to the meat industry for the possible utilization of microencapsulated or free probiotics in sucuk processing as demanded by conscientious consumers.

Key words: Sucuk, microencapsulation, probiotic, *L. rhamnosus*, response surface method

1. Introduction

Fermented meat products can be considered as a suitable alternative vehicle for probiotics. In particular, dry fermented sausages with probiotics are very much appreciated due to their functional influence and acceptable quality properties (1). To date, *Lactobacillus rhamnosus* has been extensively added to fermented meat products due to its desirable technological, sensory, and safety properties (2–5) to confer probiotic properties. The beneficial effects of probiotics on the health of the host are possible with the ingestion of probiotic lactic acid bacteria in sufficient amounts (at least 10^6–10^7 cfu/g). Probiotic cells used in dry fermented sausage production should resist the challenging conditions in the final product (low pH and water activity, curing agents, and competitive organisms and species) and gastric conditions in the human intestinal system (2,6). Gastrointestinal tract conditions and stress factors might cause important losses in the viability of probiotics. The microencapsulation method has emerged and has been developed to protect the viability of probiotics against adverse conditions (7). Sucuk, typically known as Turkish dry fermented sausage, is the most popular and widely consumed meat product in Turkey (8). Recently, a number of studies about probiotic sucuk or dry fermented sausages have been conducted, but there have been few studies about the use of microencapsulated probiotic culture in sucuk or dry fermented sausage (1,6,7,9,10).

Prebiotics, calcium alginate, gelatin, and gellan gum, when used as coating materials, may provide better protection for probiotics in food and in the intestinal tract because of the potential for synergy between probiotics and prebiotics. The selection of appropriate coating materials may offer the best protection for the probiotics in microcapsules against gastric conditions and stress factors (11–15).

The aim of this study was to select optimal coating materials for microcapsules of *L. rhamnosus* used in sucuk production and to design probiotic sucuk production with microencapsulated and free cells of *L. rhamnosus*. It was observed that the findings of this study could provide useful information for sucuk producers in the design of microencapsulated probiotic sucuk processing.

2. Materials and methods

2.1. Bacterial strain and culture condition

L. plantarum (Blessing-Biotech GmbH, Stuttgart, Germany) as starter culture and *L. rhamnosus* (Danisco Inc., USA) as probiotic culture were grown in de Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany).

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2.2. Experimental design for *L. rhamnosus*

The selection of optimal coating material combinations is necessary for highest cell viability. Therefore, the optimization of coating materials was performed according to a response surface method employed in a similar way to the work done by Chen et al. (11). To carry out the response surface modeling, regression was performed on the experimental results to construct mathematical models (Table 1) by using Design-Expert 6.02 software (16). Variables were defined as coating materials (alginate, gelatin, gellan gum, fructooligosaccharide (FOS), and peptide), and responses were viable cell counts of *L. rhamnosus* in simulated gastric fluid (SGF) and bile-salt solution (BSS). Optimal proportions of coating materials were determined as 1.89% alginate, 0.96% gellan gum, 0.15% gelatin, 1% peptide, and 1.45% FOS.

2.3. Microencapsulation of *L. rhamnosus*

L. rhamnosus was microencapsulated with 26 combinations of coating materials (Table 1) according to the extrusion technique. Approximately 1% (v/v) of the culture concentrate of *L. rhamnosus* was mixed with 50 mL of sterile coating material solution. The cell suspension was injected through a 0.11-mm needle into sterile 0.1 M $CaCl_2$. The beads, approximately 0.5 mm in diameter, were allowed to stand for 1 h for gelification and then rinsed with and subsequently kept in sterile 0.1% peptone solution (Merck, Germany) at 4 °C (14,17).

2.4. Survival of encapsulated probiotics in SGF and BSS

Resistance to SGF was determined by adding 1 g of the microencapsulated bacteria to flasks containing 10 mL of SGF, which consisted of 0.3% pepsin (Sigma, USA) and 0.5% sodium chloride (Nakalai, Kyoto, Japan), adjusted to pH 2.0 with 1 N HCl (Sigma, USA). Resistance to bile salts was determined by adding microcapsules to the BSS, which consisted of 2% ox gall powder (Sigma, USA). Both resistance treatments took place in agitated flasks (100 rpm) at 25 °C for 1 h (11).

2.5. Determination of *L. rhamnosus* viability in microcapsules

One gram of the microcapsules of *L. rhamnosus* was resuspended in 9 mL of phosphate buffer (0.1 M, pH 7.0) and homogenized for 15 min. The homogenate was serially diluted in peptone water and appropriate dilutions were cultured in duplicate. The counts of *L. rhamnosus* were enumerated on MRS agar (Merck, Germany) in anaerobic conditions (Anaerocult A, Merck, Germany) after 48 h at 30 °C (14,18).

2.6. Production of probiotic sucuk

Probiotic sucuk was manufactured using methods described by Kaban and Kaya (19) and Muthukumarasamy and Holley (6). Sucuk production was carried out with three different culture combinations at a dose of 10^7 cfu/g (A: control sample with free *L. plantarum*; B: sucuk sample with free *L. rhamnosus* + free *L. plantarum*; C: sucuk sample with microencapsulated *L. rhamnosus* + free *L. plantarum*).

2.7. *L. rhamnosus* counts and physicochemical and textural properties in the sucuk samples

From the three replications, the pH value and moisture content of the sucuk samples were determined according to TS 3136/TSE-1978 and TS 1743/TSE-1974, respectively. The water activity (aw) of the sucuk samples was measured by using aw equipment (Labmaster, Novasina, Switzerland). To determine the counts of *L. rhamnosus*, petri plates with MRS-vancomycin agar (Merck, Germany) were incubated at 43 °C for 72 h in anaerobic conditions. After incubation, white colony growth was evaluated as *L. rhamnosus* (20). For texture profile analysis (TPA), from the three replications, sausages of 40 mm in diameter were cut into cylinders and held for equilibration at room temperature (20 °C). TPA tests were performed using a Texture Analyzer (TA.XT Plus, Stable Micro Systems Ltd., Godalming, UK) to determine hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness (21).

2.8. Sensorial analysis

From three replications, sensorial analyses were performed by a group of five trained panelists with previous experience in quantitative descriptive analysis. The selected sensorial parameters of color of exterior surface, color of slice, appearance of slice, stickiness, texture, pleasant odor, unpleasant odor, and overall acceptability were evaluated using a hedonic scale with 9 points (22).

2.9. Statistical analysis

The findings of this study were given as means of triplicate data with their standard deviations. Analysis of the data was carried out using one-way ANOVA. Duncan's multiple test was also used to separate significant differences between means at the $P < 0.05$ significance level by using SPSS 15 for Windows (Chicago, IL, USA) (23).

3. Results

3.1. Survival of encapsulated probiotics in SGF and BSS

The viability of microencapsulated probiotics before and after SGF and BSS conditions is represented in Table 1. While *L. rhamnosus* counts in microcapsules were found to be at an approximate level of 10^9 cfu/g before SGF and BSS, *L. rhamnosus* counts in microcapsules ranged from 10^6 to 10^8 cfu/g after SGF and BSS. Preliminary tests have previously shown that different concentrations and types of coating materials could improve probiotic microencapsulation (24). Therefore, in this study, concentrations of coating materials changing between 0.5% and 2% for alginate, 0% and 1% for gellan gum,

Table 1. Variables and responses of the experiment.

Combination	Alginate (%)	Gellan gum (%)	Gelatin (%)	Peptide (%)	FOS (%)	LR before SGF and BSS (cfu/g)	LR after SGF (cfu/g)	LR after BSS (cfu/g)
1	2.00	1.00	0.00	1.00	0.00	9.4×10^9	8.2×10^7	1.7×10^8
2	2.00	0.00	0.00	1.00	2.00	7.0×10^9	1.2×10^8	4.0×10^8
3	0.50	0.00	0.00	0.00	0.00	1.0×10^9	1.0×10^5	5.0×10^6
4	0.50	0.00	1.00	1.00	2.00	9.2×10^9	5.4×10^7	1.6×10^7
5	0.50	1.00	1.00	0.00	2.00	6.3×10^9	4.0×10^6	4.0×10^8
6	1.25	0.50	0.50	0.50	1.00	1.5×10^9	5.0×10^7	1.7×10^8
7	1.25	0.50	0.50	0.50	2.00	7.1×10^9	4.0×10^6	1.6×10^8
8	0.50	1.00	0.00	1.00	2.00	9.2×10^9	1.4×10^8	1.5×10^8
9	1.25	0.50	0.50	1.00	1.00	2.3×10^9	1.1×10^8	2.1×10^8
10	1.25	1.00	0.50	0.50	1.00	4.5×10^9	1.7×10^8	1.4×10^8
11	2.00	1.00	1.00	0.00	0.00	1.1×10^9	6.0×10^7	1.0×10^8
12	1.25	0.50	0.00	0.50	1.00	9.5×10^9	8.0×10^7	2.7×10^8
13	1.25	0.50	0.50	0.50	1.00	7.7×10^9	1.2×10^8	7.2×10^7
14	2.00	0.00	1.00	1.00	0.00	3.1×10^9	4.0×10^7	4.0×10^7
15	1.25	0.50	0.50	0.00	1.00	2.0×10^9	2.3×10^8	4.0×10^7
16	0.50	1.00	1.00	1.00	0.00	1.7×10^9	3.0×10^6	5.0×10^7
17	1.25	0.00	0.50	0.50	1.00	1.3×10^9	1.0×10^7	2.2×10^8
18	1.25	0.50	0.50	0.50	0.00	1.2×10^9	3.0×10^6	6.0×10^7
19	2.00	0.00	1.00	0.00	2.00	4.7×10^9	1.3×10^7	1.1×10^8
20	1.25	0.50	1.00	0.50	1.00	9.2×10^9	1.2×10^7	2.2×10^8
21	2.00	1.00	0.00	0.00	2.00	1.1×10^9	3.5×10^7	3.4×10^7
22	0.50	0.50	0.50	0.50	1.00	1.0×10^9	7.0×10^6	7.4×10^7
23	2.00	0.50	0.50	0.50	1.00	8.0×10^9	1.2×10^8	6.4×10^7
24	1.25	0.50	0.50	0.50	1.00	7.7×10^9	1.1×10^8	7.1×10^7
25	1.25	0.50	0.50	0.50	1.00	7.6×10^9	1.0×10^8	6.8×10^7
26	1.25	0.50	0.50	0.50	1.00	7.6×10^9	1.1×10^8	6.9×10^7

LR: *L. rhamnosus*; SGF: simulated gastric fluid; BSS: bile-salt solution.

0% and 1% for gelatin, 0% and 1% for peptide, and 0% and 2% for FOS were tested. The optimal values found and subsequently used for the preparation of optimum microcapsules were 1.89% alginate, 0.96% gellan gum, 0.15% gelatin, 1% peptide, and 1.45% FOS.

3.2. *L. rhamnosus* counts and physicochemical and textural properties in the sucuk samples

L. rhamnosus counts, pH value, moisture content, water activity (aw), and textural properties in the sucuk samples are presented in Table 2. The production method (A, B, or

Table 2. *L. rhamnosus* counts and physicochemical and textural properties in the sucuk samples.

	A	B	C
The counts of *L. rhamnosus* (log cfu/g)	$4.55^{a} \pm 0.48$	$7.35^{b} \pm 0.87$	$8.19^{b} \pm 0.25$
pH	$4.62^{a} \pm 0.01$	$4.51^{a} \pm 0.03$	$4.54^{a} \pm 0.01$
Moisture content	$38.03^{a} \pm 2.80$	$36.10^{a} \pm 1.05$	$35.73^{a} \pm 2.10$
Water activity (aw)	$0.88^{a} \pm 0.01$	$0.88^{a} \pm 0.01$	$0.88^{a} \pm 0.01$
Hardness (N)	$101.46^{a} \pm 6.98$	$107.51^{a} \pm 8.84$	$97.49^{a} \pm 15.73$
Adhesiveness (Ns)	$-0.38^{a} \pm 0.19$	$-0.70^{a} \pm 0.47$	$-0.57^{a} \pm 0.29$
Springiness	$0.61^{a} \pm 0.01$	$0.64^{a} \pm 0.05$	$0.67^{a} \pm 0.04$
Cohesiveness	$0.59^{a} \pm 0.02$	$0.60^{a} \pm 0.02$	$0.61^{a} \pm 0.03$
Gumminess (N)	$59.93^{a} \pm 5.10$	$64.43^{a} \pm 4.46$	$60.05^{a} \pm 13.70$
Chewiness (Ns)	$36.72^{a} \pm 3.02$	$41.53^{a} \pm 6.20$	$40.69^{a} \pm 11.26$
Resilience	$0.19^{a} \pm 0.00$	$0.20^{a} \pm 0.01$	$0.19^{a} \pm 0.01$

A: Control sample with free *L. plantarum*; B: sucuk sample with free *L. rhamnosus* + free *L. plantarum*; C: sucuk sample with microencapsulated *L. rhamnosus* + free *L. plantarum*
± standard deviations; a, b: values with different letters are significantly different ($P < 0.05$).

C) had no effect on the pH, moisture content, and water activity of all sucuk samples. *L. rhamnosus* counts in the A, B, and C samples were found to be 4.55 log cfu/g, 7.35 log cfu/g, and 8.19 log cfu/g, respectively. *L. rhamnosus* counts were significantly affected by the production method. The production method did not show a significant impact on the texture profile of sucuk (hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience). Likewise, the texture profile obtained with the TPA test and texture properties of the sucuk in sensorial analyses performed by panelists were not significantly affected by the production method. Therefore, the TPA test corrected texture results in sensorial analysis performed by the panelists.

3.3. Sensorial analysis of sucuk

Sensorial analysis results of the sucuk samples are shown in Table 3. There were no significant differences between sucuk samples in terms of sensory quality ($P < 0.05$) The

Table 3. Sensorial properties of the sucuk samples.

	A	B	C
Color of exterior surface	8.27^{a}	8.20^{a}	8.07^{a}
Color of slice	7.13^{a}	7.47^{a}	7.33^{a}
Appearance of slice	7.33^{a}	8.13^{a}	7.67^{a}
Texture	7.20^{a}	7.53^{a}	7.33^{a}
Stickiness	7.87^{a}	8.13^{a}	7.73^{a}
Pleasant odor	8.13^{a}	7.93^{a}	8.00^{a}
Unpleasant odor	8.93^{a}	8.93^{a}	8.93^{a}
Overall acceptability	7.87^{a}	8.07^{a}	8.07^{a}

A: Control sample with free *L. plantarum*; B: sucuk sample with free *L. rhamnosus* + free *L. plantarum*; C: sucuk sample with microencapsulated *L. rhamnosus* + free *L. plantarum*; a, b: values with different letters are significantly different ($P < 0.05$).

production method with different culture combinations in the sucuk did not exhibit any effect on sensorial quality. All sensorial parameters in the sucuk samples were evaluated as acceptable the panelists.

4. Discussion

The proportion of prebiotic and coating materials used in microencapsulation of probiotics influences their survival against SGF and BSS (11). In this sense, the present study detected the optimal ratio of the coating materials, which offered the best protection for the probiotics in microcapsules. Similarly, according to earlier studies (12,14,17,25), blending prebiotic agents such as peptide and FOS with sodium alginate and gelatin for probiotic microencapsulation improved the viability of probiotic bacteria. Additionally, the prebiotic effects of peptide and FOS were confirmed by Chen et al. (11). As a result, the use of optimal coating materials increased the survival capacities of probiotics in sucuk, and their positive survival characteristics can contribute to safety, provide sensory and nutritional benefits, and promote health (4).

According to the Turkish Standard Institute (26), good ripened sucuk should have pH values between 4.7 and 5.4. All of the sucuk samples in our study were in this range. The starter culture provides stability for the pH value (27). The presence of both starter and probiotic culture in the sucuk samples contributed to the stability of pH between different samples. The Turkish Food Codex-Meat Products Communiqué stated that the moisture content of Turkish sucuk should be at a maximum of 40%. Moisture contents (approximately between approximately 35% and 38%) in our sucuk samples were in accordance with this limit value. It was considered that the presence of *L. rhamnosus* in the control sample (A) resulted from the improvement of spontaneous flora during fermentation. In previous studies, it was reported that *L. rhamnosus* was isolated from sucuk obtained from spontaneous fermentation (28). As seen from our results, the microencapsulation technique improves the survival of probiotic culture and contributes to the stability of probiotic culture amounts in products such as sucuk that contain intensive spice and low moisture (2,7). In addition, prebiotic additives in the coating material promote the resistance of probiotics (14). The textural properties of sucuk affect sensorial quality and sucuk acceptance by consumers (29). Rubio et al. (4) reported that changes in pH influenced the texture of the product. In accordance with this hypothesis, the textural properties of our sucuk samples did not show any significant differences as there were not any significant differences in the pH values of the sucuk samples. Production methods did not strongly affect the sensory properties of the A, B, and C samples. These results are in agreement with those found in the study of Moyano et al. (30), who reported that probiotics did not have any negative effect on the sensory property of Iberian dry-fermented sausages. Additionally, no significant difference in sensory quality was found between sucuk containing either free or microencapsulated *L. rhamnosus*. Similar observations were noted for the effect of free or microencapsulated probiotics on sensorial quality by Muthukumarasamy and Holley (6). As a result of sensorial analysis, a production method with different culture combinations did not lead to any detectable change in the sensory properties of sucuk. For consumers, a new probiotic meat product was presented with the same or similar quality characteristics as traditional sucuk (1–3,10).

In conclusion, the compositional parameters of all sucuk samples were found to be in accordance with the sucuk standards laid down in the Turkish Food Codex. The application of microencapsulated or free probiotic *L. rhamnosus*, in conjunction with starter culture *L. plantarum*, has not led to a negative impact on the quality characteristics of sucuk. In this study, a novel sucuk production method with microencapsulated probiotic culture was improved for the benefit of the industry. As a result, the probiotic sucuk produced in this study can be a pleasant and functional product for consumers demanding different probiotic products.

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