A novel and rapid method for fatty acid preparation by the lipase-catalyzed hydrolysis of Phoenix tree seeds

Shangde Sun¹ · Jinming Liu¹ · Xulei Li¹

Received: 13 June 2018 / Accepted: 4 September 2018 / Published online: 8 September 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Fatty acids are the precursors for the production of fuels, oleochemicals and special health care products. In this study, a novel rapid method for fatty acid (FA) preparation by the enzymatic hydrolysis of Phoenix tree seed, an undeveloped woody oil seed, was developed. High-temperature GC with flame ionization detector (FID) and the hydrolysis ratio were used to monitor reaction progress. Enzyme screening and the effect of reaction variables on the hydrolysis of seeds were evaluated and optimized by response surface methodology. The results showed that among the tested enzymes, Lipozyme TLIM showed the greatest amount of hydrolysis of Phoenix tree seed. FAs can be rapidly prepared by one-step hydrolysis of Phoenix tree seeds using Lipozyme TLIM as the biocatalyst. Under the optimized conditions (6% enzyme load, 1:8 mass ratio of seed to water, 47.7 °C and 16 min), the maximum hydrolysis ratio ($96.4 \pm 1.1\%$) can be achieved. The effect of reaction variables on the hydrolysis decreased in the following order: reaction time > enzyme load > substrate ratio of seed to water > reaction temperature. This work provides a novel and rapid method for FA preparation from oil seeds.

Keywords Fatty acids · Hydrolysis · Lipozyme TLIM · One-step · Phoenix tree seed · Response surface methodology

Introduction

The Phoenix tree is a tall deciduous tree that has been used as an ornamental plant for roadsides and courtyards in China, Europe and USA (Woo et al. 2016; Xie et al. 2014; Upson and Cullen 2012; Tang et al. 2010). The seed of the Phoenix tree has been used as a traditional Chinese herb for promoting digestion and treating stomachaches (Woo et al. 2016). In our previous work (Sun and Li 2016), some active ingredients of Phoenix tree seeds, such as oil, protein, moisture, ash and fiber, were found, accounting for 27.8, 19.7, 7.5, 4.4 and 31.2% (w/w) of the seed, respectively. It is a new undeveloped woody oil seed that contains 10 types of fatty

acids. The major fatty acids (FAs) were sterculic acid (S, $23.22 \pm 1.57\%$), oleic acid (O, $22.23 \pm 1.85\%$) and linoleic acid (L, $30.16 \pm 1.92\%$) (Sun and Li 2016). These FAs are valuable molecules that can be used as precursors for the production of fuels, oleochemicals and special health care products (Khoo et al. 1991; Lennen et al. 2010; Liu et al. 2010; Nickolau et al. 2008). Therefore, it is interesting to prepare FAs from Phoenix tree seeds.

Traditionally, FAs can be prepared by the hydrolysis of oil and fats using chemical catalysts (acid or base) or biocatalysts (Murty et al. 2002; Satyarthi et al. 2011; Le; Mi et al. 2017). Compared with chemical catalysts, biocatalysts have shown many advantages for FA preparation, especially for some polyunsaturated fatty acids, such as EPA and DHA (Chen et al. 2014; Chakraborty et al. 2010; Singh et al. 2016), which were attributed to mild reaction conditions (Goswami et al. 2011; Hasan et al. 2006; Leng et al. 2008; Mendes et al. 2012; Rungruang-saphakun and Keawsompong 2018; Santos et al. 2013). However, these traditional procedures required more time and a large amount of solvent to extract oil from the seeds; thus later, FAs were prepared by the hydrolysis of the extracted oil. As far as we know, there is no available

✉ Shangde Sun
sunshangde@hotmail.com

Jinming Liu
942141942@qq.com

Xulei Li
741836801@qq.com

¹ Lipid Technology and Engineering, School of Food Science and Engineering, Henan University of Technology, Lianhua Road 100, Zhengzhou 450001, Henan, People's Republic of China

information about FA preparation directly from seeds as substrate for enzymatic hydrolysis.

This work aimed to develop a novel and rapid method for FA preparation by the enzymatic hydrolysis of Phoenix tree seeds (Fig. 1). Enzyme screening and the effect of reaction variables on the hydrolysis of seeds were investigated and response surface methodology (RSM) was used to optimize the enzymatic hydrolysis.

Materials and methods

Materials

Phoenix tree seeds were provided by Jiangsu Xintai Nursery Filed (Jiangsu, China). Lipozyme TLIM (250 IU/g) was obtained from Novozymes A/S (Bagsvaerd, Denmark). Alkaline protease (35,000 U/g), acid protease (100,000 U/g) and cellulase (38,000 U/g) were all purchased from DENYKEM Biological Technology Co., Ltd. (Shanghai, China). Ethanol (95%, v/v) and Alkali Blue 6B were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). All other chemicals used were analytical grade.

Pretreatment of Phoenix tree seeds

Phoenix tree seeds were first milled and passed through a 40-mesh sieve. The seed powder had an average particle size of 0.45 nm and was dried at 105 °C for 4 h and used for the next reactions.

Enzyme screening

Enzymatic hydrolysis reactions of Phoenix tree seed powder using different enzymes were performed in 250-mL three-necked flasks. Phoenix tree seed powder (10 g) and water (100 g) were mixed by a magnetic stirrer (200 rpm) at 45 °C. Next, 5.5 g of enzyme was added to the reaction mixture, and the reaction was initiated. After 10 min, the reaction was complete. The product was first centrifuged, and then, the supernatant was transferred into the oven under vacuum to dry at 70 °C for 4 h. The dried supernatant was then transferred into 250-mL conical flask for extraction using hexane, and the product was used for further analysis.

Effect of reaction variables

The enzymatic hydrolysis reactions were performed in 250-mL three-necked flasks. Phoenix tree seed powder was first mixed with water according to different mass ratios using a magnetic stirrer (200 rpm) at different temperatures (30–55 °C). The reaction was initiated when enzyme was added into the reaction mixture. The samples (2 mL) were withdrawn at specified time intervals. The samples were treated using the same method of enzyme screening for further analysis.

Analysis methods

High temperature GC (Agilent 7890B), equipped with a DB-1H capillary column (30 m×0.25 mm, 0.1 µm of film thickness) and flame ionization detector, was used to evaluate the hydrolysis process. The detector and injector temperatures were set at 350 and 400 °C, respectively. The column initial temperature was set at 100 °C and then increased to

Fig. 1 Enzymatic hydrolysis of phoenix tree seeds to prepare fatty acids

360 °C at 20 °C/min. Helium was used as the carrier gas at 1 mL/min. The injection volume was 1 µL.

Evaluation of the hydrolysis ratio

Acid values were analyzed according to the AOCS Official Method Cd 3d-63. The hydrolysis ratio (%) was calculated from the following equation:

$$\text{Hydrolysis ratio} = \frac{AV_t - AV_0}{SV - AV_0} \times 100\%, \quad (1)$$

where AV_0 is the initial acid value of the Phoenix tree seed and AV_t is the acid value of the Phoenix tree seed at time t . SV is the saponification value of the Phoenix tree seed oil.

FA yield (%) was the mass ratio of FAs in the product to the theoretical value in the seeds.

Experimental design for RSM

A 3-level-4-factor Box–Behnken design was used to evaluate the effect of reaction variables on the hydrolysis. The factors and levels were temperature [30 °C (−1), 45 °C (0), 60 °C (+1)], enzyme load (0.1% (−1), 5% (0), 9.9% (+1), relative to the weight of all substrates), time [1 (−1), 10 (0), 19 min (+1)], and the substrate ratio of seed to water [1:2 (−1), 1:10 (0), 1:18 (+1), w/w].

Statistical analysis

A Box–Behnken design (Design-Expert 8.0) for four independent variables was used to obtain the combination of values that optimized the responses within the region of the three dimensional observation space, which allows for designing a minimum number of experimental runs. The model evaluates the effects of each independent variable on responses. 3^k factorial tests allow efficient estimation of a second-order polynomial regression. The mathematical relationship relating the variables to the response can be calculated by the quadratic polynomial equation as follows:

$$Y = \beta_0 + \sum_{i=1}^4 \beta_i X_i + \sum_{i=1}^4 \beta_{ii} X_i^2 + \sum_{i=1}^3 \sum_{j=i+1}^4 \beta_{ij} X_i X_j, \quad (2)$$

where Y is the hydrolysis rate of Phoenix tree seed; X_1 , X_2 , X_3 and X_4 represent the reaction temperature, enzyme load, reaction time and substrate ratio of seed to water. β_0 , β_i , β_{ii} and β_{ij} are the constant coefficients.

All experiments were carried out in triplicate. The results were expressed as the average $\pm$ standard error (SE). Statistical analysis was performed using Statistical Product and Service Solution (SPSS) software version 19.0. The differences in mean value were evaluated by an analysis of variance

(two-way ANOVA) method. Significance was determined at a 95% level of probability. Different letters (a, b, c and d) indicated that there are significant differences among these data. The same letters suggest that there is no significant difference in the data.

Results and discussion

Enzyme screening

Lipozyme TLIM, alkaline protease, acidic protease and cellulase were used as catalysts for the hydrolysis of Phoenix tree seeds. The order of hydrolysis activities, from greatest to least, was as follows: Lipozyme TLIM > acidic protease > Cellulase > alkaline protease (Fig. 2). Lipozyme TLIM showed the highest performance for hydrolysis. This can be explained by the fact that Lipozyme TLIM is the hydrolytic enzyme for lipids and shows increased hydrolysis activity compared to other enzymes.

Effect of hydrolysis variables

In the first 5 min, the hydrolysis rate of the Phoenix tree seed rapidly increased to 87% and the maximum hydrolysis rate (~95%) was obtained at 10 min (Fig. 3). The time (10 min) is shorter than that of the lipase-catalyzed hydrolysis of linseed oil (3.33 h) (Chen et al. 2014) and unrefined jatropha oil (8 h) (Zhou et al. 2015), which was due to the presence of the emulsification effect of protein in the seed.

Different amounts of Lipozyme TLIM from 0.5% (relative to the weight of the Phoenix tree seed) to 7% were used for the hydrolysis of Phoenix tree seed (Fig. 4). The results

Fig. 2 Enzymatic hydrolysis of Phoenix tree seeds using different enzymes. Hydrolysis conditions: 5% enzyme load, 1:10 (w/w) mass ratio of seed to water, 10 min and 45 °C

Fig. 3 Effect of reaction time on the hydrolysis of Phoenix tree seeds. Reaction conditions: 5% enzyme load, 1:10 (w/w) mass ratio of seed to water and 30 °C. Significance was represented as different letters (a, b, c and d)

Fig. 4 Effect of enzyme load on the hydrolysis of Phoenix tree seeds with 1:10 (w/w) mass ratio of seed to water at 30 °C for 10 min. Significance was represented as different letters (a, b, c and d)

showed that the hydrolysis rate of Phoenix tree seed rapidly increased with the increase in the enzyme load from 0.1 to 5% and the maximum hydrolysis rate (~95%) was obtained with a 5% enzyme load. However, the enzyme load had a minor effect on FA yields. These results can be explained by the fact that at a higher enzyme load (> 5%), enzyme aggregation was found, resulting in decreased enzymatic efficiency.

Fig. 5 Effect of mass ratio of seed to water on the hydrolysis of Phoenix tree seeds with 5% enzyme load at 30 °C for 10 min. Significance was represented as different letters (a, b, c and d)

Fig. 6 Effect of reaction temperature on the hydrolysis of Phoenix tree seeds with 5% enzyme load and 1:10 (w/w) mass ratio of seed to water for 10 min. Significance was represented as different letters (a, b, c and d)

The hydrolysis rate increased with the decrease in the substrate ratio from 1:2 to 1:10 (w/w). After reaching equilibrium, the hydrolysis rate of Phoenix tree seed increased to the maximum (~95%) (Fig. 5). However, the substrate ratio had no obvious effect on FA yield. These results showed that an appropriate amount of water is necessary for the hydrolysis of Phoenix tree seed.

The hydrolysis rate of Phoenix tree seed increased with an increase in reaction temperature from 30 to 45 °C. The maximum hydrolysis rate (~98%) was achieved at 45 °C (Fig. 6). After that, the hydrolysis ratio decreased, which

was due to the deactivation of Lipozyme TLIM at a higher temperature ($> 45\text{ }^{\circ}\text{C}$). Thus, $45\text{ }^{\circ}\text{C}$ was optimal for the hydrolysis of Phoenix tree seed.

Response surface analysis and model fitting

RSM is an empirical modeling technique used to evaluate the relationship between a set of controllable experimental factors and observed results (Shet et al. 2018; Sonawane and Arya 2017). The experimental values obtained from the model are shown in Table 1. The regression data used a quadratic polynomial equation to calculate the relationship between the hydrolysis ratio of Phoenix tree seed with reaction variables (Table 2).

A low P value ($P < 0.0001$) and high coefficient ($R^2 = 0.9496$) can be obtained from the variance (ANOVA)

analysis (Table 2). These results showed that the model and the Eq. (3) were both validated to explain the relationship between reaction variables with the hydrolysis ratio of Phoenix tree seed. The order of hydrolysis variables influencing the hydrolysis ratio of Phoenix tree seed was as follows: reaction time $>$ enzyme load $>$ substrate ratio of seed to water $>$ reaction temperature. The regression equation explaining the hydrolysis ratio of Phoenix tree seed was given as follows:

$$Y(\%) = 97.38 - 11.72X_1 + 29.66X_2 + 16.14X_3 + 4.42X_4 - 13.74X_1X_2 + 3.62X_1X_3 - 1.30X_1X_4 + 6.43X_2X_3 - 3.24X_2X_4 - 0.56X_3X_4 - 13.97X_1^2 - 31.16X_2^2 - 16.70X_3^2 - 7.88X_4^2 \quad (3)$$

Table 1 Response surface experimental design and results

Trial ^a	Temperature X_1 ($^{\circ}\text{C}$)	Enzyme load X_2 (%) ^b	Hydrolysis time X_3 (min)	Substrate of seed to water X_4 (w/w)	Hydrolysis ratio (%)
1	45 (0)	5 (0)	19 (+1)	1:18 (+1)	97.8 ± 1.2
2	45 (0)	5 (0)	10 (0)	1:10 (0)	95.2 ± 1.4
3	45 (0)	0.1 (−1)	19 (+1)	1:10 (0)	31.1 ± 2.1
4	30 (−1)	5 (0)	10 (0)	1:2 (−1)	85.1 ± 2.4
5	45 (0)	0.1 (−1)	1 (−1)	1:10 (0)	13.1 ± 1.9
6	30 (−1)	5 (0)	10 (0)	1:18 (+1)	93.6 ± 1.5
7	45 (0)	5 (0)	10 (0)	1:10 (0)	98.1 ± 1.2
8	45 (0)	5 (0)	10 (0)	1:10 (0)	98.2 ± 0.9
9	45 (0)	5 (0)	10 (0)	1:10 (0)	97.9 ± 1.7
10	60 (+1)	0.1 (−1)	10 (0)	1:10 (0)	43.0 ± 2.6
11	60 (+1)	5 (0)	10 (0)	1:2 (−1)	59.1 ± 2.4
12	45 (0)	0.1 (−1)	10 (0)	1:18 (+1)	27.1 ± 1.7
13	60 (+1)	9.9 (+1)	10 (0)	1:10 (0)	62.1 ± 1.3
14	30 (−1)	5 (0)	19 (+1)	1:10 (0)	93.7 ± 1.9
15	45 (0)	9.9 (+1)	1 (−1)	1:10 (0)	54.1 ± 2.4
16	60 (+1)	5 (0)	10 (0)	1:18 (+1)	62.4 ± 1.5
17	45 (0)	9.9 (+1)	19 (+1)	1:10 (0)	97.9 ± 1.2
18	30 (−1)	5 (0)	1 (−1)	1:10 (0)	73.1 ± 2.1
19	45 (0)	9.9 (+1)	10 (0)	1:2 (−1)	92.1 ± 1.7
20	45 (0)	9.9 (+1)	10 (0)	1:18 (+1)	98.1 ± 0.5
21	45 (0)	0.1 (−1)	10 (0)	1:2 (−1)	8.1 ± 1.4
22	45 (0)	5 (0)	1 (−1)	1:2 (−1)	51.6 ± 2.8
23	45 (0)	5 (0)	19 (+1)	1:2 (−1)	90.8 ± 2.6
24	45 (0)	5 (0)	1 (−1)	1:18 (+1)	60.9 ± 1.5
25	60 (+1)	5 (0)	1 (−1)	1:10 (0)	28.5 ± 2.9
26	60 (+1)	5 (0)	19 (+1)	1:10 (0)	63.6 ± 1.3
27	30 (−1)	0.1 (−1)	10 (0)	1:10 (0)	19.9 ± 1.7
28	45 (0)	5 (0)	10 (0)	1:10 (0)	97.5 ± 1.1
29	30 (−1)	9.9 (+1)	10 (0)	1:10 (0)	93.9 ± 1.5

^aNumbers were run in random order

^bEnzyme load (%; relative to the weight of all substrates)

Table 2 ANOVA analysis of quadratic model for the hydrolysis rate of Phoenix tree seed

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob > F ^a
Model	23952.68	14	1710.91	18.84	<0.0001
A-Reaction temperature	1646.89	1	1646.89	18.13	0.0008
B-Enzyme load	10558.37	1	10558.37	116.26	<0.0001
C-Reaction time	3124.70	1	3124.70	34.41	<0.0001
D-Substrate ratio	234.70	1	234.70	2.58	0.1302
AB	755.43	1	755.43	8.32	0.0120
AC	52.35	1	52.35	0.58	0.4603
AD	6.71	1	6.71	0.07	0.7898
BC	165.25	1	165.25	1.82	0.1988
BD	41.93	1	41.93	0.46	0.5079
CD	1.25	1	1.25	0.01	0.9081
A ²	1266.81	1	1266.81	13.95	0.0022
B ²	6297.52	1	6297.52	69.34	<0.0001
C ²	1809.56	1	1809.56	19.93	0.0005
D ²	403.16	1	403.16	4.44	0.0536
Residual	1271.45	14	90.82		
Lack of fit	1265.41	10	126.54	83.83	0.0003
Pure error	6.04	4	1.51		
Total	25224.13	28			

$$R^2 = 0.9496 \quad R_{Adj}^2 = 0.8992$$

^a $P < 0.05$ indicates statistical significance

The interactions among reaction variables were evaluated by 3D surface plots (Fig. 7a–c). The synergistic interaction of reaction temperature and enzyme load showed that the highest hydrolysis ratio (96%) of Phoenix tree seed could be obtained at the enzyme load (5–7%) and moderate temperature (40–45 °C) (Fig. 7a). The interaction between the temperature and the reaction time had a positive effect on the hydrolysis ratio (Fig. 7b). Compared with the substrate ratio of seed to water, the effect of the enzyme load on the hydrolysis ratio of the Phoenix tree seed was more significant. The maximum hydrolysis ratio of the Phoenix tree seed can be obtained at an enzyme load of 5–7% and a substrate ratio of seed to water at 1:6–1:10 (Fig. 7c).

In addition to studying the effects of the variables on the responses, the 3D surface plots indicate several optimal combinations of the variables that produced a high hydrolysis ratio. These data provide options when practical aspects are considered in real commercialization of the process. According to optimization by the 3D surface plots, the highest hydrolysis ratio ($96.4 \pm 1.1\%$) of Phoenix tree seed can be achieved under the optimized conditions: 47 °C, 16 min,

6% enzyme load, and a 1:8 mass ratio of seed to water. A good agreement between the observed hydrolysis ratio ($96.4 \pm 1.1\%$) and the predicted hydrolysis ratio (97.2%) supports the validation of the model.

Conclusion

In this study, FAs were rapidly prepared by Lipozyme TLIM to catalyze the hydrolysis of Phoenix tree seeds and RSM was used to optimize the hydrolysis reaction. A quadratic regression model between reaction variables (reaction time, substrate ratio, enzyme load and reaction temperature) and the hydrolysis ratio of Phoenix tree seeds was developed. The maximum hydrolysis ratio ($96.4 \pm 1.1\%$) of Phoenix tree seeds was obtained under the optimized conditions (47.7 °C, 16 min, 6% enzyme load and a 1:8 mass ratio of seed to water). The effects of reaction variables on the FA yield of the Phoenix tree seed were ordered as follows: reaction time > enzyme load > substrate ratio of seed to water > reaction temperature.

Fig. 7 3D surface plots between two variables for the hydrolysis of Phoenix tree seed: **a** reaction temperature and enzyme load combined with 1:10 (w/w) substrate ratio for 10 min; **b** reaction temperature and time combined with 1:10 (w/w) substrate ratio and 5% enzyme load and **c** substrate ratio and enzyme load at 45 °C for 10 min

Acknowledgements The authors gratefully acknowledge financial support from National key research and development program in 13th Five-Year (2018YFD0401102) and Provincial Key Laboratory for Transformation and Utilization of Cereal Resource (PL2016008).

Compliance with ethical standards

Conflict of interest No conflict of interest was declared.

References

- Chakraborty K, Vijayagopal P, Chakraborty RD, Vijayana KK (2010) Preparation of eicosapentaenoic acid concentrates from sardine oil by *Bacillus circulans* lipase. *Food Chem* 120(2):433–442. <https://doi.org/10.1016/j.foodchem.2009.10.032>
- Chen W, Sun S, Liang S, Peng L, Wang Y, Shen M (2014) Lipase-catalyzed hydrolysis of linseed oil: optimization using response surface methodology. *J Oleo Sci* 63(6):619–628. <https://doi.org/10.5650/jos.ess13189>
- Goswami D, Basu JK, De S (2011) Lipase applications in oil hydrolysis with a case study on castor oil: a review. *Crit Rev Biotechnol* 33:81–96. <https://doi.org/10.3109/07388551.2012.672319>
- Hasan F, Shah AA, Hameed A (2006) Industrial applications of microbial lipases. *Enzyme Microb Technol* 39:235–251. <https://doi.org/10.1016/j.enzmictec.2005.10.016>
- Khoo DE, Fermor B, Miller J, Wood CB, Apostolov K, Barker W, Williamson RCN, Habib NA (1991) Manipulation of body fat composition with stercularic acid can inhibit mammary carcinomas in vivo. *Br J Cancer* 63(1):97–101. <https://doi.org/10.1038/bjc.1991.20>
- Kumar S, Negi S (2015) Transformation of waste cooking oil into C-18 fatty acids using a novel lipase produced by *Penicillium chrysogenum* through solid state fermentation. *3 Biotech* 5:847–851. <https://doi.org/10.1007/s13205-014-0268-z>
- Leng CCS, Baharin BS, Man YC, Tan CP (2008) Optimisation of enzymatic hydrolysis for concentration of squalene in palm fatty acid distillate. *J Sci Food Agr* 88(9):1512–1517. <https://doi.org/10.1002/jsfa.3237>
- Lennen RM, Braden DJ, West RM, Dumesic JA, Pfleger BF (2010) A process for microbial hydrocarbon synthesis: overproduction of fatty acids in *Escherichia coli* and catalytic conversion to alkanes. *Biotechnol Bioeng* 106(2):193–202. <https://doi.org/10.1002/bit.22660>
- Liu T, Vora H, Khosla C (2010) Quantitative analysis and engineering of fatty acid biosynthesis in *E. coli*. *Metab Eng* 12(4):378–386. <https://doi.org/10.1016/j.ymben.2010.02.003>
- Mendes AA, Oliveira PC, Castro HFD (2012) Properties and biotechnological applications of porcine pancreatic lipase. *J Mol Catal B: Enzym* 78(6):119–134. <https://doi.org/10.1016/j.molcatb.2012.03.004>
- Mi L, Qin D, Cheng J, Wang D, Li S, Wei X (2017) Efficient production of free fatty acids from ionic liquid-based acid- or enzyme-catalyzed bamboo hydrolysate. *J Ind Microbiol Biotechnol* 44(3):419–430. <https://doi.org/10.1007/s10295-016-1888-6>
- Murty VR, Bhat J, Muniswaran PKA (2002) Hydrolysis of oils by using immobilized lipase enzyme: a review. *Biotechnol Bioproc Eng* 7(2):57–66. <https://doi.org/10.1007/BF02935881>
- Nickolau BJ, Perera MADN, Brachova L, Shanks B (2008) Platform biochemicals for a biorenewable chemical industry. *Plant J* 54(4):536–545. <https://doi.org/10.1111/j.1365-3113X.2008.03484.x>
- Rungruangsaphakun J, Keawsompong S (2018) Optimization of hydrolysis conditions for the mannoooligosaccharides copra meal hydrolysate production. *3 Biotech* 8:169. <https://doi.org/10.1007/s13205-018-1178-2>
- Santos KC, Cassimiro DMJ, Avelar MHM, Hirata DB, Castro HF, Fernández-Lafuente R, Mendes AA (2013) Characterization of the catalytic properties of lipases from plant seeds for the production of concentrated fatty acids from different vegetable oils. *Ind Crops Prod* 49:462–470. <https://doi.org/10.1016/j.indcrop.2013.05.035>
- Satyarthi J, Srinivas D, Ratnasamy P (2011) Hydrolysis of vegetable oils and fats to fatty acids over solid acid catalysts. *Appl Catal A Gen* 391:427–435. <https://doi.org/10.1016/j.apcata.2010.03.047>
- Shet VB, Palan AM, Rao SU, Varun C, Aishwarya U, Raja S, Goveas LC, Rao CV, Ujwal P (2018) Comparison of response surface methodology and artificial neural network to enhance the release of reducing sugars from non-edible seed cake by autoclave assisted HCl hydrolysis. *3 Biotech* 8:127. <https://doi.org/10.1007/s13205-018-1163-9>
- Singh R, Kumar M, Mittal A, Mehta PK (2016) Microbial enzymes: industrial progress in 21st century. *3 Biotech* 6:174. <https://doi.org/10.1007/s13205-016-0485-8>
- Sonawane SK, Arya SS (2017) Bioactive L acidissima protein hydrolysates using Box–Behnken design. *3 Biotech* 7:218. <https://doi.org/10.1007/s13205-017-0862-y>
- Sun S, Li X (2016) Physicochemical properties and fatty acid profile of Phoenix tree seed and its oil. *J Am Oil Chem Soc* 93:1111–1114. <https://doi.org/10.1007/s11746-016-2861-2>
- Tang Q, Tang X, Hu M, Li Z, Chen Y, Lou P (2010) Removal of Cd(II) from aqueous solution with activated *Firmiana simplex* leaf: Behaviors and affecting factors. *J Hazard Mater* 179:95–103. <https://doi.org/10.1016/j.jhazmat.2010.02.062>
- Upton TM, Cullen J (2012) *Firmiana simplex*. *Curtis Bot Mag* 29(2):170–181. <https://doi.org/10.1111/j.1467-8748.2012.01783.x>
- Woo KW, Suh WS, Subedi L, Kim SY, Kim A, Lee KR (2016) Bioactive lignan derivatives from the stems of *Firmiana simplex*. *Bioorg Med Chem Lett* 26(3):730–733. <https://doi.org/10.1016/j.bmcl.2016.01.008>
- Xie S, Manchester SR, Liu K (2014) *Firmiana*, (Malvaceae: Sterculioideae) fruits from the upper miocene of Yunnan, Southwest China. *Geobios* 47(4):271–279. <https://doi.org/10.1016/j.geobios.2014.03.005>
- Zhou G, Chen G, Yan B (2015) Two-step biocatalytic process using lipase and whole cell catalysts for biodiesel production from unrefined jatropha oil. *Biotechnol Lett* 37(10):1959–1963. <https://doi.org/10.1007/s10529-015-1883-4>