EVALUATION OF COMPETITIVENESS AND ADAPTATION ABILITY OF AUTOCHTHONOUS STARTER CULTURES IN SUCUK FERMENTATION

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Abstract- In this study, four different groups of sucuk were produced, using double combinations of three different autochthonous *Lactobacillus* species (*Lactobacillus sakei, Lactobacillus curvatus and Lactobacillus plantarum*) beside a control group produced without starter culture. Percent moisture, pH, water activity and lactic acid bacterial count determined in the sucuk samples during the fermentation (0., 4., 8. and 12. days). PCR-DGGE analysis was also performed in order to understand the dynamics and diversity in bacterial flora of sucuk during the fermentation. According to PCR-DGGE analysis results, in all sucuk samples while a intense *L. sakei* band was observed since the fourth day of fermentation, bands of *L. plantarum* and *L. curvatus* were only observed in the sucuk groups that they were added. In the results, autochthonous bacterial starter cultures, inoculated to sucuk formulation showed a relatively poor competitiveness and weak adaptation when comparing to *L. sakei* present in house flora. However, bacterial culture addition had significant effects on pH and LAB counts of the sucuk samples while leading to reduction at the drying times.

Key Words – PCR-DGGE, Lactic acid bacteria, Physicochemical analyses

I. INTRODUCTION

Sucuk, Turkish fermented dry sausage, is the most popular meat product in Turkey. It usually produced by mixing sheep meat and/or beef with sheep tail fat, salt, sugar, garlic, spices and nitrate and/or nitrite [1]. In traditionally produced sucuk, the fermentation which important stage for the development of desirable organoleptic characteristics and improving the safety of the final product is controlled by indigenous microflora [2]. In naturally fermented sucuk, the predominant lactic microbiota are usually composed from *L. sake, L. plantarum* and *L. curvatu*s [3]. Today, most of the sausages fermentation is carried out by inoculating microbial starter cultures mainly from both *Lactobacilli* and coagulase-negative cocci, responsible for the acidification and desirable flavor generation [4]. However, the metabolic activity and competitiveness of wild-type strains (indigenous microflora) against to undesired microflora are higher in contrast to industrial starters, which are not well-adapted to raw material and technology applied to the production [5]. Therefore, in the last decade researchers focused on to understand the microbial populations responsible for the ripening and to select potential autochthonous strains to be employed as starter culture in production. However it is required to exactly understand that whether autochthonous cultures, used in production are metabolically active and become predominant throughout the fermentation. Recently the culture-independent denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S ribosomal DNA fragments become the most suitable tools for accurately monitoring the dynamic changes in the population responsible for ripening of fermented sausages [6,7]. Therefore, in this study we aimed to evaluate the competitiveness and adaptation ability of autochthonous starter cultures in sucuk fermentation. Hence bacterial flora of sucuk samples, inoculated with different species of autochthonous lactic acid bacteria were monitored by using PCR-DGGE technique and also physicochemical characteristics were simultaneously assessed during the fermentation.

II. MATERIALS AND METHODS

Sucuk Manufacturing

In this study four different groups of sucuk were produced by using double combination of *L. sakei*, *L. plantarum* and *L. curvatus* strains,

previously isolated from the spontaneously fermented sucuk samples besides a control group, produced without LAB cultures. LAB cultures were inoculated at level of 10^7 cfu/g to sucuk mixtures, consisted of common ingredients such as powdered red pepper, garlic, salt, sugar, cumin, black pepper, pimento, antioxidant and sodium nitrite (E 250) in addition to beef, tail fat. All of the sucuk mixtures were stuffed into artificial casings and fermented for 3 days at 22°C and relative humidity (RH) 90% followed by a gradual reduction of temperature to 19 °C and RH to 80% during the next 7 days, and ripening process was completed by a step at 16 °C and 65% RH for 2 days. Sucuk samples were named by considering LAB cultures which were inoculated as PC (*L. plantarum* + *L. curvatus*), PS (*L. sakei* + *L. plantarum*) and CS (*L. sakei* + *L. curvatus*).

PCR-DGGE analysis of sucuk samples

PCR-DGGE analysis was performed for monitoring the bacterial flora of sucuk samples during the fermentation (0, 4, 8 and 12 days). Extraction of bacterial DNA from sucuk samples and pure cultures was performed using a commercial DNA isolation kit (QIAamp DNA Mini kit, Qiagen, Germany) according to the manufacturer's protocol for Gram-positive bacteria. The V1 region of the16S rDNA gene was amplified by using forward primer V1f (5′-GCGGCGTGCCTAATACATGC-3′) with GC-clamps and reverse primer V1r (5′-TTCCCCACGCGTTACTCACC-3′) [8]. All of PCR amplifications were performed in a final volume of 50µl using "touchdown PCR" parameters [9]. DGGE analysis of PCR amplicons was performed in a 1 mm polyacrylamide gel (8% [wt/vol] acrylamide-bisacrylamide [37.5:1]) essentially as described previously [10]. The DGGE bands were identified by sequencing and searching the results in the GenBank with the BLAST program.

Physico-chemical analyses

Physicochemical analyses were performed in sucuk samples during the fermentation. Moisture analysis was performed using gravimetric method. The pH value was directly measured into each sample by pH meter (inoLab®pH 720 WTW, GmbH Germany) and the water activity (a_w) values of the samples were determined using Acualab 3TE (Decagon, USA).

Enumeration of lactic acid bacteria (LAB)

The counts of LAB were determined in sucuk samples during the fermentation (0, 4, 8 and 12 days) by using agar plate method, performed on MRS agar plates with incubation at 35 °C for 48 h in anaerobic chamber.

III. RESULTS AND DISCUSSION

DGGE Profiles of Sucuk Samples

Lactic microflora of sucuk samples were monitored using PCR-DGGE analysis during the fermentation (Fig. 1). At the first day of the fermentation, the bacterial flora of the sucuk samples composed from natural meat contaminants, such as *Lactococcus piscium*, *Brochothrix thermosphacta* and *Carnobacterium divergens*. However, after 3 days the intensity of the bands of *Lc. piscium* and *C.divergens* decreased while the band of *B. thermosphacta* was disappeared. On the 4th day of fermentation the intense band of *L. sakei* appeared, revealing that this species became dominant and remained stable until the end of the ripening period in all sucuk samples even in the control group. On the other hand, the DGGE bands, belonging to *L. plantarum* and *L. curvatus* appeared in sucuk groups which only they were inoculated on the 4th day of the ripening. Although the intensity of these bands was relatively weak, they remained stable during fermentation.

Enumeration of Lactic Acid Bacteria

At the beginning of the fermentation LAB were present with counts of about 6-7 log cfu/g. They changed throughout the fermentations and reached a final numbers of 8.38-8.79 log cfu/g. The lowest counts of LAB were observed in control samples during the fermentation (Fig. 2), and a statistically significant difference was found between the sucuk groups inoculated with lactic acid bacteria and control.

Physicochemical analysis of the sucuk samples

The results of the physicochemical analysis of the sucuk samples during fermentation are showed in Figure 2.

pH

At the beginning of the fermentation the pH values were about 5.84-5.89 and then rapidly decreased as a result of the increase in LAB count in the first stages of the fermentation. After 3 days of fermentation the pH reached 4.81, which was the lowest value in all sucuk samples. The final pH of the sucuk samples ranging from 4.96 to 5.45 at the end of fermentation. The highest pH values (5.45) were observed in the control samples during fermentation. Thus, bacterial culture addition had significant effects on pH value.

Water Activity (a_w)

The a_w values were about 0.95-0.96 at the first day of the fermentation in all sucuk samples. The a_w decreased gradually during ripening and reached to 0.89–0.91 in final products. The a_w values of the PS group showed a significant difference than that of other sucuk groups.

Moisture (%)

The moisture values decreased gradually throughout the fermentation period and ranged from 39.50% to 41.50% at the end of the ripening. Addition of lactic cultures reduced the drying times of PC and PS groups as compared to the control and CS groups.

IV. CONCLUSION

This study indicates that the *L. sakei* in house flora was apparently became dominant during the sucuk fermentation. The autochthonous bacterial starter cultures inoculated sucuk formulation showed a relatively poor competitiveness and weak adaptation when compared with the *L. sakei* in house flora. On the other hand, the addition of lactic cultures in the sucuk formulation increased the counts of lactic acid bacteria while leading to faster pH decrement and reduction of drying times in the sucuk groups when compared to the control groups.

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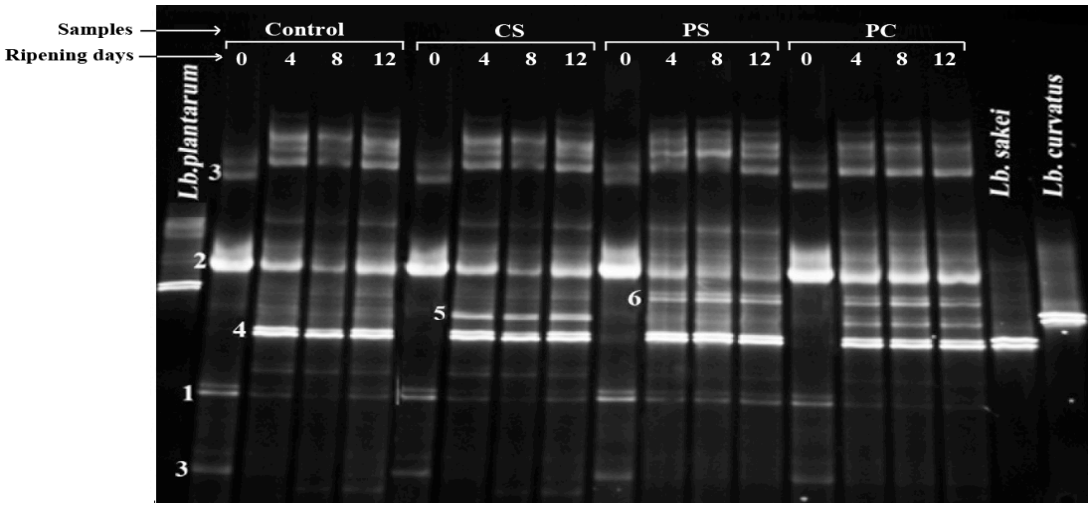

Fig 1: DGGE profiles of the PCR products obtained from sucuk samples during the ripening. PS: *L. plantarum*+ *L. sakei*; PC: *L. plantarum* + *L. curvatus*; CS: *L. curvatus*+ *L. sakei* 1: *Lc. piscium*; 3: *Brochothrix thermosphacta;* 2: *Carnobacterium divergens;* 4: *L. sakei;* 5: *L. curvatus*; 6: *L. plantarum.*

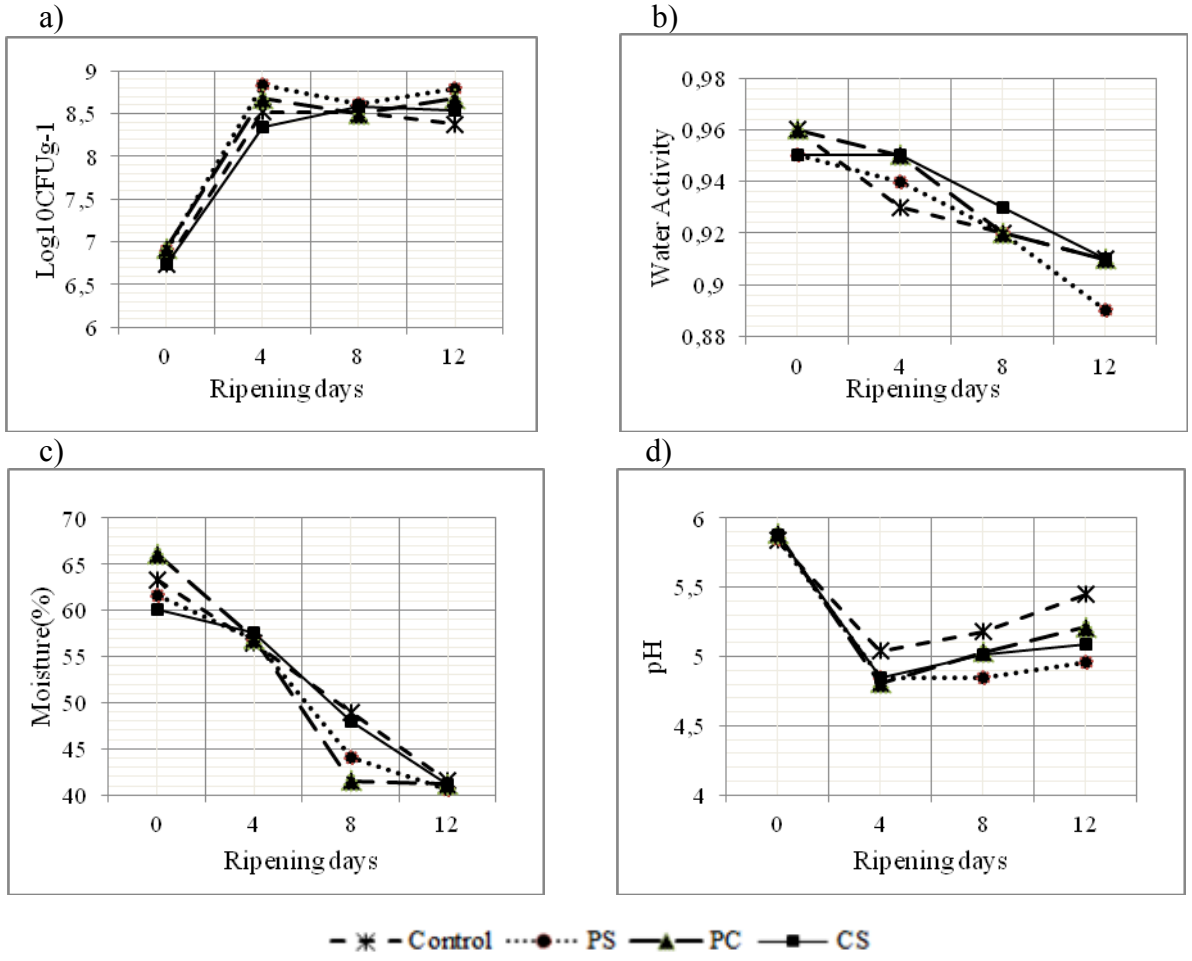

Fig. 2. LAB counts and results of physicochemical analysis during ripening in the sucuk samples. (a) LAB counts, (b) pH, (c) Water activity, (d) Moisture (%), PS: *L. plantarum*+ *L. sakei*; PC: *L. plantarum* + *L. curvatus*; CS: *L. curvatus*+ *L. sakei*