Asian Journal of Chemistry; Vol. 36, No. 7 (2024), 1511-1517

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.31515

Production of Oleic Acid from Mango Kernels Waste using Probiotic Bacteria Isolated from Marine Fishes

Rubavathi Anandan[1], Visali Kannan[1], Lavanya Sivakumar[1], Venkatajothi Ramarao[2] and Murugan Athiappan[1,*]

[1]Department of Microbiology, Periyar University, Salem-636011, India
[2]Department of Medical Microbiology, Basic Medical Sciences, Michael Chilufya Sata School of Medicine, The Copperbelt University, Ndola, Zambia

*Corresponding author: E-mail: amuruganpu@gmail.com

Received: 14 March 2024; *Accepted*: 16 April 2024; *Published online*: 29 June 2024; AJC-21668

Oleic acid is a mono-unsaturated omega-9 fatty acid that serves as an antioxidant, antibacterial, antidiabetic, anti-inflammatory, anticancer and antidepressive agents. The present study focused on the production of oleic acid from mango kernel waste as an alternate method for conventional chemical extraction. An alternate mechanism has been devised to synthesize oleic acid from agro-industrial waste using probiotic bacteria and has been found to be economical. Microbial production of oleic acid from kernels discharged as waste from mango pulp processing industrial waste is a typical example of the valorization. The results of the present study demonstrated 5.26 mg/100 mL of oleic acid production using gut bacterial isolates from marine fish. Optimization of oleic acid production with response surface methodology by using Design-Expert has shown 40 mg/100 mL at pH 6.0, 15 ºC and 25 g concentration of mango kernel substrate with 48 h of incubation. The oleic acid produced using gut bacteria have been confirmed with the GC-MS fatty acid methyl ester. Therapeutic applications of oleic acids like antioxidant, anti-inflammatory and antibacterial activities were found to be promising.

Keywords: Oleic acid, Mango kernel, Probiotic bacteria, Anti-inflammatory.

INTRODUCTION

Oleic acid is a mono-unsaturated fatty acid (MUFA) known as omega-9 fatty acid found in various animal and vegetable oil sources, such as olive oil nuts, peanuts and walnuts. The oleic acid intake accounts for about 15% of total energy intake in a standard Med Diet [1]. Oleic acid is utilized in pharmaceuticals as an excipient and in aerosol products as an emulsifying or solubilizing agent, but also for industrial lubricants, fuels and pharmaceutical raw materials [2]. In the presence of albumin, oleic acid was the only fatty acid produced and released by astrocytes, implying that this occurrence serves a purpose. Oleic acid's single double bond is sufficient to significantly improve the fluidity of biological membranes [3]. Because membrane fluidity is essential for neurons, incorporating oleic acid-derived phospholipids into a specific area of the membrane could significantly alter membrane properties [4]. In general, oleic acid is preferentially incorporated into neurite bases, suggesting that increased fluidity is required at the sites of newly emerging axons and/or dendrites. Oleic acid diminishes the expression of cholesterol transport-related proteins, decreases cholesterol absorption and decreases the oxidation of low-density lipoprotein (LDL), preventing atherosclerosis [5].

Mango (*Mangiferia indica* L.) is one of the most important tropical fruits of the tropical countries. Mangoes are native to the Indian subcontinent and Southeast Asia [4]. It is one of the most widely used fruits in terms of food, juice, flavour, smell and colour. About 17-22%, in the pulp processing industries, kernels are being thrown away as wastes. Thus, valorization of mango kernels would be an alternative way to reduce the economic burden incurred in waste disposal [6]. Polyphenols and fatty acids are abundant in mango kernel waste. Due to the high commercial cost of oleic acid, the current study focuses on producing oleic acid using mango kernels as a substrate, which could be cost-effective. It has been reported that stearic acid (58.08%), oleic acid (17.99%), palmitic acid (1.33%), linoleic acid (2.86%), myristic acid (0.17%) and oxadecanoate acid are major components of the mango kernel [7].

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

Microorganisms (bacteria and phytoplankton) are the primary producers of oleic acid and they remain a primary source for the transfer of fatty acids to the rest of the food chain. It has been reported that the bacteria represent a green source of Long-chain polyunsaturated fatty acids (LC-PUFAs), with cheaper downstream processes compared with fish oil [8]. Several marine bacteria produce omega-3 fatty acids and their metabolic pathways have been well studied. Fermentation is the technique of the biological conversion of complex substrates into simple compounds by various microorganisms, such as bacteria and fungi. In the course of this metabolic breakdown, they also release several additional compounds apart from the usual products of fermentation, such as carbon dioxide and alcohol. The previous study already reported 3.38 mg/g of oleic acid production [9]. This study examines the mechanisms by which mango waste serves as a carbon supply and fatty acid substrate for microbes obtained from fish intestines, focusing specifically on the processes involving oleic acid.

EXPERIMENTAL

Preparation of substrate: The waste mango kernels were collected from the Adhiyaman mango pulp processing industry in Krishnagiri district, India. The seeds were dried in sunlight for 3 days, followed by drying in a hot air oven at 60 ºC for 24 h and ground seeds were used for submerged fermentation.

Isolation of probiotic bacteria from marine fish: Three varieties of fish [Mackerel (Fig. 1a), Chub Mackerel (Fig. 1b) and Ladyfish (Fig. 1c)] were collected from Indian ocean at Rameshwaram coast, India. The marine fish were packed aseptically in a sterile ice bag and brought to the laboratory. To isolate the bacteria, the stomach and intestines were excised from the fish into sections no longer than 5 cm. Each separate section was then placed in a 250 mL Erlenmeyer flask containing 100 mL of PYM medium (5 g L^{-1} peptone, 3 g L^{-1} yeast, 3 g L^{-1} malt extract and 55 mM glucose) and incubated for 1 day at 15 ºC. A three-fold dilution was obtained from the sample and 10 µL was streaked onto ZoBell's marine agar. Bacterial colonies were observed after 2-4 days of incubation at 15 ºC. Isolates were re-streaked onto ZoBell's marine agar plates to check the purity of the strains before secondary screening for fatty acid production.

Primary screening for fatty acids: The reduction of 2,3,5-triphenyl tetrazolium chloride (TTC) produced red-triphenyl formazan, which indicated the synthesis of the fatty acids. The red triphenyl formazan formed was measured using a 485 nm spectrophotometer [10].

Fig. 1a. Mackerel

Fig 2b. Chub mackerel

Fig. 1c. Lady Fish

Production of oleic acid: About 100 mL of ZoBell's marine broth supplemented with 2% of pasteurized mango kernel was added and 1 mL (1.3×10^8 CFU/mL) of log culture of isolate was added to the media and incubated for 2-4 days at 15 ºC.

Separation of oleic acid: After fermentation, the biomass was removed by centrifugation at 10,000 rpm then the supernatant was collected. It was then filtered again through Whatman No. 1 filter paper. After filtration equal volume of hexane was added and stored in a dark place and allowed to stand at room temperature for the separation of organic phase and aqueous phase. Fatty acids were extracted into the organic phase, which was then separated for further analysis.

Optimization of oleic acid production using response surface methodology: The response surface methodology (RSM) of Box-Behnken design (BBD) was employed at 3 levels (–1, 0, +1) to determine optimum conditions for oleic acid production. BBD is one of the symmetrical experimental designs used to determine which of the many experimental parameters and their interactions have the greatest statistical significance. This methodology is cheaper as it reduces the number of experiments and offers an excellent opportunity for optimization purposes [11]. The Design Expert 13 software was used to simulate the experiment data to create a predictive model of maximum oleic acid production. The model was used to determine the interactions, optimize the process of oleic acid production. The number of experiments for the three levels in BBD was determined using eqn. 1:

$$N = 2k (k - 1) + Cp \quad (1)$$

where N denotes the number of experiments, Cp is the number of central points and k is the number of factors such as temperature, pH and so on.

The total number of experiments carried out in this study was 20, with twelve experiments for each factor at three levels and five central experiments. Every factor in this study was adjusted at three uniformly spaced levels (–1, 0, +1) by the BBD rule. The BBD methodology was used to generate the experimental points using the Design Expert 13 software package. The factors influencing oleic acid production under investi-

gation include temperature, pH and the percentage of mango kernel waste as a substrate. The experiments were carried out using the matrix transformation. The parameters were the independent variables (factors), while the yield of oleic acid was the dependent variable (response). A statistical software package (Design Expert 13) was used to relate the relationship between oleic acid production and the parameters under investigation. The quadratic polynomial equation was established to demonstrate the relationship between oleic acid production (response) and independent factors that are unrelated to each other. The quadratic polynomials used for response can be represented as follows [11]:

$$Y = f(A, B, C) \quad (2)$$

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C \quad (3)$$

where β_0, β_1, β_2 and β_3 are the independent variables, which are represented by the coefficients A, B and C, while the objective functions (dependent variables) represented by Y.

Characterization of oleic acid using GC-MS FAME: Direct transmethylation of the stored bacterial pellets was used to make fatty acid methyl esters (FAMEs) [12]. The biomass was mixed with 10 µL of an internal standard solution (23:0 FAME in toluene) and 0.5 mL of 1% (v/v) sodium methoxide in methanol. The mixture was then heated to 80 ºC and left there for 30 min. The incubation cycle was repeated after 0.5 mL of 5% methanolic HCl was added to the vial and it had cooled to room temperature. Hexane (1 mL) was used to extract the FAMEs, followed by hexane being evaporated with the help of argon gas. The resulting FAME residue was then redissolved in 100 mL of chloroform. Duplicate samples were examined using the gas chromatograph GCMS-QP2010 Ultra (Shimadzu, Tokyo, Japan) with a flame ionization detector (FID) and mass spectrometry (MS) detector. Using a Restek Rtx-5MS capillary column and a detector splitting system (30 m × 0.25 mm i.d, 0.25 µm) split a column flow so that signal data could be collected by both an MS detector and an FID. As a carrier gas, 1/20 split helium was used. Temperatures for the injector and detector were 280 ºC and 320 ºC, respectively. The temperature program of oven was set to begin at 70 ºC (hold for 2 min), then climb to 320 ºC at a rate of 4 ºC per min and stay there for 15 min. The MS spectra were used to locate fatty acid peaks. By regressing linearly, the area of the chromatographic peak in proportion to the peak of a known concentration of an internal standard for FAME quantification was achieved (23:0).

***In vitro* approaches to studying the bioactivity of oleic acid**

Antioxidant assay: A 1 mL of DPPH solution was mixed with 250 µL of extract at levels of 25, 50, 75 and 100 µg/mL or standards (ascorbic acid). After incubation in the dark at room temperature for 30 min, absorbance was measured at 517 nm against pure methanol as a blank. The antioxidant activity determined by the DPPH method was expressed as a percentage using the formula below:

$$\text{Antioxidant activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where A is the absorbance of the control and experimental samples.

Anti-inflammatory assay: Anti-inflammatory activity was assessed using the human red blood cell (HRBC) membrane stabilization method. The blood sample was collected from a healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks before the experiment and then transferred to heparinized centrifuge tubes at 3,000 rpm. The blood was washed three times with an equal volume of normal saline and reconstituted as a 10% v/v suspension. About 50 µL of blood cell suspension was added to different concentrations of fermented extract (25, 50, 75 and 100 µg/mL), followed by 3 mL of phosphate buffer (PBS) added to it and then incubated in a water bath at 54 ºC for 20 min. Centrifuged at 2500 rpm for 3 min to pellet the RBC and 3 mL of PBS with 50 µL of blood cell suspension were used as a control. Normal saline was used as a blank reference and aspirin was used as standard. The absorbance of the supernatants was measured at 540 nm. The following equation was used to calculate the percentage inhibition of hemolysis.

$$\text{Inhibition of hemolysis (\%)} = \left(1 - \frac{A_2}{A_1}\right) \times 100$$

where A_1 = absorbance of control and A_2 = absorbance of the test sample.

Minimal inhibitory concentration (MIC): *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* were obtained from the Bio-Line Laboratory, Salem district, India. Bacteria were transferred to Luria-Bertani broth (LB) media from slant. Aliquots of 100 µL of Mueller-Hinton broth containing a series of fermented extracts containing oleic acid at concentrations of 25, 50, 75 and 100 µg/mL were added to each well of a 96-well plate (Iwaki brand, Asahi Techno Glass, Japan). The concentrations of fatty acids in the crude extract ranged from 250 to 1250 µg/mL. Bacterial cell suspension (2 µL) of 24 h culture was added to the appropriate wells. Microplates were kept at room temperature for 24 h and the absorbance of the cell suspension was measured at 595 nm using a microplate reader (Bio-Rad Mark, CA, USA).

RESULTS AND DISCUSSION

Isolation and identification of oleic acid-producing bacteria: In order to isolate oleic acid-producing bacteria, marine fish gut samples were used as a source of probiotics. Nearly 15 different colonies were obtained from the intestines of marine fish (Mackerel, Chub Mackerel and Ladyfish) samples. Already, fatty acid-producing bacteria from marine fish gut showed that the bacterial strain isolated from fish gut exhibited antibacterial potential [13]. The marine environment represents a largely untapped source of microorganisms that may be capable of generating bioactive compounds. Similarly, 16 fish isolates, 20 shrimp isolates and 24 shellfish isolates were obtained from a similar study [14]. In order to further study their bile and acid tolerances, three strains were chosen.

Based on standard methods of screening done with 2,3,5-triphenyl tetrazolium chloride (TTC), it was indicated that out of 15 bacteria, only six, namely C1, C2, C3, C4, C5 and C6, showed positive results for fatty acid production (Fig. 2). Of

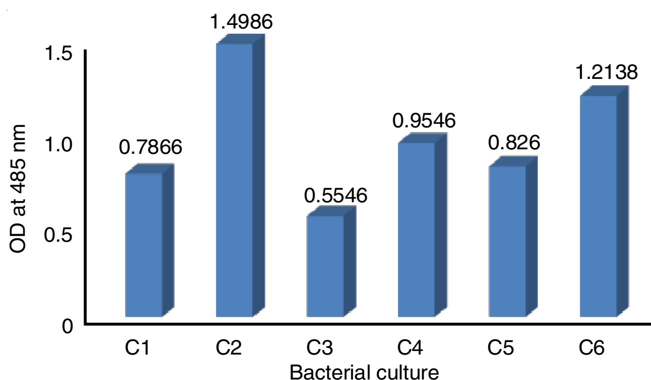

Fig. 2. Screening of fatty acids producer

these C2 strains, one showed a high production of fatty acids (Fig. 3). In previous report, 2,3,5-triphenyltetrazolium chloride (TTC) colorimetric method was used to screen for fatty acid production [10]. TTC has been applied as a screening test for the synthesis of bacterial fatty acids. This method reduced the time, effort and cost involved in screening for fatty acid production. *Bacillus toyonens* isolate demonstrated strong activity for fatty acids. Accordingly, there is a direct correlation between the ability to reduce TTC to triphenyl formazan (TF) and the ability to produce fatty acid. Reduction of the colourless TTC to the brilliant TF by the enzyme Δ^5-desaturase [12]. The 16S rRNA sequencing was used to identify the bacterial strain C2, which is responsible for oleic acid production and was identified as *Aeromonas veronii*. A 98.8% similarity with *Aeromonas veronii* was found in the NCBI blast, which suggests that the test organism is *Aeromonas veronii*.

Secondary screening for the oleic acid-producing bacteria: The isolate shown positive for the TTC assay was screened for the presence of oleic acid using GC-MS FAME analysis. Fatty acid methyl esters (FAMEs) of bacteria are the most commonly used method for classifying them using lipid profiling [15]. High oleic acid producers were identified based on the amount of oleic acid produced. An GC-MS FAME analysis of the C2 isolate (Fig. 4) showed that it made *cis*-9 oleate (C18:1) and omega-9 (5.26%). Such as previous studies, the fatty acid methyl esters (FAMEs) were then extracted with supercritical CO_2 and analyzed using GC/MS without additional treatment [16]. The *iso*- and *ante-iso* C15:0 (pentadecanoic acid) and C17:0 (heptadecanoic acid), along with C18:0 (stearic acid), were predominant in Gram-positive bacteria.

Fig. 3. Potent fatty acids producer (C2)

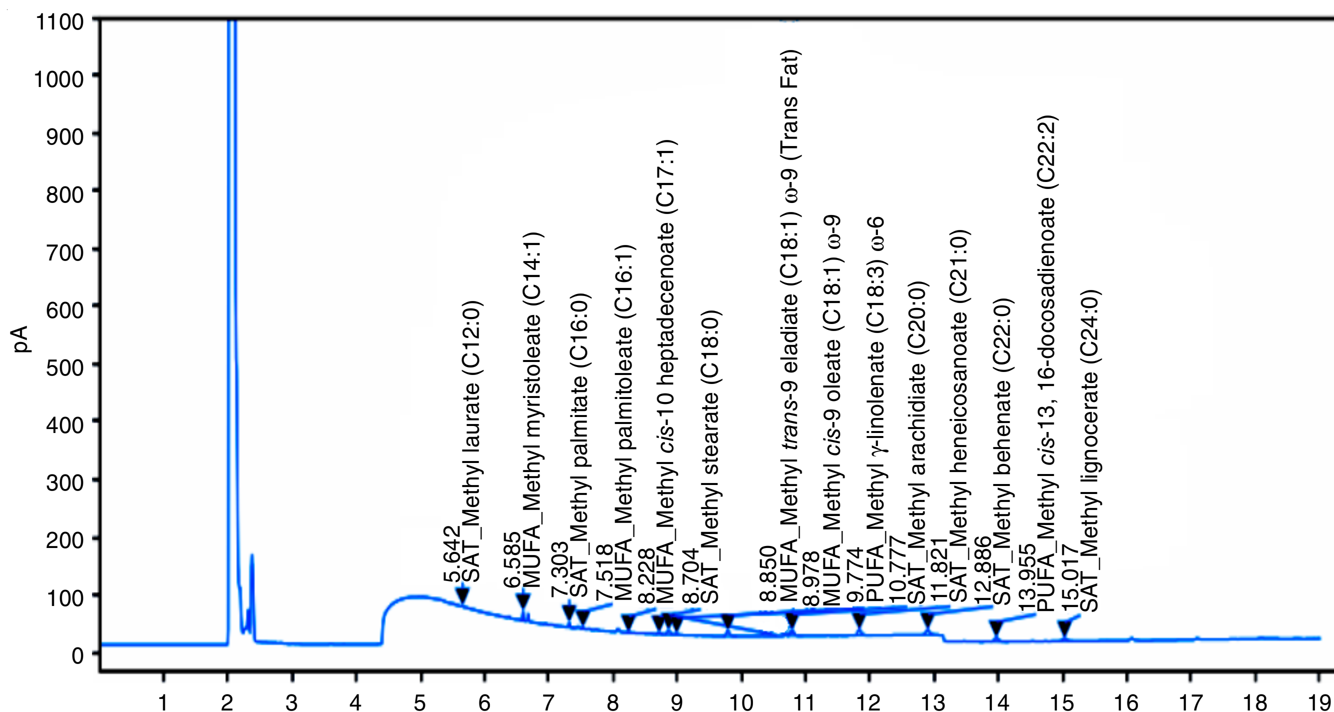

Fig. 4. GC-MS FAME analysis for fatty acid production C2

Production and optimization of oleic acid: Box-Behnken design (BBD) was evaluated based on the interaction among various factors of pH, temperature and concentration of substrate to determine their optimal level of oleic acid production. Three factors were optimized for oleic acid production *viz.* pH, temperature and substrate concentration from the lowest to highest ranges, which were analyzed at 3 levels (–1, 0 and +1) in the model. The higher oleic acid production was obtained under pH 6.0, 15 ºC and 25 g of substrate concentration (Tables 1 and 2). Higher production of (40 mg/100 mL) oleic acid by probiotic bacteria has been achieved (Fig. 5). Similarly, *Rhodotorula* sp. IIP-33 cultivated on sugarcane bagasse hydrolysate produced a maximum amount of lipids at pH 6.0 of 1.54 g L^{-1}. According to reports, *Rhodotorula* sp.

TABLE-1
EXPERIMENTAL DESIGN MATRIX FOR OPTIMIZATION OF OLEIC ACID PRODUCTION USING ENVIRONMENTAL FACTORS ACCORDING TO BBD

Run	Factor 1	Factor 2	Factor 3	Response 1: Oleic acid production mg/100 mL	
	A: Temp. (°C)	B: pH	C: Substrate concentration	Actual value	Predicted value
1	20	8	5.5	11.00	10.19
2	20	4	20	30.00	29.73
3	15	6	0.557002	5.00	5.78
4	15	6	12.75	25.00	24.50
5	20	4	5.5	12.00	10.69
6	15	6	12.75	24.00	24.50
7	23.409	6	12.75	17.00	18.73
8	10	4	5.5	13.00	13.53
9	15	9.36359	12.75	19.00	18.78
10	20	8	20	28.00	27.53
11	15	2.63641	12.75	22.00	22.14
12	6.59104	6	12.75	27.00	25.19
13	10	4	20	35.00	35.87
14	15	6	25	40.00	39.14
15	10	8	5.5	11.40	11.73
16	15	6	12.75	23.00	24.50
17	15	6	12.75	22.00	24.50
18	10	8	20	31.00	32.37
19	15	6	12.75	26.00	24.50
20	15	6	12.75	27.00	24.50

TABLE-2
ANALYSIS OF VARIANCE (ANOVA) FOR THE FITTED QUADRATIC POLYNOMIAL MODEL FOR OPTIMIZATION OF OLEIC ACID

Source	Sum of squares	Df	Mean square	F-Value	Probability (p) > F
Model	1456.96	9	161.88	52.44	< 0.0001
Lack of fit	13.37	5	2.67	0.7640	0.6125
Pure error	17.50	5	3.50		
Corrected total	1487.83	19			

R^2 = 0.9793; R^2_{adj} = 0.9606 CV (%) = 7.84

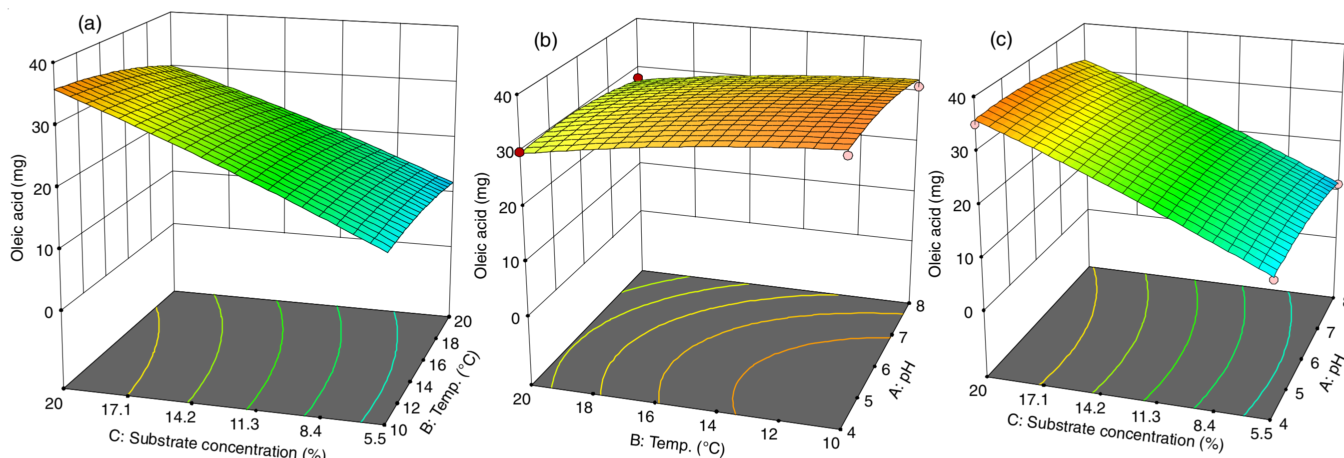

Fig. 5. 3D Optimization of different parameters for enhanced oleic acid production whereas (a) temperature *vs.* substrate concentration, (b) pH *vs.* temperature and (c) pH *vs.* substrate concentration

IIP-33 grew best in sugarcane bagasse hydrolysate at 38 °C, yielding a maximum lipid output of 2.12 g L^{-1} [17]. Likewise, as already reported with commercial oleic acid, the synthesis of estolide fatty acids was optimized through the application of the response surface methodology's Box-Behnken model [18]. The student t-test was used to statistically evaluate the parameters; no significant difference was found between the predicted and experimental yields, showing that the model was satisfactory and suitable for reflecting the expected optimization.

$$Y = + -0.4746 *A + -2.05 *B + 8.83 *C + -0.0250 *AB + -0.4000 *AC + -1.10 *BC + -2.27 *A2 + -1.39 *B2 + 2.33 *C2$$

Characterization of oleic acids: The GC-MS FAME analysis showed the variations in the metabolites of fermented broth. After 48 h, the bioconverted fermented broth was subjected to fatty acid extraction (Fig. 6). These isolates using mango kernels as substrates produced 10 metabolites (Table-3). Based on the GC-MS-FAME analysis, the predominant fatty acid is *cis*-9 oleate (C18:1) omega-9 (40.00%) in this study, which is likely to be the predominant fatty acids found in the mango kernel (Table-3). The GC-MS FAME results from a previous study showed that palmitate (13.44%), stearate (19.82%) and linoleate (14.49%) made up most of the fatty acids in coconut oil. Only palmitic acid, found to be higher in Pliek U oil, showed a difference in predominant fatty acid content. This disparity was most likely caused by the geographical location of the coconut plantations [19]. Previous experimental results revealed that biodiesel made from waste fish oil had a much higher concentration of the methyl ester group in the biodiesel sample [20]. The GC-MS FAME results revealed the existence of a significant amount of palmitic acid, oleic acid and linolenic acid, all of which are important biodiesel components.

TABLE-3
OPTIMIZED PRODUCTIONS OF FATTY ACIDS

Fatty acids	(mg/100 mL)
Methyl octanoate (C8:0)	5.546
Methyl palmitate (C16:0)	9.202
Methyl stearate (C18:0)	13.571
Methyl *trans*-9 eladiate (C18:1)	2.239
Methyl *cis*-9 oleate (C18:1)	40.00
Methyl linoleate (C18:2)	9.923
Methyl linolenate (C18:3)	0.477
Methyl arachidiate (C20:0)	1.129
Methyl behenate (C22:0)	0.865
Methyl erucate (C22:1)	0.727

Therapeutic applications of oleic acids

Antioxidant activity: Comparatively to standard ascorbic acid, the fermented mango extract shows an important relationship between concentration and radical scavenging activity by progressively increasing from 25 µL to 100 µL when compared the DPPH tube method in present study (Fig. 7). It was already reported that the effective concentration of the sample required scavenging DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of the dose-response curve plotted between the percentage of inhibition and concentrations [21].

Anti-inflammatory activity: The fatty acid-rich extract showed the highest 17.08% inhibition of hemolysis in a sample concentration of 100 µL, compared to the standard showing equal inhibition. The fermented extract showed the highest inhibition of protein denaturation at a concentration of 100 µL. For fatty acid extracts, concentrations above 100 µL were hemolytic for cells and the concentration was low, automatically

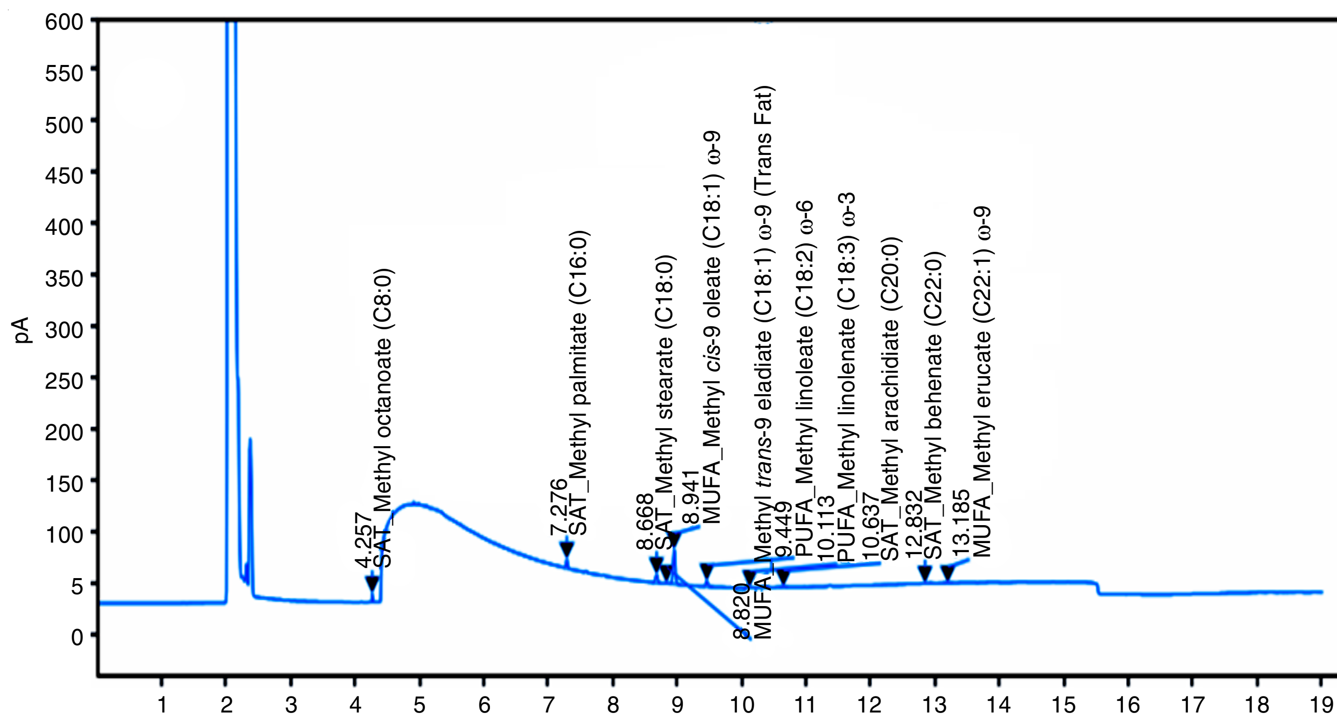

Fig. 6. GC-MS FAME analyses for oleic acids

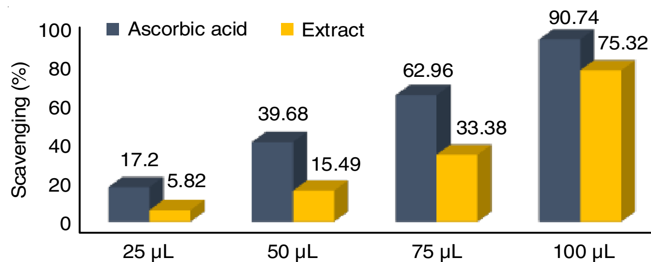

Fig. 7. Antioxidant activities of oleic acids

decreasing the hemolysis strongly; the recorded values were significantly lower ($p < 0.05$) than the control (Fig. 8). Similarly, as already reported, the fatty acid extracts showed equal inhibition that was obtained comparable with selected standards. It was already reported that inhibitory activities are closely related to radical scavenging activity and the anti-inflammatory activity of the concentrated extracts may be linked to the higher fatty acids, especially oleic acid, palmitic acid, linolenic acid and stearic acid [22]. This is consistent with the facts this study reported.

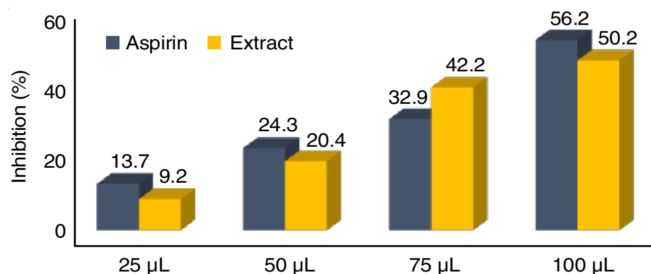

Fig. 8. Anti-inflammatory effects of oleic acids

Antibacterial activity: The MIC results of the crude oleic acids showed the significant antibacterial activity against three pathogens *e.g. P. aeruginosa*, *K. pneumoniae* and *E. coli* (Fig. 9). The measurement of the inhibition area showed that the fermented extract gave a minimum inhibitory concentration (MIC) of 1250 µg/mL of concentration to the bacterium *P. aeruginosa*, *K. pneumoniae* and *E. coli* inhibition. Oleic acid showed higher antibacterial activity against *P. aeruginosa* [23].

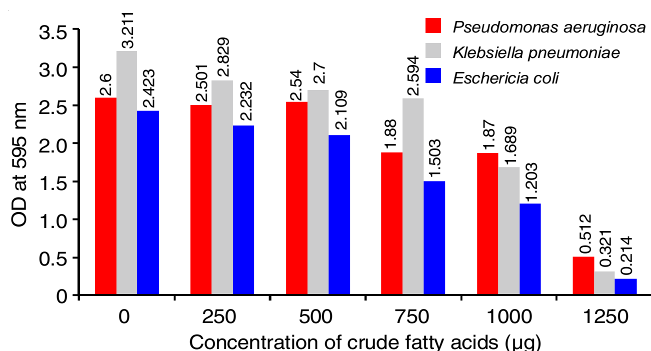

Fig. 9. Antibacterial activity of oleic acid against clinical pathogens

Conclusion

Extensive study is required to identify alternate production methods for oleic acid from the industrial wastes. The present study demonstrated that oleic acid can be produced from mango kernels using probiotic bacteria. Oleic acid has shown therapeutic applications like antimicrobial, antioxidant and anti-inflammatory activities. This can be further taken for other applications of fatty acids in pharmaceutical companies with appropriate studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. M.I. Covas, *Pharmacol. Res.*, **55**, 175 (2007); https://doi.org/10.1016/j.phrs.2007.01.010
2. J.L. Alves, A.S.C. Figueira, M. Souto, I.L. Lopes, J.C. Dionísio, R.M. Quinta-Ferreira and M.E. Quinta-Ferreira, *Energy Rep.*, **6(Suppl. 1)**, 885 (2020); https://doi.org/10.1016/j.egyr.2019.11.034
3. D. Casares, P.V. Escribá and C.A. Rosselló, *Int. J. Mol. Sci.*, **20**, 2167 (2019); https://doi.org/10.3390/ijms20092167
4. M.A. Fowomola, *Afr. J. Food Sci.*, **4**, 472 (2010).
5. C. Santa-María, S. López-Enríquez, S. Montserrat-de la Paz, I. Geniz, M.E. Reyes-Quiroz, M. Moreno, F. Palomares, F. Sobrino and G. Alba, *Nutrients*, **15**, 224 (2023); https://doi.org/10.3390/nu15010224
6. S. Kittiphoom, *Int. Food Res. J.*, **19**, 1325 (2012).
7. K.K. Yadav, N. Garg, A. Verma, S. Kumar and M. Trivedi, *Indian J. Agric. Sci.*, **87**, 943 (2017); https://doi.org/10.56093/ijas.v87i7.71925
8. E.F. Delong and A.A. Yayanos, *Appl. Environ. Microbiol.*, **51**, 730 (1986); https://doi.org/10.1128/aem.51.4.730-737.1986
9. R. Subramaniyam and R. Vimala, *Int. J. Nat. Sci.*, **3**, 480 (2012).
10. A.M. Gad, E.A. Beltagy, U.M. Abdul-Raouf, M.A. El-Shenawy and S.S. Abouelkheir, *Chem. Adv. Mater.*, **1**, 41 (2016).
11. M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar and L.A. Escaleira, *Talanta*, **76**, 965 (2008); https://doi.org/10.1016/j.talanta.2008.05.019
12. J. Ryan, H. Farr, S. Visnovsky, M. Vyssotski and G.A. Visnovsky, *J. Microbiol. Methods*, **82**, 49 (2010); https://doi.org/10.1016/j.mimet.2010.04.001
13. E.S. Bindiya, K.J. Tina, S.S. Raghul and S.G. Bhat, *Probiotics Antimicrob. Proteins*, **7**, 157 (2015); https://doi.org/10.1007/s12602-015-9190-x
14. N. Buntin, S. Chanthachum and T. Hongpattarakere, *J. Sci. Technol.*, **30**, 141 (2008).
15. J.P. Dworzanski, L. Berwald and H.L.C. Meuzelaar, *Appl. Environ. Microbiol.*, **56**, 1717 (1990); https://doi.org/10.1128/aem.56.6.1717-1724.1990
16. A.A. Gharaibeh and K.J. Voorhees, *Anal. Chem.*, **68**, 2805 (1996); https://doi.org/10.1021/ac9600767
17. S. Bandhu, D. Dasgupta, J. Akhter, P. Kanaujia, S.K. Suman, D. Agrawal, S. Kaul, D.K. Adhikari and D. Ghosh, *Springerplus*, **3**, 691 (2014); https://doi.org/10.1186/2193-1801-3-691
18. P. Sanap, D. Sonawane, S. Patil and A. Pratap, *Ind. Crops Prod.*, **188**, 115711 (2022); https://doi.org/10.1016/j.indcrop.2022.115711
19. L.R. Laureles, F.M. Rodriguez, C.E. Reano, G.A. Santos, A.C. Laurena and E.M.T. Mendoza, *J. Agric. Food Chem.*, **50**, 1581 (2002); https://doi.org/10.1021/jf010832w
20. K. Kara, F. Ouanji, E.M. Lotfi, M.E. Mahi, M. Kacimi and M. Ziyad, *J. Egypt. Pet.*, **27**, 249 (2018); https://doi.org/10.1016/j.ejpe.2017.07.010
21. T. Kuda, M. Tsunekawa, H. Goto and Y. Araki., *J. Food Compos. Anal.*, **18**, 625 (2005); https://doi.org/10.1016/j.jfca.2004.06.015
22. A. Kicel, A. Owczarek, P. Gralak, P. Ciszewski and M.A. Olszewska, *Phytochem. Lett.*, **30**, 349 (2019); https://doi.org/10.1016/j.phytol.2019.02.027
23. G. Casillas-Vargas, C. Ocasio-Malavé, S. Medina, C. Morales-Guzmán, R.G. Del Valle, N.M. Carballeira and D.J. Sanabria-Ríos, *Prog. Lipid Res.*, **81**, 101093 (2021); https://doi.org/10.1016/j.plipres.2021.101093