OPTIMIZATION OF ENHANCED CONJUGATED LINOLEIC ACID PRODUCTION BY *L. PLANTARUM* AB20-961 AND *L. PLANTARUM* DSM2601 IN MEAT MODEL SYSTEM USING RESPONSE SURFACE METHODOLOGY

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Abstract – This study was conducted to determine the optimum pH, time, temperature, the initial count of added *L. plantarum strains*, and the type and amount of added fatty acid source for high CLA production in ground beef model system. The highest CLA production (7.91 mg CLA/g fat) by *L. plantarum* AB20-961 was obtained under the optimum conditions of pH 7.93, temperature 37 °C, time 78.78 h, 5% fatty acid and 8 log cfu/g initial count of added *L. plantarum* AB20-961. Fatty acid source did not influence yield of CLA production. The highest CLA production (38.31 mg CLA/g fat) by *L. plantarum* DSM2601 was determined under the optimum conditions of pH 7.68, temperature 37 °C, time 78.78 h, 5% added fatty acid source and 8 log cfu/g initial count of added *L. plantarum* DSM2601. In addition, yield of CLA production significantly increased with using safflower oil ($P<0.05$). The results showed that enhancing CLA level in a meat model system can be achieved by using optimum condition determined in this study to satisfy consumer expectations for the meat products with high CLA content.

Key Words – conjugated linoleic acid, microbial production, optimization, beef, chicken.

I. INTRODUCTION

The most common approach of previous studies for evaluating the potential of conjugated linoleic acid (CLA) production by food grade bacteria is *in vitro* setup. However, there are only two previous studies related to enhancing the amounts of CLA in a meat system by fermentation (Gorissen, Raes, De Smet, De Vuyst, & Leroy, 2012; Ozer, Kilic, & Kilic, 2016). These previous studies indicated that many internal and external factors such as pH, time, temperature, initial number of bacteria and the amount and type of fatty acids used were effective in CLA production in a meat system by fermentation and the researchers suggested that the effects of these factors and their optimum application conditions should be determined to obtain high CLA content in meat based products. The purpose of this study was to determine the effect of pH, time, temperature, the initial count of added bacteria, the type and amount of added fatty acid source on the yield of CLA production by *L. plantarum* AB20-961 and *L. plantarum* DSM2061, which showed the ability to produce CLA *in vitro*, and in the meat model system previously (Ozer et al., 2016) and to find the optimum application conditions of these factors to produce a high yield of CLA production.

II. MATERIALS AND METHODS

L. plantarum DSM2601 was selected from the catalogue of DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and *L. plantarum* AB20-961 was isolated from human sources in Suleyman Demirel University (Turkey). Sunflower and safflower oil were hydrolyzed before used and linoleic acid was used directly. Optimization of fermentation conditions for CLA production by *L. plantarum* DSM2601 or *L. plantarum* AB20-961 was carried out using response surface methodology (RSM). The independent processing variables were pH (4.30-9.30), time (0-120 h), temperature (4-37 °C), initial number of bacteria (6-8 log cfu/g) and amount of fatty acid (0-5%). Three different fatty acid sources were linoleic acid (1), sunflower oil (2) and safflower oil (3) which were designed as a blocks. A central composite design was selected for optimization of process variables at 5 levels with 54 runs including twelve replicates at the central point. After the incubation period, the samples were taken for the determination of CLA content. Total lipid extraction and fatty acid analysis were performed by the method of Ozer et al. (2016).

Experimental data was analyzed using Minitab (Minitab 15.1.0.0., Minitab Inc., State College, PA, USA) and SPSS (SPSS 22.0.0, SPSS Inc., Chicago, IL, USA) statistical software and fitted to a second-order polynomial regression model containing the coefficient of linear, quadratic, and two factors interaction effects. Additionally, the effect of fatty acid source was not included in the model equation of response and thus a common model equation for three fatty acids source has been established. However, the effects of fatty acid source on yield of CLA production were determined. The values of R^2 and adjusted-R^2 of models were evaluated to check the model adequacies.

III. RESULTS AND DISCUSSION

CLA production by *L. plantarum* DSM 2601 and *L. plantarum* AB20-961 varied between 0-34.88 mg CLA/g fat and 0-6.72 mg CLA/g fat, respectively.

The surface response analysis for CLA production showed that the main effects of pH ($P \leq 0.05$), time ($P \leq 0.05$), temperature ($P \leq 0.05$) and type and amount of fatty acid source ($P \leq 0.05$) were significant factors for both strains. Amount of added bacteria count was significant for *L. plantarum* DSM 2601 whereas it was not significant for *L. plantarum* AB20-961. Numerous studies about *in vitro* CLA production have reported that properties of CLA producing bacteria and fermentation conditions such as time, temperature and free fatty acid tolerance of bacteria had significant effects on yield of CLA production (Kishino, Ogawa, Yokozeki, & Shimizu, 2009; Tyagi et al., 2015). All main factors had a positive effect on CLA production except added bacteria count for both strains. However, second order effects of pH and time parameters had negative effects. Therefore, after a certain time and pH value, yield of CLA production begun to fall. The results revealed that type of fatty acid source did not significantly affect yield of CLA production for *L. plantarum* AB20-961. However, yield of CLA production by *L. plantarum* DSM2601 significantly increased with using safflower oil ($P<0.05$). Similar results about effects of pH, time, type and amount of fatty acid source on CLA production *in vitro* have been reported previously (Gorissen et al., 2012).

IV. CONCLUSION

The maximum CLA production by *L. plantarum* DSM 2601 and *L. plantarum* AB20-961 were obtained as 38.31 and 7.91 mg CLA/g fat with 7.68 and 7.94 pH, and 73 and 79 h of fermentation time, respectively. In addition, a 37 °C fermentation temperature, 8 log cfu/g initial bacteria count and 5% added fatty acid source were other optimum conditions determined for the highest CLA production by both *L. plantarum* AB20-961 and *L. plantarum* DSM2061.

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