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# THE ROLE OF STARTER CULTURES IN PEPTIDE FORMATION AND ANTIOXIDANT ACTIVITY IN TURKISH FERMENTED SAUSAGE (SUCUK)

B. Arslan Solmaz[1*], S. Bağder Elmacı[2], A. Soyer[2]

[1] Erzincan B. Y. University, Erzincan, Türkiye

[2]Ankara University, Ankara, Türkiye

*Corresponding author email: betul.arslan@erzincan.edu.tr

## I. INTRODUCTION

Fermentation is a critical process in dry-cured sausages, influencing texture, flavor, and safety. Proteolysis, driven by endogenous muscle enzymes and microbial activity, plays a key role in peptide formation. Lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) contribute to proteolysis, affecting the final product's sensory and functional properties [1]. This study aims to evaluate proteolytic changes and antioxidant activity in sausages fermented with different starter cultures, including *Pediococcus pentosaceus*, *Staphylococcus carnosus*, *Lactobacillus sakei*, and *Staphylococcus xylosus*.

## II. MATERIALS AND METHODS

Fermented sausages were made with a standard formulation: control (C) without starters and three groups: S1 (*P. pentosaceus* + *S. carnosus*), S2 (*L. sakei* + *S. xylosus*), and S3 (*L. sakei* + *P. pentosaceus* + *S. carnosus* + *S. xylosus*). Peptides were extracted on days 0, 3, 6, and 12 for concentration, antioxidant activity, and protein degradation analysis. Peptide concentration was measured by the OPA method [2], while antioxidant activity was evaluated using ABTS and FRAP assays [3]. Protein degradation patterns were assessed by monitoring molecular weight changes [4].

## III. RESULTS AND DISCUSSION

Peptide concentration analysis showed a significant increase in small peptides (<3 kDa) over time, with S2 and S3 having the highest levels at day 12 (Table 1). Peptide concentration in unfractionated extracts and the <10 kDa fraction was not affected by starter culture. ABTS radical scavenging capacity increased significantly (P<0.01) over fermentation, with values rising from 0.16 to 0.23 mM TEAC/g peptide after day 3. While the starter culture effect was not significant, higher values in S2 and S3 indicated that smaller peptides had greater radical scavenging capacity [5].

Table 1 – The peptide concentration of 3 kDa peptides extracted from sucuk.

| Treatment | Ripening time (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 3 | 6 | 12 | Mean |
| C | 4.05±0.04 | 4.87±0.03 | 3.12±0.01 | 3.13±0.03 | 3.79±0.01$^{b}$ |
| S1 | 4.36±0.01 | 4.74±0.05 | 3.49±0.03 | 3.78±0.01 | 4.09±0.01$^{a}$ |
| S2 | 3.73±0.02 | 5.74±0.04 | 3.27±0.01 | 3.85±0.01 | 4.15±0.02$^{a}$ |
| S3 | 3.56±0.02 | 5.64±0.03 | 3.05±0.01 | 3.93±0.03 | 4.05±0.01$^{a}$ |
| Mean | 3.92±0.02$^{B}$ | 5.25±0.03$^{A}$ | 3.23±0.02$^{B}$ | 3.67±0.02$^{B}$ | |

Results are presented as the mean ± standard deviation of three replicates. A-C: Different letters in the same row indicate significant differences (P < 0.01). a-b: Different letters in the same column indicate significant differences (P<0.01).

Table 2 – The antioxidant activity measured by FRAP method in 3 kDa peptides extracted from sucuk.

| Treatment | Ripening time (day) | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 12 |
| C | 229.92±0.00$^{Ba}$ | 221.17±0.0$^{Ba}$ | 364.50±0.0$^{Bc}$ | 830.75±0.01$^{Ac}$ |
| S1 | 223.67±0.02$^{Ba}$ | 267.42±0.0$^{Ba}$ | 403.25±0.0$^{Ba}$ | 940.75±0.01$^{Ab}$ |
| S2 | 192.83±0.01$^{Ca}$ | 357.00±0.04$^{Ca}$ | 563.25±0.01$^{Ba}$ | 953.67±0.07$^{Ab}$ |
| S3 | 257.00±0.01$^{Ba}$ | 351.58±0.03$^{Ba}$ | 684.50±0.03$^{Bb}$ | 1245.33±0.02$^{Aa}$ |

Results are presented as the mean ± standard deviation of three replicates. A-C: Different letters in the same row indicate significant differences (P < 0.01). a-b: Different letters in the same column indicate significant differences (P<0.01).

The FRAP assay demonstrated that peptide extracts had increased ferric reducing antioxidant power over time (Table 2). By day 12, the highest FRAP values were observed in S3, followed by S2, suggesting that the presence of specific starter cultures enhances antioxidant activity. These findings align with previous studies reporting that smaller peptides contribute to higher antioxidant potential. SDS-PAGE analysis revealed that high molecular weight

proteins (180 kDa) degraded over time, with significant reductions observed by day 12 (Figure 1). The 100 kDa protein bands disappeared after day 3, while 70 and 55 kDa bands initially increased and later declined. S1 exhibited the fastest proteolytic activity, likely due to *P. pentosaceus* and *S. carnosus* adaptation and enzymatic activity. *S. xylosus* and *L. sakei* showed synergistic effects, enhancing low molecular weight peptide formation. The slow degradation in S2 and S3 suggests a more controlled proteolytic process. The presence of *S. carnosus* in S3 contributed to increased aminopeptidase activity.

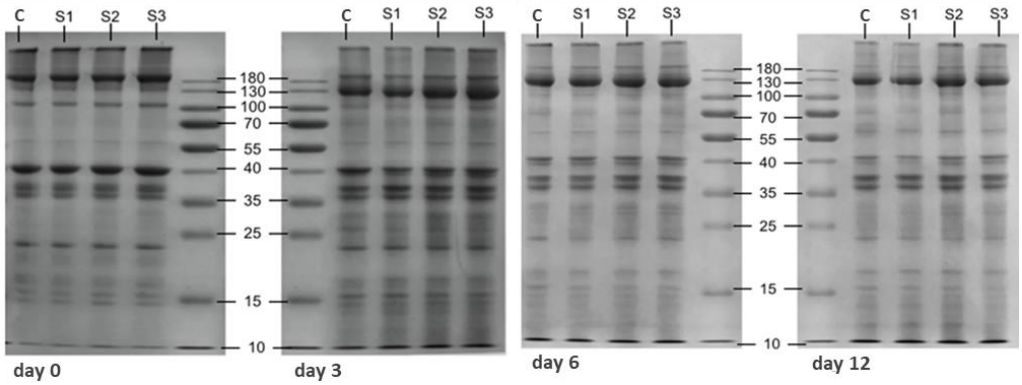



Figure 1. Proteolytic pattern of peptides extracted from sucuk.

## IV. CONCLUSION

Proteolysis varied among starter cultures, with S1 showing the most rapid peptide degradation. The combination of *L. sakei* and *S. xylosus* in S2 enhanced peptidase activity, leading to a higher abundance of low molecular weight peptides. Peptide concentration and antioxidant activity were highest in S2 and S3, highlighting the influence of microbial composition on functional properties. The ABTS and FRAP assays confirmed that smaller peptides exhibited greater antioxidant capacity. The findings emphasize the role of specific starter cultures in optimizing the biochemical and functional attributes of fermented sausages.

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