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Dual Lipases System in Transesterification of Ethyl Ferulate with Olive Oil: Optimization by Response Surface Methodology

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ABSTRACT— ***a novel and simple dual lipases system was effectively developed in attaining high performance of ferulate esters by transesterification between ethyl ferulate and olive oil. Enzyme screening revealed 1: 9 w/w of Novozym 435-Lipozyme RMIM to be the most efficient lipases ratio for the reaction synthesis. Response surface methodology (RSM) based on four-factor-five-level central composite design (CCD) was used to study interactive effects of reaction factors: time, lipase dosage, ratio substrates and temperature. A high percent conversion of 94.03 % was achieved under the optimum conditions of 12 h, 87 mg lipases, 1: 2 ratio substrates and 60 °C, which compared well with the maximum predicted value of 94.83 %.***

Keywords— Dual lipases system, transesterification, ferulate esters, optimization

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1.INTRODUCTION

The versatility of lipase (triacylglycerol acylhydrolase; EC 3.1.1.3) has paved the way for its wide applications especially in fats and oils segment. Nowadays, the used of lipases in modifying fats and oils to form structured lipids are a mature topic due to their improvable characteristics, like certain physical [1] and chemical properties [2] as well as nutritional benefits [1, 3].

Ferulic acid is a hydroxycinnamic acid naturally found in plant kingdom possessing functional and nutritional benefits [4]. Due to the hydrophilicity, however, ferulic acid exhibits low solubility and stability in various solvent systems and thus restricts its application in diverse fields. Therefore, the strategy to transesterify of the ferulic acid or its ethyl ester (ethyl ferulate) with lipophilic media (fats or oils) was a trendy idea to enhance the solubility of the compounds [5, 6, 7, 8]. The new amphiphilic molecule possesses surface activity because it contains both hydrophobic head (fatty acid moiety) and hydrophilic tail (phenolic moiety) [7]. Together, its original function properties such as antioxidant, anti-inflammatory, antiviral and UV absorbing also showed an improvement [9, 10].

Non-specific lipase of Novozym 435 from Antartic yeast *Candida Antarctica* commonly reported as an efficient biocatalyst for the synthesis of ferulate esters [6, 7, 11]. An additional problem is the industrial scale production of ferulate esters using Novozym 435 is still very restricted due to the relative high production cost of this lipase [12, 13, 14]. Combination of Novozym 435 with any specific lipase simultaneously seems to be an alternative idea to overcome such shortcoming and reveal their combined interactions.

Until recently, there have been few attempts to use combination of two lipases with different specificities, also known as dual lipases system, in the synthesis of bio-lubricants [15], transesterification of biodiesel [16, 17, 18, 19], modification of bulky oils [20], production of wax esters [21] and hydrolysis of starch [22]. This concept has been applied in two forms; immobilization of crude lipases onto one support and application of commercialize immobilized lipases concurrently. Dual lipases system demonstrates several advantages including enhances the conversion within time [17, 19], offers advance catalytic activity [18] , displays synergistic effect [20] and improves thermostability [22].

Therefore, in this study, a dual lipases system of non-specific Novozym 435 and 1, 3-specific Lipozyme RM IM (from fungi *Rhizomucor miehei*) was investigated in catalyzing transesterification of ethyl ferulate with olive oil. The effects of reaction factors (incubation time, lipase dosage, ratio substrates and temperature) were also evaluated using

RSM. The target was to maximize the conversion of ferulate esters using a dual lipases system with positive reduction of total biocatalyst cost.

2.MATERIALS AND METHODS

2.1 Materials

Substrates (ethyl ferulate and olive oil) were obtained from Sigma-Aldrich (St. Louis, USA), solvents and chemicals (toluene, ethanol, acetone and potassium hydroxide) were purchased from Merck, Germany. Commercial lipases of Novozym 435 (immobilized lipase B from *Candida antarctica*) and Lipozyme RM IM (immobilized lipase from *Rhizomucor miehei*) were procured from Sigma-Aldrich (St. Louis, USA). All chemicals were of analytical grade.

2.2 Enzymatic transesterification

Transesterification method was modified from Compton et al. [8]. 100 mg various ratios of immobilized lipases of Novozym 435 to Lipozyme RM IM were added into 25 mL screw capped vials containing a mixture of 1 g ethyl ferulate and 4 g olive oil in 5 mL toluene. The vials were placed in a controlled water -bath shaker at 60 $^{\circ}$C and shaken at 200 rpm. The mixture was continuously reacted for 12 h. All batch reactions were conducted under the above conditions unless stated elsewhere.

2.3 Analysis of reaction product

After completion of each assigned period, the reaction was terminated with 7 mL of ethanol: acetone (1: 1 v/v) and the lipases were filtered. The percent conversion (%) of ferulate esters was measured by titration with 0.3 M potassium hydroxide in an automatic tittrator (Methrom, Switzerland) [23]. All the samples were assayed in triplicate and the experiment was repeated twice. The means were used for evaluation of the results.

$$\text{Conversion of ferulate esters (\%)} = \frac{\text{Volume of KOH (without lipases)} - \text{Volume of KOH (with lipases)}}{\text{Volume of KOH (without lipases)}} \times 100$$

2.4 Response surface design

A four-factor-five-level CCD was employed, requiring 28 experiments including 16 factorial points, 6 axial points and 6 center points. The factors and levels selected based on the conventional study were as follows: reaction time (4 – 12 h), lipase dosage (60 – 90 mg), ratio substrates ethyl ferulate/olive oil (1:2 – 1:6 g/g) and temperature (50 – 70 $^{\circ}$C). Triplicate experiments were set up for each run with all 28 runs performed in random order. The data obtained were fitted to a second-order polynomial equation:

$$Y = b0 + \sum_{i=1}^{4} bi\, xi + \sum_{i=1}^{4} bii\, xi^2 + \sum_{i=j}^{3} \sum_{j=i+1}^{4} bij\, xij$$

Where Y is dependent variable (percentage conversion) to be modelled, χ_i and χ_j are the uncoded independent variables and b_0, b_i, b_{ii}, b_{ij} are the constant coefficients of the model.

2.5 Statistical analyses

The data from the experiments performed were analyzed using RSM (Design Expert version 7.1.6) and then interpreted. Three main analytical steps: analysis of variance (ANOVA), regression analysis and plotting of response surface were generated to establish an optimum condition for the transesterification.

3.RESULTS AND DISCUSSION

3.1 Effect of dual lipases system as biocatalyst

Figure 1 shows the different ratios of Novozym 435 to Lipozyme RM IM with their conversion degrees in the synthesis of ferulate esters. The results suggested that the mixtures of Novozym 435-Lipozyme RM IM at any test ratios resulted in higher transesterification degrees (>80%) compared to the use Novozyme 435 (69.71%) and Lipozyme RMIM

(70.06%) alone. These observations agree well with those of Banerjee et al. [16] and Kuo et al. [21] whom used same dual lipases system of Novozym 435-Lipozyme RM IM for better conversion of esters. The presence of solvent in the system plays a crucial role which reflects the finding ratio [24, 25]. In addition, electronic and stearic effect of hydroxylated derivatives of cinnamic acid caused lipase from *R. miehei* shown higher reaction rate and yield compare to the lipase from *C. Antarctica* [26]. Referring to our developed dual lipases system, the application of large portion of 1, 3-specific Lipozyme RMIM lipase would trigger hydrolyzation of triacylglycerol of olive oil to form diacylglycerol for rapid acyl-enzyme actions. Further addition of small quantity of nonspecific Novozym 435 would create an environment containing various specificity of lipases for the diverse fatty acids presented in the system (from olive oil), thus result in an enhanced percent conversion of ferulate esters. Considering the degree of percent conversion and economical point of view, 1: 9 Novozym 435-Lipozyme RM IM was used as an optimum ratio.

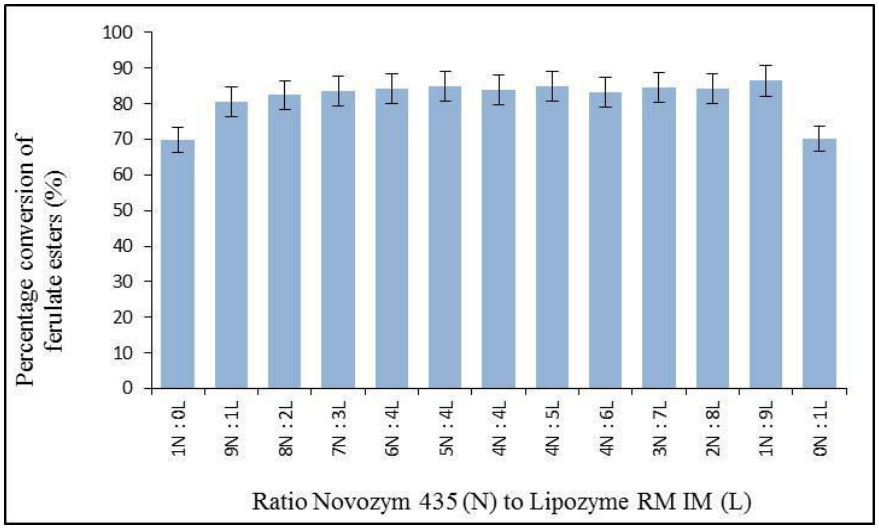

Figure 1: Screening Of Dual Lipases System in Transesterification of Ethyl Ferulate with Olive Oil

3.2 Model fitting

Modelling of factors and response was performed by RSM. All 28 of the designed experiments for enzymatic synthesis of ferulate esters with predicted value (Table 1) were seen to be sufficiently correlated to the experimental values. Among the different trials of the experimental designs, the greatest conversion (92.4 %) of ferulate esters was achieved in treatment 25 (8 h, 75 mg lipases, 1: 4 ethyl ferulate: olive oil and 60 $^{\circ}$C). Most of the treatments exhibited percent conversion between 55– 90 %, showing that Novozym 435-Lipozyme RM IM used presented a good catalyzing effect in the transesterification. Further, a mathematical model was generated based on the data obtained. Y is the predicted value for ferulate esters conversion (%) and A, B, C and D are the coded variables as describes in Table 1.

$$Y = 88.56 + 12.56\,A + 1.44\,B - 0.1\,C + 0.052\,D - 3.03\,AB - 1.83\,AC - 0.29\,AD - 1.07\,BC + 0.51\,BD + 0.39\,CD - 7.06\,A^2 - 0.84\,B^2 - 0.53\,C^2 - 6.78\,D^2$$

(Equation 1)

Table 1: CCD quadratic polynomial model, experimental data, actual and predicted values

Std	A: Time (h)	B: Lipase dosage (mg)	C: Ratio ethyl ferulate: olive oil (g/g)	D: Temperature (^{o}C)	Conversion (%)	
					Predicted	Actual
1	4 (-1)	60 (-1)	1: 2 (-1)	50 (-1)	54.07	55.65
2	12 (1)	60 (-1)	1: 2 (-1)	50 (-1)	89.48	87.94
3	4 (-1)	90 (1)	1: 2 (-1)	50 (-1)	64.13	66.37
4	12 (1)	90 (1)	1: 2 (-1)	50 (-1)	87.43	87.65
5	4 (-1)	60 (-1)	1: 6 (1)	50 (-1)	58.91	63.98
6	12 (1)	60 (-1)	1: 6 (1)	50 (-1)	86.99	85.41
7	4 (-1)	90 (1)	1: 6 (1)	50 (-1)	64.67	65.5

8	12 (1)	90 (1)	1: 6 (1)	50 (-1)	80.64	82.04
9	4 (-1)	60 (-1)	1: 2 (-1)	70 (1)	52.96	55.62
10	12 (1)	60 (-1)	1: 2 (-1)	70 (1)	87.21	85.54
11	4 (-1)	90 (1)	1: 2 (-1)	70 (1)	65.05	65.79
12	12 (1)	90 (1)	1: 2 (-1)	70 (1)	87.2	86.19
13	4 (-1)	60 (-1)	1: 6 (1)	70 (1)	59.35	58.29
14	12 (1)	60 (-1)	1: 6 (1)	70 (1)	86.27	88.09
15	4 (-1)	90 (1)	1: 6 (1)	70 (1)	67.15	72.75
16	12 (1)	90 (1)	1: 6 (1)	70 (1)	81.97	79.55
17	16 (2)	75 (0)	1: 4 (0)	60 (0)	85.42	89.42
18	8 (0)	45 (-2)	1: 4 (0)	60 (0)	82.31	81.28
19	8 (0)	105 (2)	1: 4 (0)	60 (0)	88.06	85.87
20	8 (0)	75 (0)	1: 8 (2)	60 (0)	86.24	83.01
21	8 (0)	75 (0)	1: 4 (0)	40 (-2)	61.33	58.83
22	8 (0)	75 (0)	1: 4 (0)	80 (2)	61.54	60.81
23	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	86.55
24	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	87.2
25	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	92.4
26	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	88
27	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	89.5
28	8 (0)	75 (0)	1: 4 (0)	60 (0)	88.56	87.7

3.3 ANOVA and regression analysis

P-value is employed as a tool to verify the significance of each coefficient. Low p-value less than 0.0500 indicates the significance of the corresponding coefficient is high. The model's regression F-value (30.36), with p-value <0.0001, implies that the quadratic polynomial model was significant at 95% trust level. The obtained value for the determination coefficient (R^2 = 0.9681) indicated that 96.81 % of the experimental response variability can be explained by the previously discussed model (Equation 1). Low value of coefficient of variation (CV = 5.068) was obtained. The CV is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if the CV is not greater than 10% [27]. The predicted R-squared of 0.807 was in reasonable agreement with adjusted R-squared of 0.936 suggested a satisfactory representation of the model. The insignificant lack of fit (LOF = 0.0547) confirmed that the model explains very well the experimental data in the chosen intervals. The analysis of ANOVA also shows that the order of reaction factors affecting on the transesterification by dual lipases system were reaction time > lipase dosage > ratio substrates > temperature.

3.3 Response surface analysis

Equation 1 was then used to facilitate plotting of 3D response surfaces (Figure 2) where two parameters were plotted at any one time with other two remaining parameters were fixed at zero level.

Figure 2a shows the response surface plots as a function of reaction time [A] versus lipase dosage [B] on percent conversion of ferulate esters. Theoretically, increase the levels of both factors have increased the percent conversion of ferulate esters. Two molecules of enzymes acting independently will transform twice as much substrate in a specific time [28]. A slight decrease in percent conversion was predicted at highest level of lipase dosage (90 mg) and reaction time (12 h). Too much lipase molecules in a system caused mass transfer limitation due to the agglomeration of the lipases even at longer incubation period.

Figure 2b shows the response surface plots as a function of reaction time [A] versus ratio substrates [C]. The first step in the mechanism of lipase-catalyzed reaction is attack of the catalytic serine (Ser^{120}) on the carbonyl carbon of the acyl donor (ethyl ferulate) to form the acyl-enzyme intermediate [29]. Longer reaction time may increase the chance of contact between enzyme and substrate, but, further increment of olive oil molecules in this study may distant the active site of the lipases from the limited concentration of ethyl ferulate.

In contact with reaction temperature, generally, lipase catalyzed reaction systems exhibit dome shaped plot as shown in Figure 2c (reaction time [A] versus temperature [D]). From the plot, 60 °C appeared to be a critical temperature. At below 60 °C, the reaction conversion was rather low due to viscosity of reaction system. At this condition, active sites of the lipases difficult to find and attach to the substrates. Beyond the 60 °C, however, the benefits of low viscosity reaction system were offset by operational instability of the lipases. The critical temperature was clearly observed in Figure 2d (lipase dosage [B] versus temperature [D]).

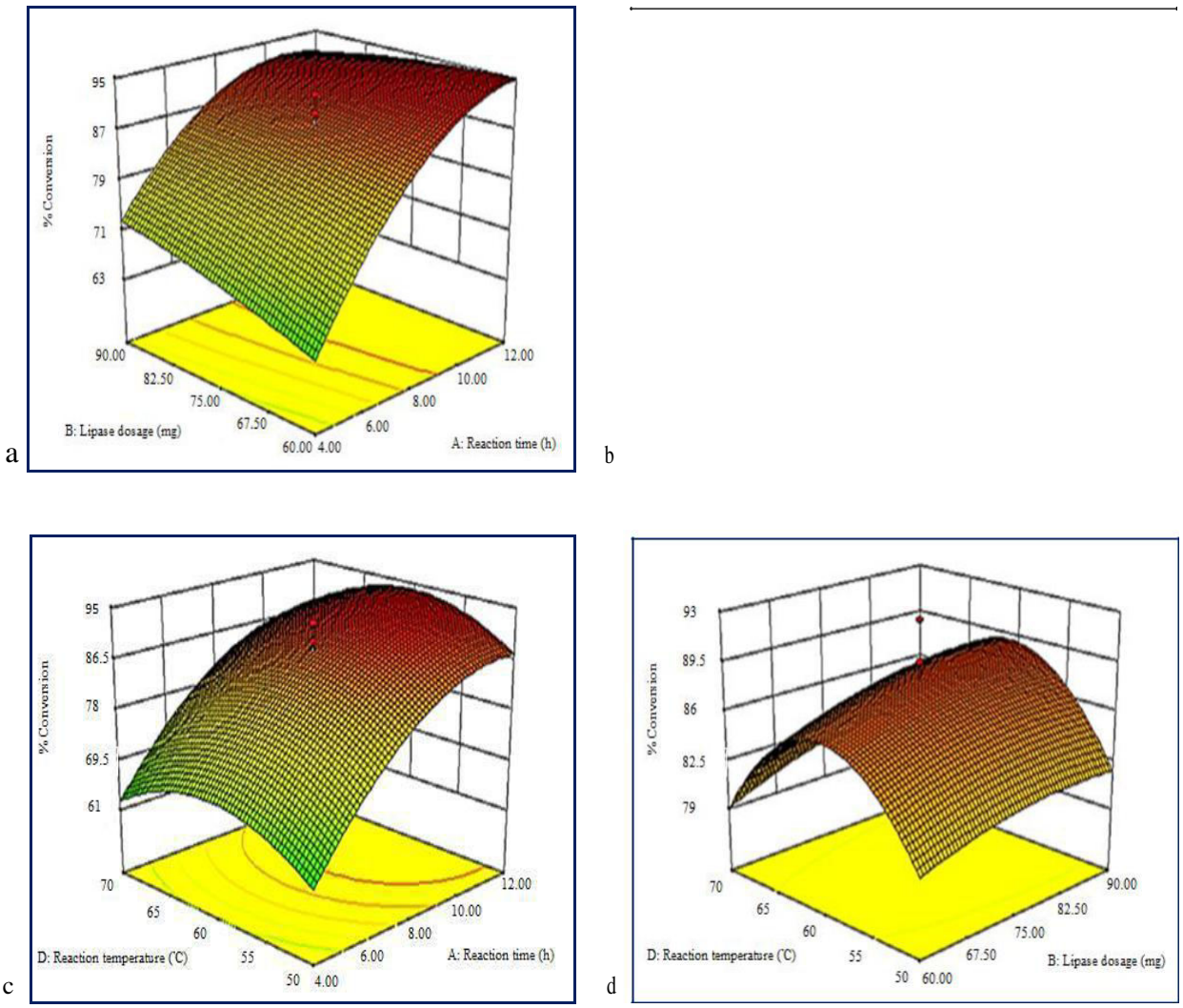

Figure 2: Response Surface Plots Between Any Two Factors with Other Parameters Fixed at Their Center Points and Conversion of Ferulate Esters. Conditions: A) Ratio Substrates=1:4, Temperature=60°C; B) Lipase Dosage=75mg, Temperature= 60°C; C) Lipase Dosage=75mg, Ratio Substrates=1:4; D) Reaction Time=8h, Ratio Substrates=1:4

3.5 Optimum conditions

The optimum conditions for the conversion of ferulate esters by Novozym 435-Lipozyme RM IM were predicted using RSM as follows: 12 h, 87 mg lipases, 1: 2 ratio substrates and 60 °C. Under these optimal conditions, ~94% of percent conversions were obtained. Significantly, much shorter of reaction time was required to convert to ferulate esters by Novozym 435-Lipozyme RM IM with high percent conversion as compared to the traditional synthetic methods by using single lipase [7, 8, 30]. The optimum conditions can be used for future upscale synthesis of ferulate esters.

4. CONCLUSION

A dual lipases system of 1: 9 Novozym 435-Lipozyme RM IM was developed and found to be a promising biocatalyst offering myriad possibilities in enhancing the ferulate esters synthesis. The larger portion of Lipozyme RM IM compare to the Novozym 435 in an optimum ratio of the dual lipases system consequently provides positive reduction for the total biocatalyst costs. According to the optimum conditions, the conversion of ferulate esters were able to reached 94%. Therefore, optimization of ferulate esters synthesis by using dual lipases system was successful.

5.ACKNOWLEDGEMENT

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REFERENCES

[1] Palla CA, Carrin ME, "Kinetics Modelling of The Acidolysis with Immobilized Rhizomucoe Miehei Lipases for Production of Structured Lipids from Sunflower Oil", Biochemical Engineering Journal, vol. 90, pp. 184-194, 2014.
[2] Ciftci D, Saldana MDA, "Enzymatic Synthesis of Phenolic Lipids Using Flaxseed Oil and Ferulic Acid in Supercritical Carbon Dioxide Media", Journal of Supercritical Fluids, vol. 72, pp. 255-262, 2012.
[3] Ma N, Hong SI, Zhao TT, No DS, Kim CT, Kim Y, Kim IH, "Enrichment of Docosahexaenoic Acid from Tuna Oil via Lipase-Mediated Esterification under Pressurized Carbon Dioxide", Journal of Supercritical Fluids, vol. 87, pp. 28-33, 2014.
[4] Barone E, Calabrese V, Mancuso C, "Ferulic Acid and Its Therapeutic Potential as a Hormetin for Age-Related Diseases", Biogerontology, vol. 10, pp. 97-108, 2009.
[5] Sun S, Zhu S, Bi Y, "Solvent-Free Enzymatic Synthesis of Feruloylated Structured Lipids by the Transesterification of Ethyl Ferulate with Castor Oil", Food Chemistry, vol. 158, pp. 292-295, 2014.
[6] Sun S, Song F, Bi Y, Yang G, Liu W, "Solvent-Free Enzymatic Transesterification of Ethyl Ferulate and Monostearin: Optimized by Response Surface Methodology", Journal of Biotechnology, vol. 164, no. 2, pp. 340-345, 2013.
[7] Yang Z, Glasius M, Xu X, "Enzymatic Transesterification of Ethyl Ferulate with Fish Oil and Reaction Optimization by Response Surface Methodology", Food Technology and Biotechnology, vol. 50, no. 1, pp. 88-97, 2012.
[8] Compton DL, Laszlo JA, Berhow MA, "Lipase-Catalyzed Synthesis of Ferulate Esters", Journal of the American Oil Chemists' Society, vol. 77, no. 5, pp. 513-519, 2000.
[9] Lin FH, Lin JY, Gupta RD, Tournas JA, Burch JA, Selim MA, Monteiro-Riviere NA, Grichnik JM, Zielinski J, Pinnell SR, "Ferulic Acid Stabilizes a Solution of Vitamins C and E and Doubles Its Photoprotection of Skin", Journal of Investigative Dermatology, vol. 125, pp. 826-832, 2005.
[10] Murakami A, Nakamura Y, Koshimizu K, Takahashi D, Matsumoto K, Hagihara K, Taniguchi H, Nomura E, Hosoda A, Tsuno T, Maruta Y, Kim HW, Kawabata K, Ohigashi H, "FA15, A Hydrophobic Derivative of Ferulic Acid, Suppresses Inflammotary Responses and Skin Tumor Promotion: Comparison with Ferulic Acid", Journal of Cancer Letters, vol. 180, pp. 121-129, 2002.
[11] Xin JY, Chen LL, Zhang YX, Zhang E, Xia CG, "Lipase Catalyzed Transesterification of Ethyl Ferulate with Triolein in Solvent-Free Medium", Food and Bioproducts Processing, vol. 89, pp. 457-462, 2011.
[12] Chattopadhyay S, Sen R, "Development of a Novel Integrated Continuous Reactor System for Biocatalytic Production of Biodiesel", Bioresourse Technology, vol. 147, pp. 395-400, 2013.
[13] Reyes-Duarte D, Lopez-Cortes N, Torres P, Comelles F, Parra JL, Peña S, Ugidos AV, Ballesteros A, Plou FJ, "Synthesis and Properties of Ascorbyl Esters Catalyzed by Lipozyme TL IM Using Triglycerides as Acyl Donors", Journal of American Oil Chemists' Society, vol. 88, pp. 57-64, 2011.
[14] Fjerbaek L, Christensen KV, Norddahl B, "A Review of the Current State of Biodiesel Production Using Enzymatic Transesterification", Biotechnology and Bioengineering, vol. 102, no. 5, pp. 1298–1315, 2009.
[15] Malhotra D, Mukherjee J, Gupta MN, "Lipase Catalysed Tranesterification of Castor Oil by Straight Chain Higher Alcohols", Journal of Bioscience and Bioengineering, vol. x, pp. 1-4, 2014.
[16] Banerjee A, Singh V, Solanki K, Mukherjee J, Gupta MN, "Combi-Protein Coated Microcrystals of Lipases for Production of Biodiesel from Oil From Spent Coffee Grounds", Sustainable Chemical Processes, vol. 1, no.1, pp. 14, 2013.
[17] Lee JH, Kim SB, Kang SW, Song YS, Park C, Ok S, Seung H, Kim W, "Biodiesel Production by A Mixture of Candida Rugosa and Rhizopus Oryzae Lipases Uing A Supercritical Carbon Dioxide Process", Bioresource Technology, vol. 102, pp. 2105-2108, 2011.
[18] Rodrigues RC, Ayub MAZ, "Effects of the Combined Use of Thermomyces Lanuginosus and Rhizomucor Miehei Lipases for the Transeterification and Hydrolysis of Soybean Oil", Process Biochemistry, vol. 46, pp. 682-688, 2011.

[19] Guan F, Peng P, Wang G, Yin T, Peng Q, Huang J, Guan G, Li Y, "Combination of Two Lipases More Efficiently Catalyzes Methanolysis of Soybean Oil for Biodiesel Production in Aqueous Medium", Process Biochemistry, vol. 45, no. 10, pp. 1677-1682, 2010.
[20] Ibrahim NA, Guo Z, Xu X, "Enzymatic Interesterification of Palm Stearin and Coconut Oil by a Dual Lipase System", Journal of American Oil Chemists' Society, vol. 85, pp. 37-45, 2008.
[21] Kuo CH, Chen HH, Chen JH, Liu YC, Shieh CJ, "High Yield of Wax Ester Synthesized from Cetyl Alcohol and Octanoic Acid by Lipozyme RM IM and Novozym 435", International Journal of Molecular Sciences, vol. 13, pp. 11694-11704, 2012.
[22] Roy I, Gupta MN, "Hydrolysis of Starch by a Mixture of Glucoamylase and Pullulanase Entrapped Individually in Calcium Alginate Beads", Enzyme and Microbial Technology, vol. 34, no. 1, pp. 26-32, 2004.
[23] Syamsul KMW, Salina MR, Siti SO, Hanina MN, Basyaruddin MAR, Kamaruzaman Jusoff, "Green Synthesis of Lauryl Palmitate via Lipase-Catalyzed Reaction", World Applied Sciences Journal, vol. 11, no 4, pp. 401-407, 2014.
[24] Salis A, Solinas V, Monduzzi M, "Wax Esters Synthesis from Heavy Fraction of Sheep Milk Fat and Acetyl Alcohol by Immobilized Lipases", Journal of Molecular Catalysis B: Enzymatic, vol. 21, no. 4-6, pp.167-174, 2003.
[25] Oguntimein GB, Anderson WA, Moo-Young M, "Synthesis of Geraniol Esters in a Solvent-Free System Catalysed by Candida Antarctica Lipase", Biotechnology Letters, vol. 17, pp. 77-82, 1995.
[26] Stamatis H, Sereti V, Kolisis FN, "Enzymatic Synthesis of Hydrophobic Derivatives of Natural Phenolic Acids in Organic Media", Journal of Molecular Catalysis B: Enzymatic, vol. 11, no. 4-6, pp. 323-328, 2001.
[27] Beg Q, Sahai V, Gupta R, "Statistical Media Optimization and Alkaline Protease Production from *Bacillus Mojavensis* in Bioreactor", Process Biochemistry, vol. 39, no. 2, pp. 203-209, 2003.
[28] Dixon M, Webb E, Enzymes, Longman, London, 1979.
[29] Carvalho CML, Serralheiro MLM, Cabral JMS, Aires-Barros MR, "Applications of Factorial Design to the Study of Transesterification Reactions using Cutinase in AOT-Reversed Micelles", Enzyme and Microbial Technology, vol. 21, pp. 117-123, 1997.
[30] Xin JY, Zhang L, Chen LL, Zheng Y, Wu XM, Xia CG, "Lipase-Catalyzed Synthesis of Ferulyl Oleins in Solvent-Free Medium", Food Chemistry, vol. 112, pp. 640-645, 2009.