# BIOPRODUCTION OF CONJUGATED LINOLEIC ACID IN SUCUK (TURKISH DRY FERMENTED SAUSAGE)

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**Abstract – In this study, twenty three *Lactobacillus plantarum* strains were screened in-vitro for CLA-producing ability and *L. plantarum* AA1-2 and *L. plantarum* AB20-961 were selected as a potential strains for CLA production. Optimum conditions for these strains to produce high levels of CLA were determined by evaluating the amount of added hydrolyzed sunflower oil (HSO) and pH in a nutrient medium. These strains were used as starter culture in sucuk manufacture. Control group was also manufactured without starter culture. Two different initial pH levels were applied in treatment groups. Study results indicated that *L. plantarum* AB20-961 produced CLA isomers in sucuk dough at different initial pH levels during first 24 hours of fermentation period. CLA isomers concentration decreased in all sucuk groups during fermentation period ($p<0.05$) but did not alter during the storage ($p>0.05$). On the other hand, there were no significant differences among groups regarding quality parameters of final product.**

**Key Words – Sucuk, CLA, Microbial production, Starter culture**

## I. INTRODUCTION

Biosynthesis of CLA is the strategy for enhancing CLA level in foods. Numerous researchers have reported that several food grade bacterias such as lactobacilli, bifidobacteria and propionibacteria can form CLA isomers from linoleic acid [1]. And many of these bacteria strains occur naturally in meat or are used as starter cultures for meat products. Furthermore, CLA producing potential microorganisms may be used as starter cultures in meat products and may lead to health-promoting meat products [1].

The aim of the present study was to assess if enhanced CLA concentrations can be obtained in fermented sucuk inoculated with L. plantarum strains that are capable of producing CLA. In addition, lipid oxidation, fatty acid composition, water activity, color, textural, chemical, microbiological and sensory properties of sucuk were investigated.

## II. MATERIALS AND METHODS

*L. plantarum* LMG 11405 and *L. plantarum* LMG 23521 selected from the catalog of BCCM/LMG in University of Ghent and 21 *L. plantarum* strains isolated from human sources [2] were used. Among 23 *Lactobacillus plantarum* strains, *L. plantarum* AA1-2 *and L. plantarum* AB20-961 were found to be a potential strains for CLA production. Certain probiotic properties of these strains were determined previously [2]. In addition, optimum conditions for high CLA production were determined. Experimental sucuk production was carried out according to the results of the optimization experiment. Control group (C) was manufactured without the use of starter culture. While two treatment groups were manufactured with *L. plantarum* AA1-2 at two different initial pH levels (A1 and A2), two other sucuks were produced with *L. plantarum AB20-961* at two different initial pH levels (B1 and B2). Sunflower oil was used as substrate on production of CLA because of its high linoleic acid content. Each group was prepared with 2% HSO and 1N NaOH was used to adjust pH to 6.0 in A2 and B2 groups. The experiment was replicated three times.

Fat, protein, ash and moisture contents were determined at the end of ripening period [3,4,5]. Water activity, pH and color were determined during 0, 15 and 30 days of storage [4]. TBARSvalues were determined at manufacturing day, at the end of fermentation and during 5, 10, 15 and 30 days of storage [6]. Total lipid was extracted [7] and fatty acid methyl esters were produced [8]. Fatty acids methyl esters were measured with gas chromatograph (7820A; Agilent Inc.).

## III. RESULTS AND DISCUSSION

The results of in vitro CLA production study indicated that *L. plantarum* AA1-2 *and L. plantarum* AB20-961 were the highest CLA producers when compared to other *L. plantarum* strains. *L. plantarum* AA1-2 and *L. plantarum* AB20-961 strains produced 0.19 % and 0.60 % total CLA isomers from hydrolyzed sunflower oil. Further optimization of reaction conditions showed that the highest CLA production of selected two *L. plantarum* strains used in this study was achieved with pH 6.0 and 2% HSO. Because of the inhibition effect of free linoleic acid, CLA production decreased with increasing concentration of linoleic acid due to higher amount of added HSO.

The results indicated that the average fat, ash, moisture and protein content of sucuk samples before and after fermentation showed non-significant differences among treatment groups (Table 1) except control group which had higher moisture content at the end of fermentation ($p<0.05$). In general, using starter culture had significant effect on the moisture content of sucuks ($p<0.05$).

Table 1. Chemical composition of Sucuk groups.

| Groups | Fat % | | Protein % | |
|---|---|---|---|---|
| | Manufacturing day | End of fermentation period | Manufacturing day | End of fermentation period |
| C | 22,63±1,12$^{aB}$ | 31,14±1,01$^{bcA}$ | 17,01±1,05$^{aB}$ | 21,83±1,04$^{aA}$ |
| A1 | 21,23±2,01$^{aB}$ | 32,79±0,51$^{abA}$ | 16,08±1,78$^{aB}$ | 20,29±2,09$^{aA}$ |
| A2 | 22,17±1,86$^{aB}$ | 33,03±1,38$^{aA}$ | 16,73±1,27$^{aB}$ | 20,64±0,33$^{aA}$ |
| B1 | 22,86±1,25$^{aB}$ | 32,25±0,34$^{abc/}$ | 17,19±1,64$^{aB}$ | 20,79±1,99$^{aA}$ |
| B2 | 21,37±0,74$^{aB}$ | 32,60±1,35$^{abc/}$ | 16,48±1,29$^{aB}$ | 22,21±0,49$^{aA}$ |

| | Moisture % | | Ash % | |
|---|---|---|---|---|
| | Manufacturing day | End of fermentation period | Manufacturing day | End of fermentation period |
| C | 58,69±1,32$^{aA}$ | 39,77±1,16$^{aB}$ | 2,84±0,30$^{aAB}$ | 3,32±0,18$^{aA}$ |
| A1 | 58,54±1,10$^{aA}$ | 35,03±2,63$^{bcB}$ | 2,78±0,27$^{aAB}$ | 3,20±0,12$^{aA}$ |
| A2 | 60,16±2,03$^{aA}$ | 36,15±1,97$^{bcB}$ | 2,71±0,19$^{aAB}$ | 3,36±0,61$^{aA}$ |
| B1 | 58,62±1,76$^{aA}$ | 36,12±2,18$^{bcB}$ | 2,84±0,18$^{aAB}$ | 3,28±0,29$^{aA}$ |
| B2 | 57,26±1,60$^{aA}$ | 36,57±1,75$^{bcB}$ | 2,85±0,31$^{aAB}$ | 3,22±0,10$^{aA}$ |

pH in all sucuk groups decreased during fermentation period ($p<0.05$). pH of sucuks after fermentation ranged from 4.69 to 4.92. The highest pH level was determined in control group which did not contain starter culture ($p<0.05$).

The results indicated that the fatty acid profile of HSO used in this study was consisted of 49.71% linoleic acid, 33.77 % oleic acid, 7.15% butyric acid, 5.51% palmitic acid, 3.12% stearic acid and 0.68% behenic acid. Table 2 shows the fatty acid profiles of sucuk groups. A1, A2, B1 and B2 groups showed higher CLA content compared to the control ($p<0.05$).

Table 2. Fatty acid composition of Sucuk groups.

| Fatty acids | C | A1 | A2 | B1 | B2 |
|---|---|---|---|---|---|
| C4:0 | 0,17$^{b}$ | 0,58$^{a}$ | 0,54$^{a}$ | 0,52$^{a}$ | 0,59$^{a}$ |
| C14:0 | 0,06$^{a}$ | 0,06$^{a}$ | 0,06$^{a}$ | 0,06$^{a}$ | 0,06$^{a}$ |
| C14:1 | 0,24$^{a}$ | 0,25$^{a}$ | 0,25$^{a}$ | 0,26$^{a}$ | 0,26$^{a}$ |
| C15:0 | 0,75$^{a}$ | 0,78$^{a}$ | 0,76$^{a}$ | 0,77$^{a}$ | 0,78$^{a}$ |
| C16:0 | 27,17$^{b}$ | 27,34$^{a}$ | 27,36$^{a}$ | 27,30$^{a}$ | 27,31$^{a}$ |
| C16:1 | 1,38$^{a}$ | 1,41$^{a}$ | 1,39$^{a}$ | 1,40$^{a}$ | 1,40$^{a}$ |
| C17:0 | 2,67$^{a}$ | 2,65$^{a}$ | 2,68$^{a}$ | 2,68$^{a}$ | 2,66$^{a}$ |
| C17:1 | 0,18$^{a}$ | 0,17$^{a}$ | 0,18$^{a}$ | 0,18$^{a}$ | 0,17$^{a}$ |
| C18:0 | 28,09$^{a}$ | 27,86$^{a}$ | 28,06$^{a}$ | 27,97$^{a}$ | 28,05$^{a}$ |
| C18:1 | 28,86$^{b}$ | 29,16$^{a}$ | 29,23$^{a}$ | 29,11$^{a}$ | 29,20$^{a}$ |
| C18:2 | 1,75$^{b}$ | 2,02$^{a}$ | 1,98$^{a}$ | 2,05$^{a}$ | 2,04$^{a}$ |
| C18:3 | 0,26$^{a}$ | 0,25$^{a}$ | 0,26$^{a}$ | 0,26$^{a}$ | 0,26$^{a}$ |
| C20:0 | 0,08$^{a}$ | 0,08$^{a}$ | 0,08$^{a}$ | 0,08$^{a}$ | 0,08$^{a}$ |
| C21:0 | 0,46$^{a}$ | 0,48$^{a}$ | 0,47$^{a}$ | 0,47$^{a}$ | 0,45$^{a}$ |
| C22:0 | 4,18$^{a}$ | 4,18$^{a}$ | 4,18$^{a}$ | 4,18$^{a}$ | 4,18$^{a}$ |
| ∑SFA | 65,74 | 65,47 | 65,52 | 65,49 | 65,48 |
| ∑UFA | 34,26 | 34,53 | 34,48 | 34,51 | 34,52 |
| ∑MUFA | 32,30 | 32,18 | 32,16 | 32,11 | 32,14 |
| ∑PUFA | 2,17 | 2,36 | 2,32 | 2,40 | 2,38 |

Changes in the CLA content in sucuk groups during the fermentation and storage period are presented in figure 1 and 2. The average CLA content in sucuk dough samples showed non-significant differences among groups. However, there were differences for the CLA contents among groups during 24 h fermentation period. During fermentation period, there was a constant decreasing trend in CLA content of the control group.

*L. plantarum* AA1-2 strain did not produce any CLA isomers in both initial pH levels. However, *L. plantarum* AB20-961 strain was able to produce CLA isomers (cis9-trans11 and trans10-cis12) in both pH levels. Especially, the amount of CLA

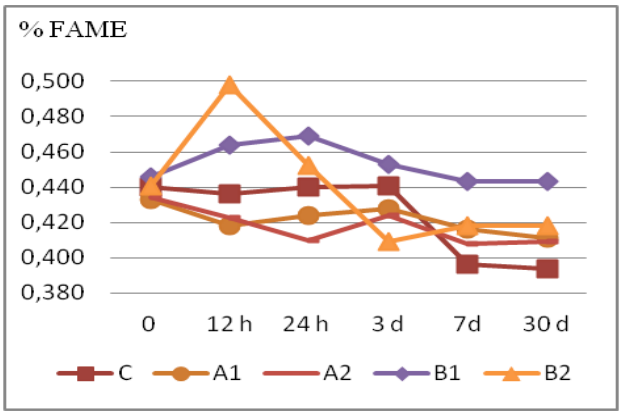

Figure 1. Changes of cis9-trans11 CLA isomer in sucuk.

produced was higher in pH 6.0 during the first 12 and 24 hours of fermentation period ($p < 0.05$). Cis9-trans11 isomer of CLA in B2 increased by 12.5% compared to its initial amount at the beginning of fermentation ($p<0.05$) (Fig.1). Similar result was determined for B1 samples. However, the increase continued until 24th h of the fermentation. Cis9-trans11 isomer of CLA in B1 samples increased by 5% compared to its initial amount ($p<0.05$). Similar increasing trend was also determined for trans10-cis12 isomer of CLA in B1 and B2 groups (Fig. 2).

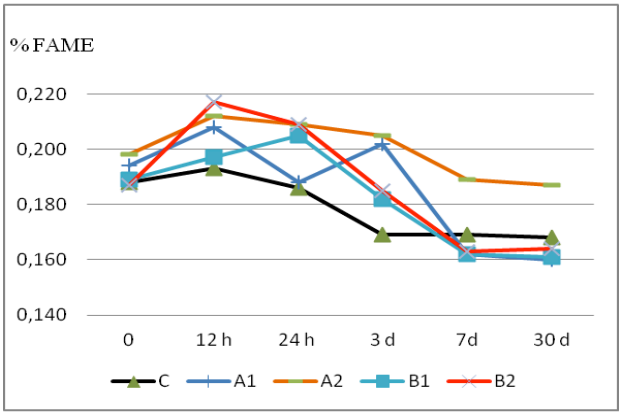

Figure 1. Changes of trans10-cis12 CLA isomer in sucuk

In the first 12 h of the fermentation, trans10-cis12 isomer of CLA in B1 and B2 samples increased by approximately 5% and 17% respectively compared to their initial amount at the beginning of fermentation ($p<0.05$). Statistical analysis results showed that initial pH of sucuk dough has an effect on production of CLA isomers ($p<0.05$). The CLA production in B2 (pH 6.00) was higher than B1 (pH 5.84) after 12 h of fermentation ($p<0.05$).

There were no differences in TBARS among treatment groups at the manufacturing day. The TBARS values of the all sucuk samples increased during fermentation and storage period ($p<0.05$). However, TBARS values were significantly lowered by use of starter culture at end of fermentation and storage ($p<0.05$). Control group had higher TBARS than that of other treatments on 1, 7, 21 and 30 days of storage ($p<0.05$). The changes in amount of CLA during the fermentation period might affect TBARS values at the end of the fermentation. TBARS values of B1 and B2 which had higher CLA production were lower than that of A1 and A2 ($p<0.05$). Considering addition of starter culture and CLA content, this difference is thought to be associated with CLA content.

It is determined that there was a non-significant difference among the L * value of sucuk groups at the end of the storage. Results indicated that a* values of all sucuks slightly increased during the first 3 days of fermentation and decreased during fermentation and storage period ($P<0.05$). Use of starter culture did not influence a* and b* values in sucuk. Furthermore, changes in the amount of CLA in sucuk did also not influence a* and b* values at the end of fermentation and the storage.

Textrure results revealed that use of starter cultures and changes of CLA content had non-significant impacts on textural properties.

Total viable counts increased during fermentation, however, it showed decreasing trend during the storage period due to drying, the low ambient temperature and a decrease in pH. On the other hand, yeast and mold counts in all sucuk groups increased and coliform bacteria counts decreased during fermentation and storage periods.

Raw samples were evaluated for cross-sectional surface color, odor and surface appearance. Cooked samples were evaluated for color, ease of fracture, flavor, odor and the overall acceptability. Sensory evaluation of groups showed that use of starter cultures and changes of CLA content had no effect on sensory properties compared to the control. There were also no differences among groups and all groups received high overall acceptability scores ranging 6-8 score from panelists.

## IV. CONCLUSION

This study concluded that *L. plantarum* AB20-961 with probiotic properties may be used in sucuk manufacture to enhance CLA content without any adverse effects on quality of final product.

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## REFERENCES

1. Gorissen, L. (2011). Production of conjugated fatty acids by bifidobacteria and lactobacilli. PhD thesis. Ghent University and Vrije University Brussel, Belgium, 257 pages.
2. Başyiğit Kılıç, G. & Karahan, A.G. (2010). Identification of lactic acid bacteria isolated from the fecal samples of healthy humans and patients with dyspepsia and determination of their pH, bile and antibiotic tolerance properties. Journal of Molecular Microbiology and Biotechnology. 18, 220-229.
3. Anonymous (2000). Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC. Washington, DC.
4. Anonymous (1995). Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) International. 16th edition, vol. 2, Arlington, Virginia.
5. Chouliara, E., Karatapanis, A., Savvaidis, I.N & Kontominas, M.G. (2007). Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 ºC. Food Microbiology, 24(6), 607-617.
6. Kilic, B & Richards, M.P. (2003). Lipid oxidation in poultry döner kebab: Pro-oxidative and anti-oxidative factors. Journal of Food Science, 68(2), 686–689.
7. Bligh, E.G.& Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 37, 911-917.
8. He, M.L., Mir, P.S., Okine, E.K. & Hapadajlo, H. (2009). Effect of conjugated linoleic acids from beef or industrial hydrogenation on growth and adipose tissue characteristics of rats. Nutrition and Metabolism, 6,19.