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Research Article

Optimization of Conjugated Linoleic Acid and Eicosapentaenoic Acid Production from Sesame Waste by Response Surface Methodology

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Conjugated linoleic acid (CLA) and eicosapentaenoic acid (EPA) have been recognized for their physiological functions and potential as dietary supplements. This study is aimed at investigating the production and enhancing the efficiency of these two acids from *Lactiplantibacillus plantarum* using sesame waste as a natural, cost-effective, and readily available culture medium. Response surface methodology (RSM) was employed to optimize the production of CLA and EPA in a solid bed system using sesame waste paste as the fermentation substrate. The main processing parameters, including three humidity levels (60%, 70%, and 80%), three inoculum percentages (2%, 4%, and 6% *v*/*v*), and three fermentation temperatures (30°C, 37°C, and 44°C), were optimized. An experiment was conducted to validate the determined conditions, and a strong correlation was observed between the experimental results and the predictions made by the software. The optimal conditions for CLA and EPA production were determined to be an inoculation level of 6% with 80% humidity and at 37°C. Gas chromatography analysis of the fermented sesame waste medium revealed that the highest yields of CLAc9t11 and CLAt10c12, as well as eicosapentaenoic acid, were obtained in the medium fermented with 80% humidity and 6% inoculation at 37°C. The respective percentages of these fatty acids in the total fatty acid composition were found to be 0.351% (*w*/*v*) and 0.1% (*w*/*v*) for CLA and 0.139% (*w*/*v*) for EPA under the optimized conditions. These findings contribute to the understanding of CLA and EPA production and highlight the potential of *L. plantarum* and sesame waste as a fermentation substrate for their efficient production.

1. Introduction

In recent years, there has been a growing interest in the production of healthy and safe food products, with consumers seeking natural foods that can improve their health through active ingredients. Fats, being a major component of foods, have raised concerns due to their association with cardiovascular diseases, diabetes, and obesity. However, certain types of fats, such as conjugated linoleic acid (CLA) and eicosapentaenoic acid (EPA), have demonstrated beneficial physiological effects on humans' health [1].

CLA is a naturally occurring unsaturated trans-fatty acid found in the meat and milk fat of sheep, goats, and deer [2, 3]. It is categorized as nontrans according to the FDA and is

considered safe (GRAS) [4]. EPA, on the other hand, is a highly unsaturated omega-3 fatty acid found in cold-water fish and certain seaweed [5]. A group of bacteria like *Gammaproteobacteria* and *Shewanella* and *Bifidobacterium lactis* can biosynthesize this fatty acid [6, 7]. It has been associated with beneficial effects on the cardiovascular system, and EPA supplements have shown the potential to reduce triglyceride levels in individuals with high blood triglyceride levels [8, 9].

CLA, specifically the isomers CLAc9t11 and CLAt10c12, has been found to possess anticancer activity, while CLAc12t10 and CLAt9c11 have been associated with reducing body fat, losing weight, and increased energy consumption [10–12]. However, the chemical methods traditionally used to produce CLA often lead to the unexpected production of different isomers. Therefore, there is a need to identify a healthy, safe, and selective process for CLA production [13, 14].

Microbial production of CLA offers an alternative approach, where microorganisms can biocatalyze linoleic acid to produce CLA or be used to ferment foods to produce CLA. *Lactiplantibacillus plantarum* is a genus of lactic acid bacteria (LAB) known for its adaptability and widespread presence in various fermented foods [15–19]. *L. plant*arum has been shown to have health benefits, including reducing gastrointestinal infections, inflammatory bowel diseases, and exhibiting immune-stimulating effects [20, 21].

The development of economic technologies to increase the nutritional value and bioactive compounds of natural has attracted considerable attention in recent years [22]. Every year, large quantities of residues are produced in the agricultural and food industries; if recycling of this waste is well managed, it could have many economic and environmental benefits [23]. In addition, the use of metabolites can generate new sources. The waste of food oil factories is a problem in developed countries; it is of particular importance to researchers due to environmental issues [24] as the seeds of oil products are a significant share the production. So, it is considered as a source. Moreover, for proper evaluation, waste recycling can be important in terms of economic, environmental, social, and ecological aspects. Therefore, conversion of biomass into high value-added compounds can be very beneficial [23].

Sesame seeds contain high amount of oil (42-56%) and protein (20-25%) and contain high levels of calcium, magnesium, and minerals. Major fatty acids in sesame seeds include linoleic acid (40.5-47.9%), oleic acid (35.9-42.3%), palmitic acid (7.9-12%), and stearic acid [16, 18]. Sesame seeds are mechanically pressed to extract oil by cold pressing or chemically processed with organic solvents such as hexane. The cold press method leads to products free of organic solvents [25]. After the production of oil from oil seeds, valuable by-products (cakes/meal) rich in proteins, few lipids, carbohydrates, and bioactive compounds may be obtained [26]. In addition, they could be considered as a rich source of protein, as they contain amino acids, oligosaccharides, vitamins B and E, and minerals [27]. Also, they contain isoflavones that can promote the growth of microorganisms. So, during the past years, chicken skin and soybean meal have been used as rich sources to produce CLA [7, 28]. Sesame waste is considered an economical source rich in linoleic acid, making it a promising substrate for CLA production [7]. However, to the best of our knowledge, no study has investigated the potential application of sesame waste for CLA production using *L. plantarum*.

One of the oldest processes applied by humans is the solid-state fermentation (SSF) used for food [4]. Solid fermentation is a biological process with a high potential for bioenhancing the conversion of plant wastes into many valuable compounds [22]. SSF has many advantages, including cost-effectiveness, less volume of equipment, high efficiency production per unit volume and easier aerobic process. In addition, there is an increase of oxygen diffusion rate in wet solids [29, 30]. The selection of bacteria capable of synthesizing CLA or CLNA under in vitro condition is the first step required to evaluate the possible effect of their production during food fermentation or their impact on the surface of the intestine. Conversion and isomer patterns depend on various factors [30]. In this regard, *Lactiplantibacillus plantarum* have attracted much attention. There is also some evidence showing that the ability to convert linoleic acid (LA) to CLA is strain specific [31] and that the conversion rates vary, depending on growth conditions and matrix [6]. Few studies have been, however, performed on the production of fatty acids with the help of a group of bacteria such as *Lactiplantibacillus plantarum* and *Bifidobacterium* species, which are mainly used as probiotics [32]. Most strains of *Lactiplantibacillus plantarum* are more efficient at synthesizing CLA and conjugated linolenic acid (CLNA) [33]. Not only they have the potential to accelerate the production or recovery of CLA and CLNA from LA and A-linolenic acid (ALA) [6, 30, 33], but also they have the ability to grow in sesame waste and used carbohydrates by modifying the substrate to increase its nutritional and functional properties [34, 35]. By producing the enzyme, A-galactosidase or B-glucosidase, and also hydrolyzing the proteins in it, lactic acid can be produced with a decrease in pH. Producing functional foods enriched in conjugated fatty acids by using them as starter or adjunct culture can be considered a promising topic for further development and study [6].

Innovation has always been the key to success. We should make the optimal use to ensure future progress and success. Given the rate of obesity and mortality due to cardiovascular diseases, diabetes, and cancer, especially in the young generation [8], and considering the potential health benefits of CLA and EPA, as well as their applications in medicine, pharmacy, and the food industry, understanding their production and optimization is crucial [13, 14]. Sesame waste can be considered as an excellent, natural, low-cost, and cost-effective substitute serving as a substrate for use in SSF for the production of fatty acids [14]. By identifying and optimizing biological reactions, it becomes possible to develop safe and efficient processes for producing these bioactive fatty acids [16, 17]. In this study, to optimize and maximize the production of CLA, a response surface model (RSM) design was employed using a solid bed system of sesame waste. The solid fermentation substrate used a homogeneous sesame paste with varying degrees of humidity, ranging from 60% to 80%. The factors considered for

optimization were the amount of inoculation (2%, 4%, and 6%), humidity (60%, 70%, and 80%), and temperature (30°C, 37°C, and 40°C). The maximum production of CLA was also optimized. The per capita consumption of fish and seafood in Iran is relatively low compared to developed countries, making the investigation of EPA and CLA production and its potential application in the country particularly relevant [36]. The aim of this study was, therefore, to produce beneficial fatty acids (CLA and EPA) by *Lactiplantibacillus plantarum* on a sesame waste-based substrate as a natural, rich, suitable, inexpensive, and available environment to return part of the waste of food oil factories in the production cycle.

2. Materials and Methods

2.1. Preparation of Materials. In this study, cold-pressed sesame waste was obtained from Tehran Afsaneh Oil Company. *Lactiplantibacillus plantarum* (DSMZ 20174) was obtained from the Microbial Bank of the Faculty of Agriculture, Tehran University. MRS media were sourced from Canadian Quelab Company (Canada), and chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Preparation of Microbial Culture

2.2.1. Bacterial Enumeration. In this process, the bacterium was linearly cultured twice, each for 72 h, in an MRS agar medium containing 0.5 gram per liter L-cysteine, and the plate was sealed with paraffin and incubated at 37° C for 72 hours. To enrich bacteria, they were first put in an MRS broth medium and incubated three times, each for 24 h, at 37°C. The cells were collected at the end of the growth phase in the MRS broth through the centrifuge and rinsed twice to obtain $1.5 * 10^7$ in 0.5 McFarland standard; then, the turbidity of the bacterial suspension was adjusted to the 0.5 McFarland standard ($1.5 * 10^8$). The inoculation size was optimized to support solid bed fermentation; thus, dilutions of 2, 4, and 6% were made. To draw the growth kinetics, curve was made by the first-degree equation ($rx = dCx/dt = \mu Cx - KdCx$) [34]. After bacteria transfer to the broth media, the agar rate absorption at 0, 1, 2, 3, 4, 12, 18, 24, 30, 36, 48, 60, and 72 h was measured to draw the curve (Figure 1).

2.2.2. Preparation of the Fermentation Medium. To culture *Lactiplantibacillus plantarum* in the fermentation medium, the sesame waste was soaked in water for 12 hours. Subsequently, the humidity of the medium was adjusted within the range of 60-80% using sterile distilled water. The mixture was then homogenized thoroughly. It is important to note that the moisture content of the sesame waste after sterilization served as the baseline humidity. Next, 100 g of the resulting paste was transferred into an Erlenmeyer flask and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was cooled to 35°C in preparation for fermentation [7].

2.2.3. Inoculation of Bacteria into the Culture Medium. In order to adjust the density of the inoculated microbial suspension, the bacteria were harvested from the logarithmic phase and transferred to a tube containing the sterile physiological serum. The resulting turbidity was compared with the standard McFarland standard solution [37].

To confirm the turbidity of the microbial suspension prepared according to the half-McFarland standard, 3×10^{-7}, 6×10^{-7}, and 9×10^7% inoculates were cultured in plates containing the sterile culture medium of sesame waste and then incubated in the incubators at 30, 37, and 44°C for 48 hours [37].

2.3. CLA Production Optimization. To optimize and maximize the production of CLA, a response surface model (RSM) design was employed using a solid bed system of sesame waste. The solid fermentation substrate used was a homogeneous sesame paste with varying degrees of humidity, ranging from 60% to 80%. The factors considered for optimization were the amount of inoculation (2%, 4%, and 6%), humidity (60%, 70%, and 80%), and temperature (30°C, 37°C, and 40°C) [35]. The pH changes of the samples after inoculation time were recorded and reported as responses, after 48 h incubation, rapid spectrophotometry based on UV absorption of their growth ability producing CLA according to [5], response 2. The Design-Expert software was utilized to analyze the data and create a mathematical model (Table 1). The analysis of variance and regression model confirmed the statistical acceptability of the production model.

2.3.1. Evaluation of the Nutritive and Inhibitory Capacity of the Culture Medium. The nutritive capacity of the culture medium was assessed by diluting the initial suspension with normal saline or distilled water at a ratio of 1 : 100. Subsequently, 0.01 mL of the diluted suspension was inoculated into the culture medium. To analyze the inhibition capacity of the culture medium, the initial suspension was diluted with normal saline or sterile distilled water at a ratio of 1 : 10. Then, 0.01 mL of the diluted suspension was inoculated into the culture medium [38].

2.4. Investigation of the pH of the Culture Medium after Fermentation. The pH changes of the sesame waste during a 48-hour fermentation period were evaluated using a pH meter (Metrohm, model 827, Switzerland). The pH meter was used to measure the pH of the culture medium at different time points during the fermentation process (Table 1).

2.5. Spectrophotometric Analysis of CLA in the Supernatant. To assess the production of CLA by *Lactiplantibacillus plantarum*, the method proposed by Raimondi et al. [31] was employed. A fast spectrophotometer (WPA, model, UK) device from the Health Management Company was used for this purpose. The culture sample was centrifuged at 13,000 rpm for 5 minutes, and 1 mL of the supernatant was mixed with 2 mL of isopropanol. To the sample, 1.5 mL of hexane was added, followed by thorough mixing and incubation for 5 minutes. The hexane layer was collected, and the absorbance value was recorded at 233 nm using the spectrophotometer. This analysis allowed for the screening of CLA production by *L. plantarum* (Table 1).

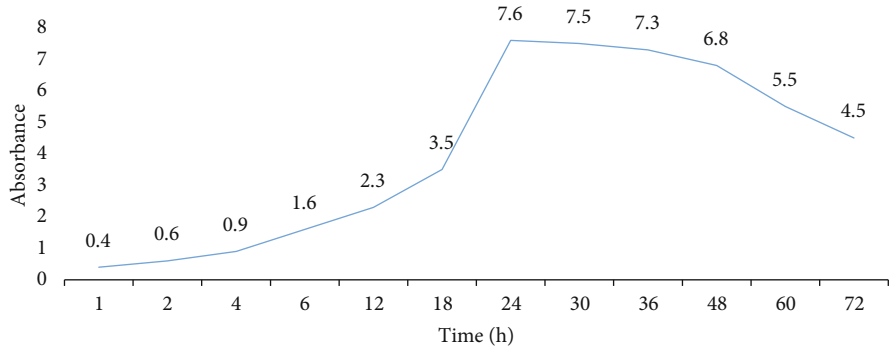


Figure 1: *Lactiplantibacillus plantarum* growth curve.

Table 1: Independent variables and response data in the response surface methodology (RSM) method.

Real Run	Value x_1	x_2	x_3	Response 1 pH	Response 2 Absorbance
1	37 (0)	2 (-1)	60 (-1)	4.98	0.65
2	30 (-1)	4 (0)	60 (-1)	4.8	0.7
3	37 (0)	4 (0)	70 (0)	4.82	0.8
4	44 (+1)	2 (-1)	70 (0)	5.2	0.2
5	30 (-1)	4 (0)	80 (+1)	4.95	2.5
6	37 (0)	4 (0)	70 (0)	4.83	0.43
7	37 (0)	4 (0)	70 (0)	4.82	0.3
8	37 (0)	2 (-1)	80 (+1)	4.9	1.5
9	37 (0)	6 (+1)	60 (-1)	4.97	0.5
10	37 (0)	4 (0)	70 (0)	4.85	0.47
11	30 (-1)	2 (-1)	70 (0)	4.98	0.9
12	44 (+1)	4 (0)	60 (-1)	5.2	0.2
13	37 (0)	4 (0)	70 (0)	4.75	0.4
14	37 (0)	6 (+1)	80 (+1)	4.7	2.5
15	44 (-1)	4 (0)	80 (+1)	4.8	0.3
16	30 (-1)	6 (+1)	70 (0)	5	0.8
17	44 (+1)	6 (+1)	70 (0)	5	0.3

x_1: temperature; x_2: inoculation; x_3: humidity. Average and standard deviation for pH and absorbance are 4.91 ± 0.142 and 0.64 ± 0.720, respectively.

2.6. Determination of Fatty Acid Profile by Gas Chromatography (GC). To extract and separate the oil, 5 g of the sample was mixed with 100 mL of a chloroform-methanol mixture in a volume ratio of 2 : 1. The mixture was then centrifuged for 3 minutes at 3000 rpm using a SIGMA 30 K-3 centrifuge (model company, UK). After centrifugation, the solution was mixed twice with filter paper and filtered. Distilled water (5 mL) was added twice to the filtrate and vigorously stirred. The mixture was then centrifuged again at 5000 rpm for 5 minutes, resulting in the formation of two layers. The upper layer, containing water and methanol, was discarded, while the bottom layer, containing chloroform and fatty acids, was concentrated using a rotary evaporator (IKA ∗ RV10, Germany).

Further analysis of the extracted fat was conducted using gas chromatography. The extracted fat was first methylated by adding 5.0 mL of 1 N methanol sodium to the fat extract and vortexing for 1 minute. The mixture was then kept at 70°C for 15 minutes. Afterward, 1 mL of boron trifluoride containing 14% methanol was added, and the mixture was gently shaken at room temperature for 10 minutes. Following this, 2 mL of hexane was added, and the mixture was vortexed for 1 minute and centrifuged at a high speed. The supernatant was removed, and the pellets were washed with 5 mL of distilled water and centrifuged at 11,000 rpm. Additional water rinsing was performed, and the organic phase was separated. Anhydrous sodium sulfate was added to the organic phase and filtered through filter paper. Two to four microliters of the fatty acid methyl ester solution were injected into the liquid gas chromatography injection site. A capillary column, Dikmacap-2330, with a length of 60 meters and an inner diameter of 25 mm, was used. The injection site temperature was set at 250°C, and the detector temperature was set at 260°C. Hydrogen gas with a purity of

Table 2: Independent variables and their values.

Independent variables	Symbol	Code levels		
		+1	0	-1
Temperature	x_1	30	37	44
Inoculation	x_2	2	4	6
Humidity	x_3	60	70	80

99.99% and a flow rate of 2 mL/min was used as the carrier gas. The initial column temperature was set at 60°C and held for 2 minutes. It was increased to 240°C with a gradient of 10°C per minute and held at that temperature for 7 minutes to allow for sufficient elution of the fatty acids from the column [34]. The identification of fatty acids was performed by comparing the retention times of pure fatty acid standards, and the results were expressed as percentages.

2.7. Statistical Analysis. The main and interaction effects of the experimental factors were analyzed using the response surface methodology (RSM) statistical design. In RSM, a model is defined for each dependent variable to express the main and interaction effects of factors on each variable separately. The multivariate model is represented by Equation (1), where Y represents the predicted response, b_0 is the constant coefficient, b_i represents the linear effects, b_{ii} represents the square effects, and b_{ij} describes the interaction effect.

$$Y_n = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_{ij} + \sum_{i \neq j=1}^{3} b_{ij} X_i X_j. \quad (1)$$

In this study, the box-bank design (BBD) was employed as a part of the RSM method to assign codes to the independent variables. The codes assigned to the independent variables in the experiment are described in Table 2. After implementing the RSM method and producing CLA and EPA, Excel software was used to generate the diagrams. The statistical analysis was performed using SPSS software, version 16.

3. Results and Discussion

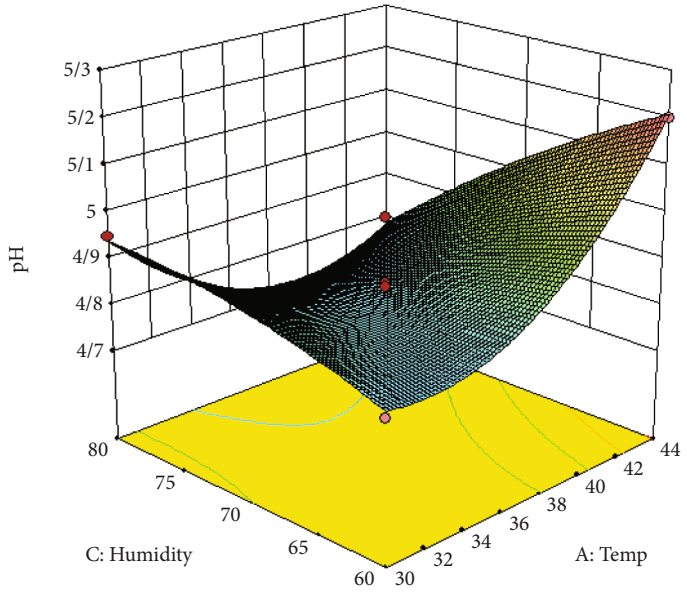
3.1. Growth Rate of L. plantarum. The growth kinetics of *L. plantarum* in the sesame waste during a 72-hour fermentation period at 37°C is plotted in Figure 1. To assess the growth patten of the microorganisms, they are cultured in MRS agar for 72 h at 37°C. So, pH, temperature, and microbial growth stages are the most critical factors in the biological viscosity that inoculated linoleic acid. The growth of *Lactiplantibacillus plantarum* on the sesame waste was evaluated in different conditions including pH, temperature, humidity, and inoculum percentage. The results showed that microorganisms could grow well in substrate at different humidity, temperature, and inoculum levels. The bacterial count did not show a significant increase during the early stages of the fermentation process, due to the adaptation of the microorganisms to the new growth environment, and there was no significant change in cell population and pH.

Table 3: Analysis of the variance of the effect of independent variables on pH and absorption.

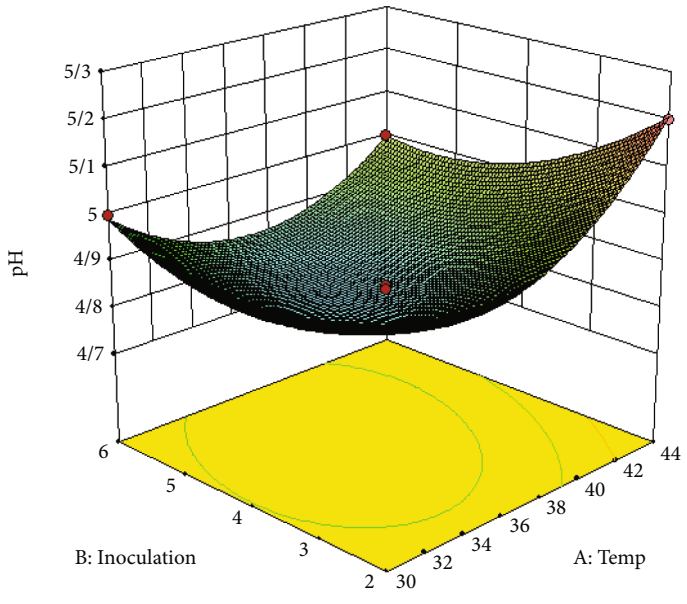
Source	df	pH	Abs
Model 1	9	0.3^{***}	59.7^{**}
x_1	1	0.028^{***}	1.90^{***}
x_2	1	0.019^{***}	0.090
x_3	1	0.045^{***}	2.82^{**}
x_1x_2	1	0.012^{***}	0.010
x_1x_3	1	0.076^{***}	0.72^{*}
x_2x_3	1	$9.025E\text{-}0.035^{***}$	0.33
x_1^2	1	0.0073^{***}	0.10
x_2^2	1	0.028^{***}	0.18
x_3^2	1	$2.8E\text{-}0.03^{*}$	1.42^{**}
Residual	7	$2.155E\text{-}0.03$	0.62
Lack of fit	3	$1.475E\text{-}0.03^{ns}$	0.48^{ns}
Pure error	4	$6.800E\text{-}0.04$	0.14
Corrected total	16	0.30	8.21
R^2		0.92	
R^2 (adj)		0.82	

*, **, and *** represent nonsignificance and significance at the probability level of 5, 1, and 0.001%, respectively. ns, *, **, and ***: nonsignificant and significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$ probability levels, respectively. x_1: temperature; x_2: inoculation; x_3: humidity.

However, after 6 h of fermentation, there was a sharp decrease in pH; in addition, the microbial population began to increase significantly as the microorganisms adapted to the conditions of the medium. The highest growth of *L. plantarum* in the medium was observed between 18 and 24 hours, and the bacterium entered the logarithmic phase, reaching its maxim population. In the last hour of fermentation (48 h), the decrease in the bacteria count was apparent by a lower drop of pH, at all levels. In contrast, the pH of the control sample remained unchanged. In general, the fermentation process of *Lactiplantibacillus plantarum* was within 6-48 h; however, in this experiment, the bacteria growth occurred within 24-48 h after inoculation. After the complete fermentation of the substrate, the bacteria growth rate in the sesame waste followed a descending trend. After approximately 48 to 72 h, it stopped, leading to the death of some bacteria. These findings are consistent with the study conducted by Raimondi et al. [31]. Martha et al. [3] suggested that the slow growth of bacteria in the initial hours of fermentation could be attributed to their limited proteolytic activity [39]. It is worth noting that sesame proteins can be effectively hydrolyzed during fermentation, resulting in the formation of polypeptides and peptones, such as soluble nitrogen [40]. The hydrolysis of proteins in the fermented sesame waste depends on the type of bacterial strain and the substrate humidity content [41]. It was found that an increase in the produced lactic acid volume in the culture medium decreased the pH rate. *Lactiplantibacillus plantarum* in the sesame waste exhibited high compatibility at 80% humidity and 6% inoculation level, with the lowest

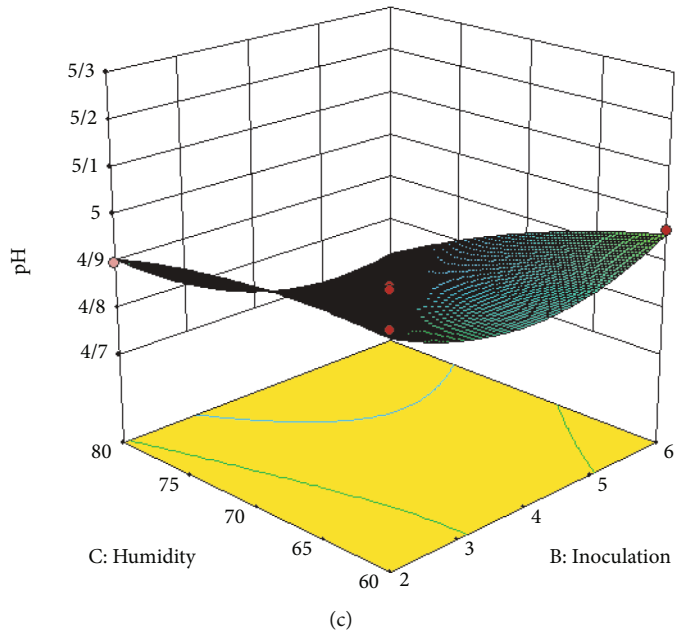


(a)



(b)

Figure 2: Continued.



(c)

Figure 2: Interaction effect of (a) temperature and humidity, (b) inoculation rate and temperature, and (c) humidity and inoculation rate on the pH of the medium.

pH of 4.7 at 37°C (Table 1). The bacteria count reduction rate after 48 h was due to the decrease in the pH and volume of the nutrients [35].

3.2. Interaction Effects of the Independent Variables on the pH and Absorption Based on RSM. Table 3 displays the adjusted R^2 and R values, indicating that the designed response surface methodology (RSM) models were highly accurate. The high values of these parameters suggest a strong correlation between the observed and predicted values. The F-value obtained from the response coefficients indicated that most of the quadratic linear coefficients and interactions were significant at the 1% and 5% probability levels. The analysis of variance through the response surface methodology demonstrated the significance of these models for both pH and absorption (Table 3). Based on the fitted models, the equations for pH (Equation (2)) and absorption (Equation (3)) are as follows:

$$\text{pH} = +4.83 + 0.059x_1 - 0.049x_2 - 0.075x_3 - 0.055x_1x_2 - 0.14x_1x_3 - \frac{0}{047x_2x_3} + 0.13x_1^2 + 0.082x_2^2 - 0.026x_3^2, \quad (2)$$

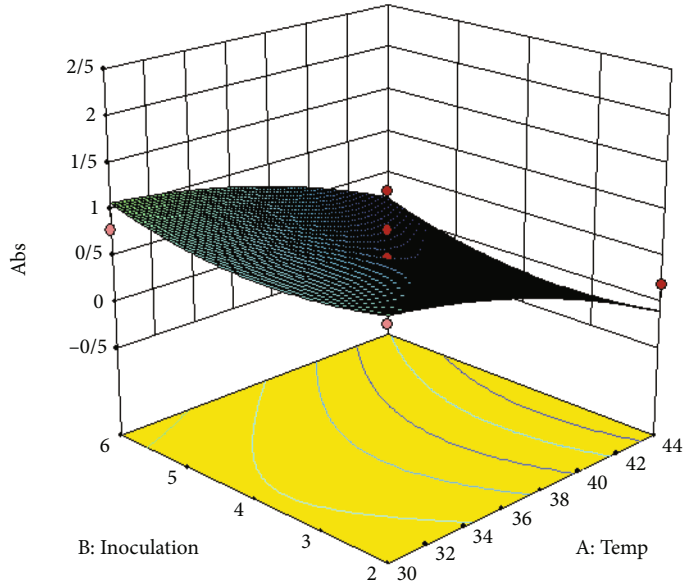
$$\text{Abs} = +0.50 + 0.49x_1 + 0.59x_3 - 0.43x_1x_3 + 0.58x_3^2. \quad (3)$$

Temperature (x_1), inoculation rate (x_2), and humidity (x_3) showed a significant effect on pH ($p < 0.001$). Additionally, temperature ($p < 0.001$) and humidity ($p < 0.01$) had a significant effect on absorption, while the inoculation rate did not show a significant effect. Table 3 reveals that the quadratic effects of temperature and inoculation factors ($p < 0.001$) and humidity ($p < 0.05$) on pH were significant. The quadratic effect of moisture content ($p < 0.01$) on absorption was also significant. Furthermore, the interaction effects of temperature and inoculation (x_1x_2), temperature and humidity (x_1x_3), and humidity and inoculation (x_2x_3) on pH were found to be significant ($p < 0.001$). However, only the interaction of temperature and humidity (x_1x_3) had a significant effect on absorption ($p < 0.05$).

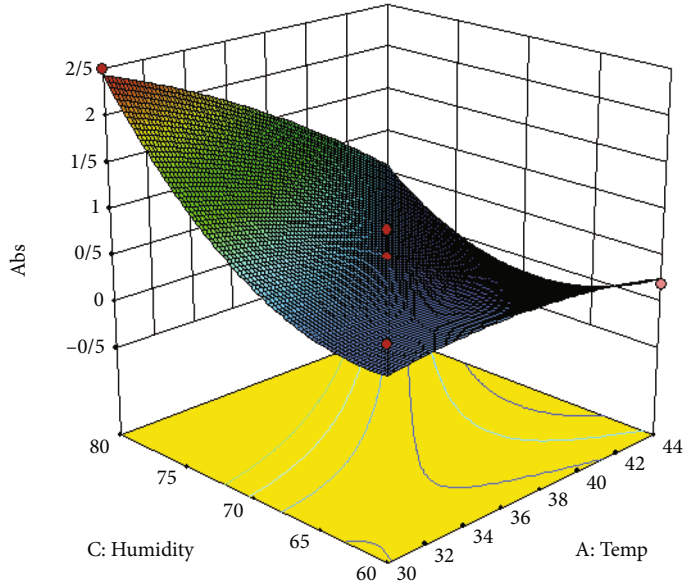
The diagram illustrating the changes in the pH of the medium, based on the variables x_1 (temperature), x_2 (inoculation rate), and x_3 (humidity), is presented in Figure 2. Each diagram represents the interaction of the independent factors on the measured pH. The maximum value observed in each sample corresponds to the optimal interaction of two independent factors on the pH parameter. pH was measured immediately after the inoculation of *L. plantarum* into the culture medium, as well as at 24 and 48 hours.

In all samples, a significant decrease in pH was observed after 24-48 hours of the fermentation process, while no significant change in pH was observed in the control sample. The analysis of the effect of the three independent factors (temperature, inoculation rate, and humidity) on pH revealed that pH decreased with increasing temperature and decreasing inoculation rate and moisture content.

The analysis of the effect of temperature and inoculation rate on pH demonstrated that the lowest pH was obtained at a temperature of 37°C and an inoculation rate of 6, while the highest pH was achieved at a temperature of 44°C and an inoculation rate of 2 (Figure 2(a)). Similarly, the study of temperature and humidity indicated that the highest pH was observed at 44°C with 60% humidity, while the lowest pH was observed at 37°C with 80% humidity (Figure 2(b)). Furthermore, the investigation of the effect of humidity and inoculation rate on pH revealed that increasing humidity and inoculation rate led to a decrease in pH (Figure 2(c)).

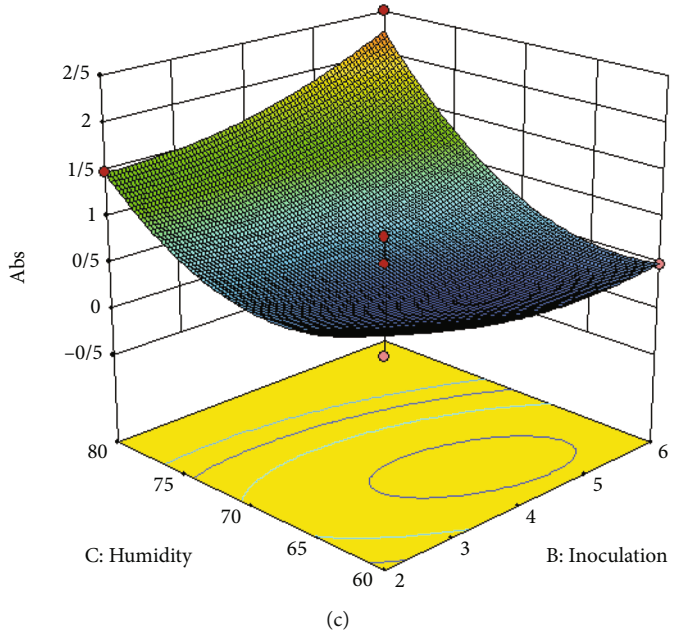


(a)



(b)

Figure 3: Continued.



(c)

Figure 3: Interaction effect of (a) temperature and inoculation rate, (b) temperature and humidity, and (c) humidity and inoculation rate on the amount of absorption.

Table 4: Optimal values of parameters and the response variable under optimal conditions of CLA production.

	Predicted value	Experimental value
pH	4.71	4.7 ± 0.2
Absorbance	2.5	2.5 ± 0.3

During the fermentation process, the growth of bacteria in the medium resulted in the consumption of carbohydrates and their conversion into organic acids, leading to a decrease in pH [33]. The pH of the samples was approximately 6 before the fermentation process, but it decreased to nearly 5 after 24 hours and below 5 after 48 hours due to the action of *L. plantarum*. These findings are consistent with the study conducted by Vinderola and Reinheimer [42], which showed a decrease in pH after 24 hours of inoculating *L. plantarum* into milk samples [43]. Additionally, Robert et al. [44] demonstrated that *L. plantarum* (optional heterofermentative) led to a faster reduction in pH compared to obligatory heterofermentative species. However, after 20 hours of fermentation, the final pH was higher with the obligatory heterofermentative species. Hence, the acidification properties during the fermentation process depend on the specific species and strain of LAB [44]. It is important to note that CLA production is dependent on the enzymatic conversion of linoleic acid, which may be influenced by the pH conditions.

The diagram of the absorption rate based on the three independent variables is shown in Figure 3. Evaluation of the effect of three independent factors including temperature (x_1), inoculation rate (x_2), and humidity (x_3) on the absorption rate showed that increasing the temperature and decreasing inoculation and humidity reduced the absorption. The evaluation of the combined effect of temperature and inoculation on pH also showed that the highest adsorption was related to the temperature of 37°C and the inoculation rates of 2 and 6%, as well as the temperature of 30°C and the inoculation rate of 2 (Figure 3(a)). The study of the two factors of temperature and humidity also showed that the highest rate of absorption was at 37°C with 80% humidity (Figure 3(b)). Further, examination of two factors, humidity and the inoculation rate, revealed that in 80% humidity and the inoculation rate of 2, 4, and 6%, absorption was at its highest level; in all three inoculation rates, adsorption was increased with raising humidity (Figure 3(c)). As shown in Figure 3, inoculation rate, temperature, and humidity had a significant effect on CLA production by *L. plantarum* in the sesame waste culture medium. Terán et al. [45] also stated that the substrate concentration had a significant effect on conversion (LA/LNA), even if bacterial growth was not affected [45]. Therefore, it could be stated that the substrate concentration was effective on the total amount of CLA produced by *L. plantarum* [13].

3.3. Optimization Confirmation. The main objective of the experimental design was to optimize the production of CLA. The optimization results are presented in Table 4. It can be observed that the experimental results are in good agreement with the values predicted by the model under the optimal conditions. This confirms the reliability and accuracy of the optimization process.

3.4. Fatty Acid Composition. The gas chromatography (GC) analysis of CLA production in the sesame waste culture medium by *L. plantarum* is shown in Figure 4. As indicated in Figure 4, in addition to the ability of *L. plantarum* strain

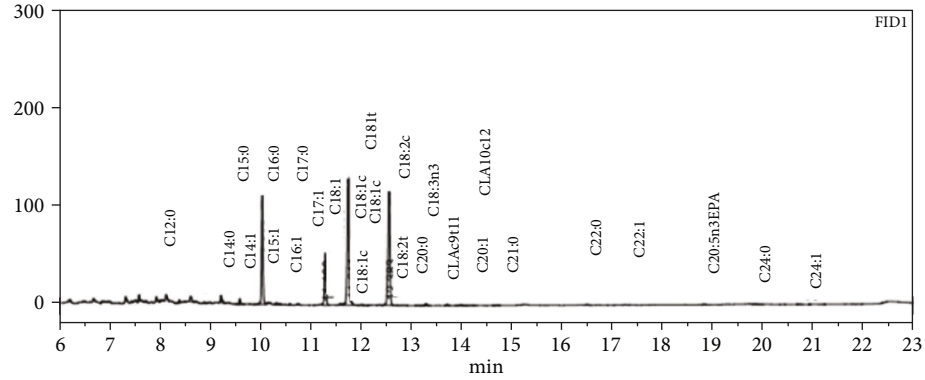


Figure 4: Gas chromatogram depicting the fatty acid profile in the fermented sesame waste by *L. plantarum.*

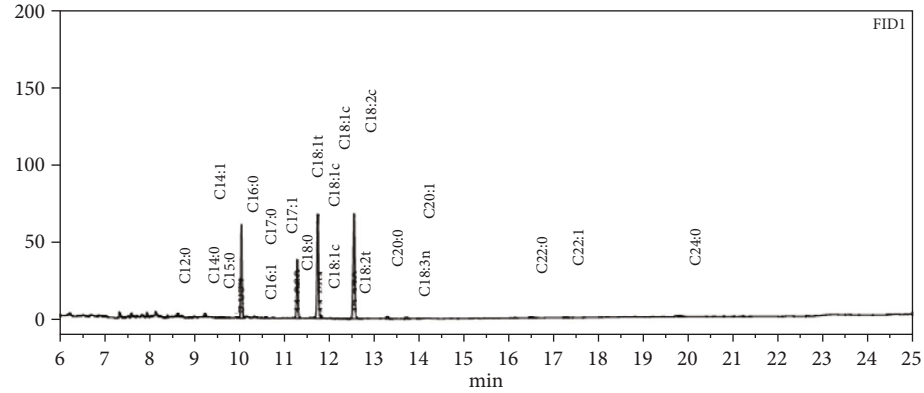


Figure 5: Gas chromatogram illustrating the fatty acid profile of the nonfermented sesame waste.

to grow in the sesame waste, it could produce CLAc9t11 by 0.35% and CLAt10c12 by 0.1%. Meanwhile, in the control sample, CLA was not detected (Figure 5). There were also significant changed in the concentration of other FFAs: the fatty acids C14:0, C15:0, C15:1, C16:0, C16:1, C18:1t, C18:1c, C18:2t, C21:0, and C24:1 were increased significantly, whereas fatty acids C14:0, C17:0, C18:1c, C18:2c, C20:0, C18:3n3, C20:1, C22:0, and C24:0 were decreased significantly. Also, C17:1 and C22:1 were obtained (Table 5). The results of our studies indicated that the proportion of CLAc911 and CLAt10-12 was different, with their ratio being approximately (4 : 1).

Lactiplantibacillus plantarum is a bacterial strain that can easily adapt to using substrate nutrients, for its growth. This bacterium can convert linoleic acid to CLA with the linoleate isomerase enzyme [31]. Furthermore, it breaks down the proteins of the sesame waste, producing free amino acids and other compounds, with the substrate having more buffering capacity. On the other hand, fermentation of various carbohydrates in the production of Lactic acid reduces the pH value of the culture medium of *Lactiplantibacillus plantarum* [14].

The concentration of CLA in the medium initially increased but decreased as biohydrogenation progressed, eventually being converted to saturated fatty acids by bacteria [14]. Previous studies have shown that the production of CLA by lactic acid bacteria is genus-dependent, while the type of isomer produced is species-dependent, indicating the importance of specific bacterial strains [1]. The substrate concentration strongly affected LA/LNA to CLA containing the produced oil conversion rate through the *Lactiplantibacillus plantarum* [46]. The CLA production was variable due to the response of *Lactiplantibacillus plantarum* to the substrate fatty acid level and composition [29]. pH and temperature could be, therefore, regarded as important environmental parameters that change the structure and affect the diversity of distribution [47]. Another researcher has also mentioned that the FA proportion and their isomer types are dependent on pH, temperature, microbial inoculation level, substrate concentration, and activation method used for bacterial strain [46, 48]. Gorissen et al. considered pH and temperature as two limiting factors in the study of microbial production of conjugated linoleic acid in fermented foods [49]. In contrast to some reports, it has been founding that certain lactic acid bacteria strains can effectively utilize the sesame waste as a substrate without the need for additional carbohydrates or nutrients [14, 35, 40].

In a similar study, Ando et al. [50] investigated the production of CLA from castor oil using *L. plantarum*. The strain utilized in their study demonstrated the ability to produce both *cis*-9 and *trans*-11 isomers of CLA simultaneously, which is a rare characteristic among bacterial

Table 5: Fatty acid composition (% total fatty acids) of the sesame waste with (B) and without (A) *Lactiplantibacillus plantarum*.

Name	A	B	Inoculation change (Δ^*)
C12:0	1.546	1.546^{ns}	0
C14:0	1.560	1.560^{ns}	0
C14:1	0.470	0.130^{**}	-0.34
C15:0	0.262	0.995^{**}	+0.733
C15:1	0.286	0.303	+0.017
C16:0	18.567	19.357^{**}	+0.79
C16:1	0.211	0.256	+0.045
C17:0	0.220	0.185	-0.035
C17:1	ND	0.019	+0.019
C18:0	13.352	10.813^{**}	-2.539
C18:1t	0.344	0.678^{**}	+0.334
C18:1c	27.112	31.215^{**}	+4.103
C18:1c	0.229	0.226	-0.003
C18:1c	0.099	0.366^{**}	+0.267
C18:2t	0.125	0.164	+0.039
C18:2t	0.165	ND^{**}	-0.165
C18:2c	30.412	29.709^{**}	-0.703
C20:0	0.681	0.470^{**}	-0.211
C20:1	0.133	0.049^{**}	-0.084
C21:0	0.126	0.270^{**}	+0.144
C22:0	0.513	0.234^{**}	-0.279
C24:0	0.556	0.129^{**}	-0.427
C24:1	0.068	0.168^{**}	+0.1
C22:1	ND	0.166^{**}	+0.166
CLA9t11	ND	0.354^{**}	+0.354
ClA10c12	ND	0.102^{**}	+0.102
C20:5n3EPA	ND	0.139^{**}	+0.139

$^*\Delta = B - A$. ND: not detectable. Average and standard deviation for A and B are 3.59 ± 7.20 and 3.689 ± 6.88, respectively. ns and $**$: nonsignificant and significant at $p < 0.05$.

strains. Additionally, L. plantarum exhibited the capability to produce EPA, a biologically active omega-3 fatty acid, with a yield of 0.139 [7]. The growth of L. plantarum not only influenced the overall fatty acid content in the sesame waste but also resulted in a change in the composition of beneficial fatty acids, particularly the production of EPA and CLA.

4. Conclusions

This study was performed to evaluate the potency of the sesame waste substrate, considering sesame waste as one of the most sources for bioactive compound. Extracted oil may contain these healthy materials although the huge waste of sesame oil production can supply the unexpensive and safe substrate for CLA and EPA production [7]. The use of sesame waste increased lactic acid during solid-state fermentation. Due to the intrinsic properties of sesame waste, it increased the substrate fatty acids, thus producing CLA and EPA. It was revealed that the oil produced from plant waste could be adopted and the microbial isomerization method could be used to produce bioactive compounds and to make valuable substances; in addition to reducing the food production costs, it could decrease such outlets' expenses. Overall, this research contributes to our understanding of the production and optimization of CLA and EPA using *L. plantarum* in a natural and sustainable manner. The findings have implications for the development of functional food products enriched with these valuable fatty acids, offering potential health benefits to consumers. Further studies can focus on exploring downstream applications and evaluating the sensory and nutritional attributes of the produced CLA- and EPA-enriched products.

Data Availability

Data are available on request from the corresponding author.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

M. H. developed the original idea and the protocol, abstracted and analyzed the data, and is a guarantor. M. M., Z. I. M., M. J., and H. K. contributed to the development of the protocol, abstracted the data, and prepared the manuscript.

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References

[1] A. S. Salsinha, L. L. Pimentel, A. L. Fontes, A. M. Gomes, and L. M. Rodríguez-Alcalá, "Microbial production of conjugated linoleic acid and conjugated linolenic acid relies on a multienzymatic system," *Microbiology and Molecular Biology Reviews*, vol. 82, no. 4, article e00019, 2018.

[2] M. Afarin, I. Alemzadeh, and Z. K. Yazdi, "Conjugated linoleic acid production and optimization via catalytic reaction method using safflower oil," *International Journal of Engineering*, vol. 31, no. 8, pp. 1166–1171, 2018.

[3] R. Martha, T. Toharmat, N. Rofiah, and D. Anggraeni, "Comparison of extraction methods for fatty acid and conjugated linoleic acid quantification in milk," in *Proceedings of the IOP Conference Series: Materials Science and Engineering*, no. article 42022, 2019IOP Publishing, Bristol, 2019.

[4] C. P. Van Nieuwenhove, V. Teran, and S. Nelina, "Conjugated linoleic and linolenic acid production by bacteria: development of functional foods," in *Probiotics*, E. C. Rigobelo, Ed., InTech, London, 2012.

[5] T. R. Dhiman, L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa, "Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic

and linolenic acid," *Journal of Dairy Science*, vol. 83, no. 5, pp. 1016–1027, 2000.

[6] T. Aziz, A. Sarwar, Z. Daudzai et al., "Conjugated fatty acids (CFAS) production via various bacterial strains and their applications. A review," *Journal of the Chilean Chemical Society*, vol. 67, no. 1, pp. 5445–5452, 2022.

[7] S. R. Azari, M. Hojjatoleslamy, Z. E. Mousavi, H. Kiani, and S. M. A. Jalali, "Production and optimization of conjugated linoleic and eicosapentaenoic acids by *Bifidobacterium lactis* in cold-pressed soybean cake," *Frontiers in Nutrition*, vol. 9, article 916728, 2022.

[8] H. Erinc and I. H. Isler, "Optimization of conjugated linoleic acid production from safflower oil and purification by low temperature crystallization," *Acta Alimentaria*, vol. 48, no. 1, pp. 37–46, 2019.

[9] M. R. Shayanmehr, A. H. Elhamirad, and M. Armin, "Optimization of conjugated linoleic acid (Cla) synthesis conditions applying it in milk enrichment," *Journal of Research and Innovation in Food Science and Technology*, vol. 9, pp. 375–388, 2021.

[10] G. C. Kuhl and J. D. D. Lindner, "Biohydrogenation of linoleic acid by lactic acid bacteria for the production of functional cultured dairy products: a review," *Food*, vol. 5, no. 1, p. 13, 2016.

[11] Y. Wang, Q. Lin, P. Zheng, L. Li, Z. Bao, and F. Huang, "Effects of eicosapentaenoic acid and docosahexaenoic acid on chylomicron and VLDL synthesis and secretion in Caco-2 cells," *BioMed Research International*, vol. 2014, Article ID 684325, 10 pages, 2014.

[12] J. Zhang and J. G. Burgess, "Enhanced eicosapentaenoic acid production by a new deep-sea marine bacterium Shewanella electrodiphila MAR441T," *PLoS One*, vol. 12, no. 11, article e0188081, 2017.

[13] A. E. Shaikh, A. Pawar, H. Parmar, R. N. Vadgama, A. M. Lali, and A. A. Odaneth, "Conjugated linoleic acid production by lactic acid bacteria: a bio-transformation study in media with oil hydrolysates," *Journal Of Applied Biology & Biotechnology*, vol. 5, no. 5, pp. 321–327, 2018.

[14] M. A. Correa Deza, A. Rodríguez de Olmos, and M. Selva Garro, "Solid state fermentation to obtain vegetable products bio-enriched with isoflavone aglycones using lactic cultures," *Revista Argentina de Microbiología*, vol. 51, no. 3, pp. 201–207, 2019.

[15] H. B. MacDonald, "Conjugated linoleic acid and disease prevention: a review of current knowledge," *Journal of the American College of Nutrition*, vol. 19, Supplement 2, pp. 111S–118S, 2000.

[16] B. Kim, H. R. Lim, H. Lee, H. Lee, W. Kang, and E. Kim, "The effects of conjugated linoleic acid (CLA) on metabolic syndrome patients: a systematic review and meta-analysis," *Journal of Functional Foods*, vol. 25, pp. 588–598, 2016.

[17] M. A. Villar-Tajadura, L. M. Rodríguez-Alcalá, V. Martín et al., "Production of conjugated linoleic and conjugated a-linolenic acid in a reconstituted skim milk-based medium by bifidobacterial strains isolated from human breast milk," *BioMed Research International*, vol. 2014, Article ID 725406, 6 pages, 2014.

[18] T. E. Lehnen, M. R. da Silva, A. Camacho, A. Marcadenti, and A. M. Lehnen, "A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism," *Journal of the International Society of Sports Nutrition*, vol. 12, no. 1, p. 36, 2015.

[19] B. Yang, H. Chen, C. Stanton et al., "Review of the roles of conjugated linoleic acid in health and disease," *Journal of Functional Foods*, vol. 15, pp. 314–325, 2015.

[20] M. C. De Vries, E. E. Vaughan, M. Kleerebezem, and W. M. de Vos, "Lactobacillus plantarum—survival, functional and potential probiotic properties in the human intestinal tract," *International Dairy Journal*, vol. 16, no. 9, pp. 1018–1028, 2006.

[21] F. Tabatabai and L. Nasiraei, "Isolation and identification of bacteria in the lactic acid microbial flora of breast milk by 16 ribosomal method," *J Res DNA Seq.*, vol. 21, pp. 167–177, 2011.

[22] Y. Chen, Y. Wang, J. Chen et al., "Bioprocessing of soybeans (Glycine max L.) by solid-state fermentation with Eurotium cristatum YL-1 improves total phenolic content, isoflavone aglycones, and antioxidant activity," *RSC Advances*, vol. 10, no. 29, pp. 16928–16941, 2020.

[23] S. Şahin and E. A. A. Elhussein, "Valorization of a biomass: phytochemicals in oilseed by-products," *Phytochemistry Reviews*, vol. 17, no. 4, pp. 657–668, 2018.

[24] Food and Agriculture Organization of the United Nations [FAO], "Food wastage footprint impacts on natural resources," Technical Report. FAO, Rome, 2013.

[25] A. Durazzo, M. Fawzy Ramadan, and M. Lucarini, "Editorial: cold pressed oils: a green source of specialty oils," *Frontiers in Nutrition*, vol. 8, article 836651, 2022.

[26] V. M. Del Carmen, M. J. Serradilla, A. Martín, E. Ordiales, S. Ruiz-Moyano, and M. De Guía Córdoba, "Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application," *Journal of the Science of Food and Agriculture*, vol. 96, pp. 2116–2124, 2016.

[27] S. Popoviæ, N. Hromiš, D. Šuput, S. Bulut, R. Romaniæ, and V. Laziæ, "Valorization of by-products from the production of pressed edible oils to produce biopolymer films," in *Cold Pressed Oils*, M. F. Ramadan, Ed., pp. 15–30, Elsevier, Amsterdam, 2020.

[28] G. B. Var and C. O. Özer, "Valorization of waste chicken skin for conjugated linoleic acid (CLA) production: optimization using response surface methodology," *Environmental Science and Pollution Research International*, vol. 29, no. 21, pp. 32279–32286, 2022.

[29] M. F. Ramadan-Hassanien, "Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay," *Grasas y Aceites*, vol. 59, no. 3, pp. 254–259, 2008.

[30] A. L. Fontes, L. Pimentel, L. M. Rodríguez-Alcalá, and A. Gomes, "Publisher correction: effect of Pufa substrates on fatty acid profile of *Bifidobacterium breve* Ncimb 702258 and CLA/CLNA production in commercial semi-skimmed milk," *Scientific Reports*, vol. 9, no. 1, p. 4671, 2019.

[31] S. Raimondi, A. Amaretti, A. Leonardi, A. Quartieri, C. Gozzoli, and M. Rossi, "Conjugated linoleic acid production by bifidobacteria: screening, kinetic, and composition," *BioMed Research International*, vol. 2016, Article ID 8654317, 8 pages, 2016.

[32] D. M. Linares, C. Gómez, E. Renes et al., "Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods," *Frontiers in Microbiology*, vol. 8, p. 846, 2017.

[33] A. Senizza, M. L. Callegari, B. Senizza et al., "Effects of linoleic acid on gut-derived Bifidobacterium breve DSM 20213: a

transcriptomic approach," *Microorganisms*, vol. 7, no. 12, p. 710, 2019.

[34] L. W. Su, Y. H. Cheng, F. S. H. Hsiao, J. C. Han, and Y. H. Yu, "Optimization of mixed solid-state fermentation of soybean meal by lactobacillus species and Clostridium butyricum," *Polish Journal of Microbiology*, vol. 67, pp. 297–305, 2018.

[35] A. Rodríguez de Olmos, M. A. Correa Deza, and M. S. Garro, "Selected lactobacilli and bifidobacteria development in solid state fermentation using soybean paste," *Revista Argentina de Microbiología*, vol. 49, pp. 62–69, 2017.

[36] A. Cano-Medina, H. Jiménez-Islas, L. Dendooven, R. P. Herrera, G. González-Alatorre, and E. M. Escamilla-Silva, "Emulsifying and foaming capacity and emulsion and foam stability of sesame protein concentrates," *International Food Research Journal*, vol. 44, no. 3, pp. 684–692, 2011.

[37] M. Hadad Khodaparast, M. Habibi Najafi, A. Elhami Rad, and N. Divandari, "Process optimization of sesame milk according to Iranian prefrences," *Iranian Food Science and Technology Research Journal*, vol. 2, no. 1, 2006.

[38] M. Timonen, D. Horrobin, J. Jokelainen, J. Laitinen, A. Herva, and P. Räsänen, "Fish consumption and depression: the Northern Finland 1966 birth cohort study," *Journal of Affective Disorders*, vol. 82, no. 3, pp. 447–452, 2004.

[39] M. Ghamari, Y. F. Tabatabaei, I. Alemzade, M. Vosooghi, M. Varidi, and H. Safari, "Optimization of culture medium containing date waste for lipase production by Aspergillus niger using RSM method," *Journal of Food Science and Technology*, vol. 14, pp. 85–96, 2017.

[40] D. E. Bauman, L. H. Baumgard, B. A. Corl, and D. J. Griinari, "Biosynthesis of conjugated linoleic acid in ruminants," *Proc Am Soc Anim Sci.*, vol. 77, pp. 1–14, 2000.

[41] N. Farazandehnia, "The evaluation of antimicrobial effect of fermented probiotic milk produced Lactobacillus casei, Lactobacillus plantarum, Bifidobacterium and Bifidobacterium angultum," *Iranian Journal of Medical Microbiology*, vol. 10, pp. 31–38, 2016.

[42] C. G. Vinderola and J. A. Reinheimer, "Enumeration of Lactobacillus casei in the presence of L. acidophilus, bifidobacteria and lactic starter bacteria in fermented dairy products," *International Dairy Journal*, vol. 10, no. 4, pp. 271–275, 2000.

[43] S. Zhao, N. Hu, J. Huang, Y. Liang, and B. Zhao, "High-yield spore production from Bacillus licheniformis by solid state fermentation," *Biotechnology Letters*, vol. 30, no. 2, pp. 295–297, 2008.

[44] H. Robert, V. Gabriel, D. Lefebvre, P. Rabier, Y. Vayssier, and C. Fontagné-Faucher, "Study of the behaviour of Lactobacillus plantarum and Leuconostoc starters during a complete wheat sourdough breadmaking process," *LWT-Food Science and Technology*, vol. 39, no. 3, pp. 256–265, 2006.

[45] V. Terán, P. L. Pizarro, M. F. Zacarías, G. Vinderola, R. Medina, and C. Van Nieuwenhove, "Production of conjugated dienoic and trienoic fatty acids by lactic acid bacteria and bifidobacteria," *Journal of Functional Foods*, vol. 19, pp. 417–425, 2015.

[46] F. Salimi, M. Fattahi, and J. Hamzei, "Optimization of celery seed essential oil extraction and its antioxidant activity using response surface methodology and ultrasound-assisted," *Journal of Horticulture Science*, vol. 32, pp. 171–183, 2018.

[47] M. Alhajabdalla, H. Mahmoud, M. S. Nasser, I. A. Hussein, R. Ahmed, and H. Karami, "Application of response surface methodology and Box-Behnken design for the optimization of the stability of fibrous dispersion used in drilling and completion operations," *ACS Omega*, vol. 6, pp. 2513–2525, 2021.

[48] A. Nasrollahzadeh, S. M. Tavani, E. Arjeh, and S. M. Jafari, "Production of conjugated linoleic acid by lactic acid bacteria; important factors and optimum conditions," *Food Chemistry: X*, vol. 20, article 100942, 2023.

[49] L. Gorissen, K. Raes, S. De Smet, L. De Vuyst, and F. Leroy, "Microbial production of conjugated linoleic and linolenic acids in fermented foods: technological bottlenecks," *European Journal of Lipid Science and Technology*, vol. 114, no. 4, pp. 486–491, 2012.

[50] A. Ando, J. Ogawa, S. Kishino, and S. Shimizu, "CLA production from ricinoleic acid by lactic acid bacteria," *Journal of the American Oil Chemists' Society*, vol. 80, no. 9, pp. 889–894, 2003.