

The effect of natural microbiota on colour, texture and sensory properties of sucuk during the production

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Summary: Fermentation occurs with the effect of starter cultures or microorganisms which origin from the raw material and environment during the sucuk (Turkish style fermented sausage) production named as ‘‘Chance Inoculation’’. These microorganisms settle on sucuk dough better than commercial microorganisms because of their metabolic activities and properties of competition. This is one of the way for the development of desirable flavour and aroma in sucuks. In this study, *Lactobacillus plantarum*, *Lactobacillus sake*, *Lactobacillus curvatus* and *Staphylococcus xylosus* were isolated and molecularly identified from the traditionally produced sucuk doughs. Fermented sucuk was produced using various combinations of these microorganisms. It was aimed to determine the most desirable microorganism combination on colour, texture and sensory properties of sucuks. It was indicated that colour measurements were not affected by microorganisms ($p>0.05$). Hardness, cohesiveness and resilience values were significantly affected by microorganisms in texture profile analysis ($p<0.05$). One-way analysis of variance displayed that texture property was affected by microorganisms in raw sucuk groups at sensory evaluation ($p<0.05$). In cooked sucuk groups, colour of outer and inner surface ($p<0.01$), texture ($p<0.01$) and property of acceptability ($p<0.05$) were significantly different in intergroup analysis. As a result of the evaluation, it was detected that *Lactobacillus sake*, *Lactobacillus curvatus* and *Staphylococcus xylosus* were the most proper microorganism combination for consumer preference.

Keywords: Colour, natural microbiota, sensory evaluation, sucuk, texture.

Sucuk üretiminde doğal floranın renk, tekstür ve duyuşsal özellikler üzerine etkisi

Özet: Sucuk üretiminde fermentasyon aşaması starter kültürlerin katkısıyla veya ‘‘Chance Inoculation’’ olarak adlandırılan üretim sırasında ham maddeden ve çevreden kaynaklı mikroorganizmalarla bulaşma sonucu gerçekleşmektedir. Ham madde ve çevre kaynaklı bu mikroorganizmalar metabolik aktiviteleri ve yarışmacı özellikleri sayesinde buldukları ortamda endüstriyel kültürlerle göre daha iyi adapte olmakta ve arzu edilen duyuşsal özelliklerin oluşumunda etkin rol oynamaktadırlar. Çalışmada, geleneksel metotlarla üretimi yapılan sucuk hamurlarından izole ve moleküler olarak tanımlanmış *Lactobacillus plantarum*, *Lactobacillus sake*, *Lactobacillus curvatus* ve *Staphylococcus xylosus* suş kombinasyonları kullanılarak yapılan sucuk üretiminde renk, tekstür ve duyuşsal özellikler üzerinde tüketici damak zevkine en uygun mikroorganizma kombinasyonunun belirlenmesi amaçlandı. Renk analiz sonuçlarına göre gruplar arası farkın önemli olmadığı sonucuna varıldı ($p>0.05$). Tekstür profil analizinde sertlik, iç yapışkanlık ve elastikiyet özellikleri istatistiksel fark gösterdi ($p<0.05$). Duyusal değerlendirmede ise çiğ sucuklar için sadece tekstür özelliği açısından istatistiksel fark belirlenirken ($p<0.05$); pişmiş sucuk değerlendirmesinde kesit yüzey rengi ($p<0.01$), dış yüzey rengi ($p<0.01$), tekstür ($p<0.01$) ve genel kabul edilebilirlik özellikleri ($p<0.05$) bakımından gruplar arası fark önemli bulundu. Değerlendirmeler sonucu, tüketici damak zevkine en uygun mikroorganizma kombinasyonunun *Lactobacillus sake*, *Lactobacillus curvatus* ve *Staphylococcus xylosus* olduğu sonucuna ulaşıldı.

Anahtar sözcükler: Doğal mikrobiyota, duyuşsal değerlendirme, renk, sucuk, tekstür.

Introduction

The first stage of sucuk production is fermentation. It occurs with the contribution of starter cultures or microbial contamination from raw material and environment called ‘Chance Inoculation’. The next stage is drying which is under control of environmental conditions in traditional sucuk production. However, it is under the control of atmospheric conditions in commercial sucuk production. The ripening period mostly consists of these two stages. Physical quality properties like texture,

colour, flavour and odor (7, 38, 41) peculiar to sucuk consist of natural microbiota activity (31). Microorganisms that derived from meat and environment, are called as ‘house microbiota’ (36). These microorganisms are also known as a wild type microorganism. Moreover, they can easily adapt to sucuk dough better than commercial cultures because of their metabolic and competitor activity (26, 40). In Europe, house microbiota can easily be colonized to sucuk dough than commercial culture, as commercial culture does not

have a sufficient effect on desired sensory properties (35). Therefore, traditional sucuks have better desired properties than commercial sucuks which are fermented under the controlled and processed with starter culture (28). Small business operators still produce traditional sucuks without using starter culture via natural fermentation to get desired flavour and aroma (36). In this study, it has been aimed to investigate how natural microbiota affect sucuk's physical and sensory properties.

Materials and Methods

Lactobacillus plantarum ATCC 8014, *Lactobacillus sake* ATCC 15521, *Lactobacillus curvatus* ATCC 25601 and *Staphylococcus xylosum* ATCC 29971 were used as reference strains. *L. plantarum*, *L. sake* and *L. curvatus* were isolated (27, 34, 39) from traditional sucuk doughs and they were evaluated in terms of gas production from glucose (15), growth at different temperature (20), growth at different salt concentrations (20), growth ability at 3.9 pH (20), the Voges-Proskauer test, metil red test (23), arginine test (9) and carbohydrate fermentation test (API CHL 50, Biomerieux). Then *L. plantarum*, *L. sake* and *L. curvatus* were molecularly identified according to Berthier and Ehrlich (5), Aymerich et al. (4). *S. xylosum* was isolated from pure cultures (39), evaluated according to oxidase test (3), sensitivity of furazolidone and lysostaphin (41), gelatine hydrolysis test (3) and growth ability on Cimmon Citrat Agar (3) and identified as molecularly (17, 29, 30). After verification, these microorganisms were compared with 1:1 and were used for sucuk producing three different ways. Each sucuk group was produced in the form of 10 kg and it was replicated three times and named as control, group I (GI), group II (GII), group III (GIII). Sucuk groups are listed in Table 1.

Table 1. Combination of starter culture.
Tablo 1. Starter kültür kombinasyonları.

| Group | Combination of starter culture |
|-----------|--|
| Control | Sucuk dough + Starter culture - |
| Group I | Sucuk dough + <i>L. plantarum</i> , <i>L. sake</i> , <i>S. xylosum</i> |
| Group II | Sucuk dough + <i>L. plantarum</i> , <i>L. curvatus</i> , <i>S. xylosum</i> |
| Group III | Sucuk dough + <i>L. curvatus</i> , <i>L. sake</i> , <i>S. xylosum</i> |

Control group had no starter culture. Other groups were inoculated with starter culture, and ratios of starter culture were 10^7 cfu/g for *L. plantarum*, *L. sake* and *L. curvatus*; 10^6 cfu/g for *S. xylosum*. Dilutions were prepared by physiological brine. Each dilution was inoculated to media to get 10^6 - 10^7 cfu/g level and enumerated. After obtaining the 10^6 - 10^7 cfu/g level, it was determined which McFarland turbidity gave 10^6 - 10^7 cfu/g level (33).

Sucuk production was made at Afyonkarahisar İkbâl Gıda Inc. Production was made using formulation and method which were announced by Gökâlç et al. (19). For this purpose, 1 kg sucuk doughs including 800 g beef and 200 g fat were prepared. It was applied basing on ripening conditions of Kaban and Kaya (25).

Colour analyses of the samples were calculated by Minolta (CR-A70, Japan) colorimetry at the final product. It was based on criteria of Commission Internationale de l'Eclairage (CIE). Lightness (L^*), redness (a^*) and yellowness (b^*) were measured. Measuring was made twice on both inner and outer surfaces for each sample. The results were evaluated by five different measurements (2).

Texture profile analysis was made on final product with the instrument (Stable Micro Systems TA.XT2, Texture Technologies Corp., Robbinsville, NJ). After getting away from sucuk's casing, they were sliced in 1 cm. This analysis was made considering the method of Bourne (6).

Sensory evaluation of samples was made with a total of 25 uneducated academics, administrative and other staff who work at the Veterinary Medicine Faculty of Afyon Kocatepe University. Evaluation was made using the hedonic scala. According to this scala, point 9 symbolizes 'very good' and point 1 symbolizes 'bad' (1, 37).

Statistical analyses: Sucuks used in this study were produced three times for each group. Testing of normal distribution for datasets was evaluated by the Kolmogorov-Smirnov and Shapiro-Wilk tests. According to these tests, it was seen that datasets did not fit normal distribution except for sensorial evaluation datasets. The sensorial evaluation datasets were analysed by one-way ANOVA. Multiple comparison test was applied by Duncan test related with sensorial evaluation datasets. The other datasets were analysed by Kruskal-Wallis test.

Results

According to CIE, statistical results of L^* , a^* and b^* values are shown in Table 2. There were no statistical differences between the groups ($p>0.05$).

The results of texture profile analysis are summarized in Table 3. Hardness values of the groups were detected between 2519.19-3391.25 g. In terms of hardness, cohesiveness and resilience, there were statistical differences between control (3391.75 g) and GI (2521.04 g)-GII (2519.19 g) groups; GIII (0.74) and GI (0.70); GIII (0.28) and GI (0.25), respectively ($p<0.05$). There were no statistical differences detected between groups with regard to adhesiveness (29.70-10.28 g.sec), springiness (0.70-0.78) and chewiness (1263.43-1742.17 g) ($p>0.05$).

Table 2. Results of outer and inner surface colour values.

Tablo 2. Dış yüzey ve kesit yüzey renk değerleri analiz sonuçları.

| Colour values | Group | N | q ₂ ^a | q ₁ ^a | q ₂ ^b | q ₁ ^b |
|-------------------|-------|---|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| <i>L</i> * values | C | 3 | 41.56 | 38.58 | 51.79 | 51.16 |
| | GI | 3 | 41.01 | 38.51 | 52.45 | 51.54 |
| | GII | 3 | 41.19 | 38.54 | 52.56 | 51.39 |
| | GIII | 3 | 42.33 | 37.31 | 51.59 | 51.35 |
| <i>a</i> * values | C | 3 | 13.93 | 13.60 | 17.43 | 16.85 |
| | GI | 3 | 10.16 | 9.99 | 17.35 | 17.00 |
| | GII | 3 | 10.45 | 10.06 | 17.11 | 16.56 |
| | GIII | 3 | 11.04 | 10.69 | 17.63 | 17.63 |
| <i>b</i> * values | C | 3 | 12.26 | 12.01 | 17.75 | 17.09 |
| | GI | 3 | 10.87 | 10.75 | 18.44 | 17.42 |
| | GII | 3 | 11.39 | 10.74 | 18.36 | 17.28 |
| | GIII | 3 | 10.69 | 10.53 | 18.02 | 17.57 |

N: number of analysis; q₂: median; q₁: quarter of %25; a: outer surface; b: inner surface; p of *L*_a* value: 0.863; p of *a*_a* value: 0.063; p of *b*_a* value: 0.075; p of *L*_b* value: 0.935; p of *a*_b* value: 0.204; p of *b*_b* value: 0.963.

N: analiz sayısı; q₂: medyan; q₁:%25'lik çeyreklik; a: dış yüzey; b: kesit yüzey; *L*_a* değeri p: 0.863; *a*_a* değeri p: 0.063; *b*_a* değeri p: 0.075; *L*_b* değeri p: 0.935; *a*_b* değeri p: 0.204; *b*_b* değeri p: 0.963.

Table 3. Results of texture profile analysis of control and other sucuk groups.

Tablo 3. Kontrol ve deneme gruplarının tekstür analiz sonuçları.

| Texture Values | Group | N | q ² | q ¹ | q ³ |
|---------------------|-------|---|-----------------------|----------------|----------------|
| Hardness (g) | C | 9 | 3391.75 ^a | 2677.64 | 3725.54 |
| | GI | 9 | 2521.04 ^b | 2158.50 | 2855.66 |
| | GII | 9 | 2519.19 ^b | 2214.69 | 2839.43 |
| | GIII | 9 | 2779.47 ^{ab} | 2669.93 | 3155.62 |
| Adhesiveness (g.sn) | C | 9 | 29.70 | 61.93 | 9.17 |
| | GI | 9 | 20.09 | 92.14 | 13.58 |
| | GII | 9 | 12.49 | 23.30 | 7.95 |
| | GIII | 9 | 10.28 | 19.56 | 7.48 |
| Springiness | K | 9 | 0.78 | 0.74 | 0.83 |
| | GI | 9 | 0.76 | 0.71 | 0.79 |
| | GII | 9 | 0.75 | 0.65 | 0.81 |
| | GIII | 9 | 0.70 | 0.61 | 0.74 |
| Cohesiveness | C | 9 | 0.71 ^{ab} | 0.68 | 0.74 |
| | GI | 9 | 0.70 ^a | 0.66 | 0.70 |
| | GII | 9 | 0.73 ^{ab} | 0.69 | 0.78 |
| | G III | 9 | 0.74 ^b | 0.72 | 0.77 |
| Chewiness (g) | C | 9 | 1742.17 | 1410.93 | 2252.39 |
| | GI | 9 | 1263.43 | 1066.27 | 1453.74 |
| | GII | 9 | 1448.97 | 974.92 | 1652.89 |
| | GIII | 9 | 1462.71 | 1302.41 | 1589.79 |
| Resilience | C | 9 | 0.25 ^{ab} | 0.24 | 0.29 |
| | GI | 9 | 0.25 ^a | 0.23 | 0.26 |
| | GII | 9 | 0.26 ^{ab} | 0.23 | 0.29 |
| | GIII | 9 | 0.28 ^b | 0.27 | 0.30 |

N:number of analysis; *p<0.05; **p<0.01; q₂: median; q₁: quarter of %25; q₃: quarter of %75; ^{a-b}: letters on the same column compare values of texture profile analysis for each property; different letters represent a statistical difference intergroup (p<0.05; p<0.01); same letters do not represent a statistical difference (p>0.05); p of hardness: 0.011**; p of adhesiveness: 0.184; p of springiness: 0.100; p of cohesiveness: 0.023*; p of chewiness: 0.052; p of resilience: 0.018**.

N: analiz sayısı; *p<0.05; **p<0.01; q₂: medyan; q₁:%25'lik çeyreklik; q₃: %75'lik çeyreklik; ^{a-b}: Her bir özellik için ayrı ayrı, aynı sütundaki harfler tekstür profil analiz değerleri karşılaştırılmasıdır. Farklı harfler örnekler arasında istatistiksel fark (p<0.05; p<0.01) olduğunu gösterirken; aynı harflerle gösterilenler örnekler arasında istatistiksel fark bulunmadığını göstermektedir (p>0.05); sertlik p: 0.011**; dış yapışkanlık p: 0.184; esneklik p: 0.100; iç yapışkanlık p: 0.023*; çiğnenbilirlik p: 0.052; elastiklik p: 0.018*

Table 4. Results of the sensory evaluation in raw sucuk.
Tablo 4. Çiğ sucuk duyuşal analiz sonuçları.

| Results of the Sensory Evaluation | Group | N | Average± Standard Deviation | F | P |
|-----------------------------------|-------|----|-----------------------------|-------|---------------|
| Colour of inner surface | C | 25 | 5.56±1.60 | 1.236 | 0.301 |
| | GI | 25 | 5.24 ±1.56 | | |
| | GII | 25 | 5.76±1.78 | | |
| | GIII | 25 | 6.12±1.66 | | |
| Colour of outer surface | C | 25 | 6.36±1.46 | 0.650 | 0.585 |
| | GI | 25 | 6.08±1.35 | | |
| | GII | 25 | 6.12±1.69 | | |
| | GIII | 25 | 6.60±1.44 | | |
| Typical sucuk odor | C | 25 | 6.76±1.80 | 0.917 | 0.436 |
| | GI | 25 | 5.92±1.95 | | |
| | GII | 25 | 6.52±2.08 | | |
| | GIII | 25 | 6.60±1.80 | | |
| Texture | C | 25 | 7.24 ^a ±1.26 | 2.970 | 0.036* |
| | GI | 25 | 6.24 ^b ±1.47 | | |
| | GII | 25 | 6.40 ^{ab} ±1.52 | | |
| | GIII | 25 | 7.08 ^{ab} ±1.44 | | |
| Acceptability | C | 25 | 7.32±1.28 | 1.696 | 0.173 |
| | GI | 25 | 6.48±1.89 | | |
| | GII | 25 | 6.92±1.60 | | |
| | GIII | 25 | 7.32±1.28 | | |

N: number of panellist; *p<0.05; ^{a-b}: Letters on the same column compare values of the sensory evaluation in raw sucuk; different letters represent statistical difference intergroup (p<0.05); same letters do not represent a statistical difference (p>0.05).

N: panelist sayısı; *p<0.05; ^{a-b}: Aynı sütundaki harfler çiğ sucuk duyuşal analiz değerleri karşılaştırılmasıdır. Farklı harfler, örnekler arasında istatistiksel fark (p<0.05) olduğunu gösterirken; aynı harflerle gösterilenler örnekler arasında istatistiksel fark bulunmadığını göstermektedir (p>0.05).

Table 5. Results of the sensory evaluation in cooked sucuk.
Tablo 5. Pişmiş sucuk duyuşal analiz sonuçları.

| Results of the Sensory Evaluation | Group | N | Average±Standard Deviation | F | P |
|-----------------------------------|-------|----|----------------------------|-------|----------------|
| Colour of inner surface | C | 25 | 7.36 ^a ±1.22 | 4.111 | 0.009** |
| | GI | 25 | 6.56 ^b ±1.44 | | |
| | GII | 25 | 7.00 ^{ab} ±1.15 | | |
| | GIII | 25 | 7.68 ^a ±0.85 | | |
| Colour of outer surface | C | 25 | 7.56 ^{ab} ±1.12 | 5.237 | 0.002** |
| | GI | 25 | 6.64 ^c ±1.52 | | |
| | GII | 25 | 7.12 ^{bc} ±1.01 | | |
| | GIII | 25 | 7.88 ^a ±0.97 | | |
| Typical sucuk flavour and aroma | C | 25 | 7.56±0.91 | 1.866 | 0.140 |
| | GI | 25 | 6.84±1.67 | | |
| | GII | 25 | 7.04±1.56 | | |
| | GIII | 25 | 7.60±1.25 | | |
| Texture | C | 25 | 6.56 ^a ±1.95 | 4.399 | 0.006** |
| | GI | 25 | 6.20 ^a ±1.60 | | |
| | GII | 25 | 6.48 ^a ±1.58 | | |
| | GIII | 25 | 7.68 ^b ±0.85 | | |
| Acceptability | C | 25 | 7.44 ^{ab} ±0.96 | 3.637 | 0.016* |
| | GI | 25 | 6.80 ^b ±1.75 | | |
| | GII | 25 | 7.12 ^b ±1.48 | | |
| | GIII | 25 | 8.00 ^a ±1.00 | | |

N: number of panelist; *p<0.05; **p<0.01; ^{a-b}: Letters on the same column compare values of the sensory evaluation in cooked sucuk; different letters represent statistical difference intergroup (p<0.05; p<0.01); same letters do not represent a statistical difference (p>0.05).

N: panelist sayısı; *p<0.05; **p<0.01; ^{a-b}: Aynı sütundaki harfler, pişmiş sucuk duyuşal analiz değerleri karşılaştırılmasıdır. Farklı harfler, örnekler arasında istatistiksel fark (p<0.05; p<0.01) olduğunu gösterirken, aynı harflerle gösterilenler örnekler arasında istatistiksel fark bulunmadığını göstermektedir (p>0.05).

Results of sensory evaluation are indicated in Table 4 for raw sucuk and in Table 5 for cooked sucuk. For raw sucuks, there were no statistical differences in terms of properties for colour of inner and outer surface, typical sucuk odor, acceptability except for texture property ($p < 0.05$). If groups were sorted from smooth to rough texture, there would be control, GIII, GII and GI groups, respectively.

In cooked sucuk, there were statistical differences in inner and outer surface colour, texture ($p < 0.01$) and acceptability ($p < 0.05$). GI (6.56) and control group (7.36); GIII (7.68) and GI were statistically different from each other in inner surface colour. Control (7.56) and GI (6.64); GIII (7.88) and GI-GII (7.12) groups were statistically different in regard to outer surface colour. GIII was statistically different from other groups with regard to property of texture ($p < 0.01$). GIII (8.0) was statistically different from GI (6.80) -GII (7.12) in property of acceptability ($p < 0.05$).

Discussion and Conclusion

In this study, values of L^* , a^* and b^* were similar to the findings of previous studies (40, 18, 24, 11, 14, 33). Moreover, it was observed that using starter culture could not affect colour parameters as stated by the earlier findings of Essid and Hassouna (14), Bozkurt and Bayram (7) and Casaburi et al. (8) found that as observed in this study.

In texture profile analysis, hardness values of groups with starter culture were lower than the control group which had no starter culture. This difference between the control group and GI-GII groups was related with the starter culture. For the cohesiveness property, *L. sake*, *L. curvatus* and *S. xylosus* which present in GIII were more effective when compare to other microorganism combinations. During the production, cohesiveness change in accordance with sucuk formulation and it is not preferable property in sucuks (12). *L. sake*, *L. curvatus* and *S. xylosus* could be effective in resilience property. According to panelist evaluations, microorganism combination of GIII was more desirable than the others as for the resilience property.

At sensory analyses, the control group had a more smooth texture than the other groups in raw sucuk groups. While GIII was more preferable in texture profile analysis, it was second line in sensory evaluation. Related with this result, subjective evaluation was not confirmed instrumental results for raw sucuk groups. Conversely, subjective evaluation was confirmed instrumental results for cooked sucuk groups. The values of texture profile analysis in this study were similar with the findings of the earlier studies considering hardness (7, 14), adhesiveness (7, 12, 33), chewiness (7) and resilience (33). The results

of texture profile analysis differ due to raw material, starter culture, processing method and environment in fermented meat products (7). Therefore, it is hard to find similar studies. However, scientific studies investigating texture profile analysis have increased day by day. Sense of texture is related with age, food culture and nutritional habit of consumers (13).

In cooked sucuk sensory analysis, GIII had the smoothest texture. So, it was verified with instrumental analysis. Panellists commented that GI had more sour flavour than the others had. Nevertheless, all of the groups had the same formulation. The reason of sour flavour was presence of *L. plantarum* in GI. This could be the reason why GI is less accepted by the panellists. This study was similar with the studies of Öztürk (33) (texture, typical sucuk odor and acceptability) and Kaban (24) (typical sucuk odor).

Hammes and Hertel (21) reported that *L. sake* and *L. curvatus* were the best microorganisms at meat fermentation. Hugas and Monfort (22) pointed that *L. sake* and *L. curvatus* inhibited unwanted microbiota because of the best adaptation feature to meat environment. By this way, microorganisms prevent undesirable odor and aroma. Garriga et al. (16) suggested that *L. plantarum* and *L. curvatus* were more effective than *L. sake* on odor improvement. *L. plantarum* is more attractive than *L. sake* and *L. curvatus* on rancid aroma development (16). Özdemir (32) claimed that *L. sake* and *L. curvatus* were dominant microbiotas in fermented sucuks, and the amount of *L. sake* was directly proportional to organoleptic quality. Dinçer et al. (10) reported that sensorial quality of fermented sucuks were the best way when using as a culture of *L. sake*, *L. curvatus* and *S. xylosus*. In this study, the most proper microorganism combination was mixture of *L. sake*, *L. curvatus* and *S. xylosus* for the sensory quality.

Fermented sucuk is special for Turkey. Natural microbiota of sucuks are different according to the area where sucuk is produced. Natural microbiota of sucuk should be investigated and its safety for public health should be evaluated and determined technologically for using as a starter culture. In order to achieve this aim, researchers can benefit from microbiota which is isolated traditional sucuks and environment. This microbiota is better adaptable to sucuk than commercial microorganisms. These microorganisms called starter culture can speed up production, have less additives and have a proper palate for consumer. Following that, starter cultures having these properties should be offered to the market. To this purpose, except for pathogen microorganisms, we should contribute to the development of starter cultures special to Turkey.

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