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Utilization of coconut and jojoba wastes for lipases production by *Aspergillus niger* and applied it for biodiesel production

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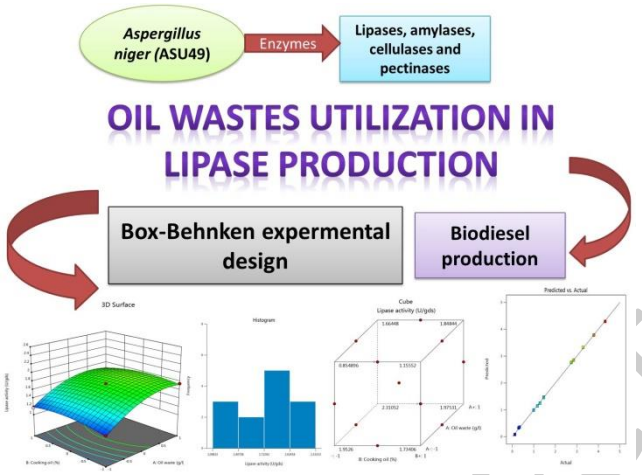
Graphical abstract



Abstract

Industrial wastes represent a huge issue for the environment and precious materials for microbial growth. Utilizing these wastes in different fermentation process decrease the costs of production, materials, energy, and represent an eco-friendly way of waste recycling. *Aspergillus niger* (ASU49) is promising isolate produce several hydrolytic enzymes as lipases, amylases, cellulases and pectinases. Lipase enzyme was optimized by Box-Behnken experiential design using coconut, jojoba and cooking oil wastes. Extracted crude lipase enzyme and lypholized one were compared and utilizing in biodiesel production. Coconut and jojoba wastes were promising in lipase production and maximum experimental values of lipase enzyme from both wastes were 4.32, 2.33 U/gds (predicted values was 4.29, 2.36 U/gds for jojoba and coconut wastes, respectively) obtained in run number (3) using Oil waste (2, (0) g/l) (A), Waste cooking oil (0, (-1) %) (B), and Temperature (30, (-1) °C) (C). The design was considerable, and efficacious to clear the interaction between factors; R^2 values of lipase activity 0.998, 0.999 and adjusted R^2 value 0.993, 0.998 for jojoba and coconut wastes, respectively. Extracted crud lipase (81.95% of total FAMEs) was more efficient than lyophilized lipases (56.14% of total FAMEs) in biodiesel production. Different oil wastes represent excellent sheep, nutritional, and effective sources for enzyme production using solid state fermentation especially with high producing microbial isolates and good optimizing designs.

Key words: wastes, recycling, biodiesel, lipase, enzymes, fungi.

1. Introduction

Filamentous fungi are now represented as biological manufactures of various metabolites and were significant in the biotechnology research (Filho *et al.,* 2019). Saprophytic nutritional mode of fungi involved secretion of various enzymes for organic matter absorption like proteases, cellulases, pectinases, amylases, and lipases to the medium to hydrolyze the polysaccharides complex (Cherry and Fidantsef, 2003). These fungi-based hydrolytic enzymes have huge applications in the food, biofuel, paper, fodder, soap, and even in medicinal field (Meyer *et al.,* 2020). The majority of enzymes currently used in industrial processes (more than 75%) are hydrolases (Prakash *et al.,* 2013).

Lipases represent the third most commercialized enzymes, after carbohydrases and proteases (Hasan *et al.,* 2006). Lipase enzyme (EC 3.1.1.3) represents a group of hydrolytic enzymes that are capable to catalyze the hydrolysis of long chain triacylglycerols into short chains at the interphase between water and lipid (Fang *et al.,* 2006; Bayoumi *et al.,* 2007). Lipases produced by bacteria, yeast and fungi especially are believed as the excellent source of extracellular lipase for mass production at industrial level. However, the production costs of lipase are critical issue in the industrial processes. Many ways tested to minimize the costs especially the production substrate (Smaniotto *et al.,* 2012). The solid state fermentation (SSF) technique involves a solid substrate (like waste materials) and is executed in low moisture content. The substrate being used should have appropriate moisture and required nutritional components to start the proper growth of microorganism (Singhania *et al.*, 2009). Filamentous fungi are among several groups of microorganisms employed in SSF because of their capacity to grow all inside and outside of variable substrates depicting a close contact with the substrate. Moreover, from the ecological and economical

viewpoints, the key advantage of SSF is the possibility of executing this procedure even in non-sterile settings (Dutra *et al.,* 2008).

Major filamentous genera of fungi included in lipase production are *Aspergillus, Eurotrium, Mucor*, *Ashbya, Rhizomucor, Fusarium, Acremonium,* and *Alternaria,* (Costa *et al.*, 2012). The fungal species that are the principal manufacturers of commercial lipases are *Aspergillus oryzae* (Celligoi *et al.,* 2017), *A. niger* (Contesini *et al.,* 2010; Knob *et al.*, 2020), *A. carneu* (Kaushik *et al.,* 2006), *A. awamori* (Basheer *et al.*, 2011), *A. flavus* (Skagerlind *et al.,* 2007), *Fusarium solani* NFCCL 4084 (Fickers *et al.*, 2005), *F. solani* FS1 (Maia *et al.,* 2001), *Rhizomucor miehei*, *Mucor miehei* (Marion and Oliver, 2013), *Trichoderma lanuginosus* (Adrio and Demain, 2014), *Penicillium roquefortii* (Mhetras *et al.*, 2009), *Penicillium abeanum* (Khambhaty, 2020), *Rhizopus nodosus (*Gonçalves *et al.*, 2009), and *R. arrhizus* (Rozalen *et al.*, 2009).

For optimizing the lipase production; one factor-at-a-time (old) and statistical experimental designs (recent) were used. The disadvantages of one factor-at-a-time method are time-consuming, expensive, and often lead to misinterpretation of results when interactions between different components are present. However statistical experimental designs minimize the error in determining the effect of parameters, and it allows simultaneous, systematic, and efficient variation of all parameters (Mahmoud *et al.*, 2021). These statistical experimental designs can be adopted at various optimization processes, such as for comparative objectives, for screening experiments, or for finding the optimal conditions. The response surface methodology (RSM) including Box-Behnken designs (BBD) is a collection of mathematical and statistical techniques for designing experiments, building models (Naveena *et al.,* 2005), evaluating the effects of several factors and obtaining optimum condition of factors for desirable responses (Park *et al.*, 2005), which provides the relationship between one or more measured dependent responses and a number of input factors (Perez *et al.,* 2003). It has some advantages that include a less number of experiments that need be executed resulting in lower reagent consumption and considerably less laboratory work (Francis *et al.*, 2003); suitability for multiple variables can reveals possible interactions between variable, search for relativity between multiple variables and finding of the most suitable correlation and forecast response (Mahmoud and Bashandy, 2021).

Increased environmental pollution challenges such as climate change, greenhouse gas emissions, and rising fossil fuel prices have prompted researchers to look into improving biofuel/biodiesel technologies made from sustainable resources (Klek, 2016). Several types of feedstocks are being investigated for biodiesel production, including edible plant oils and animal fats, as well as non-edible oils (Pinzi *et al.,* 2014). Lipolytic enzymes not only serve to alleviate the large amount of lipid waste substances in a sustainable and environmentally beneficial manner, but they also pose a threat to energy security and might be used to replace fossil fuels (Dauvergne and Neville, 2009). Esterification and transesterification reactions are catalysed by lipase, resulting in methyl esters (Fan, 2012). Lipases were discovered to be very significant biocatalysts due to their excellent bio physiochemical characteristics.The focus has shifted to biocatalysts as a result of biotechnological applications (Hafd *et al.*, 2017). Microorganisms are preferred for lipase enzyme manufacturing because they have the quickest generation time. Other advantages of microorganisms include high substrate conversion productivity, environmental flexibility, and ease of genetic operation and harvesting scenarios (Pariza and Johnson, 2001). The aim of the prest research is to utilize different oil industry wastes for lipase production by *Aspergillus niger* (ASU49), optimizing the production using statistical experimental design (Box-Behnken designs) utilizing the cooking oil waste and using the extracted lipases for biodiesel production.

2. Materials and methods

2.1. Fungal isolation and their enzymatic capabilities

Aspergillus niger (ASU49) was recovered from soybean seeds (*Glycine max* L.) on 1% tween 80 Czapek's Oil agar medium (CzOA) as lipase specific isolation medium, and incubated at 28°C for 7 days. The isolate then was cultured in CzO broth medium at incubated at 28°C for 4 days, then 50 µL of fungal spore suspension was used to inoculate tubes containing the CzO supplemented with $CaCl_2.2H_2O$, 0.2 g/l and incubated at 28°C for 7 days (Figure 1). The lipolytic activity confirmed by formation of white precipitate of calcium polysorbates which released by the enzyme (Mahmoud *et al.*, 2020).

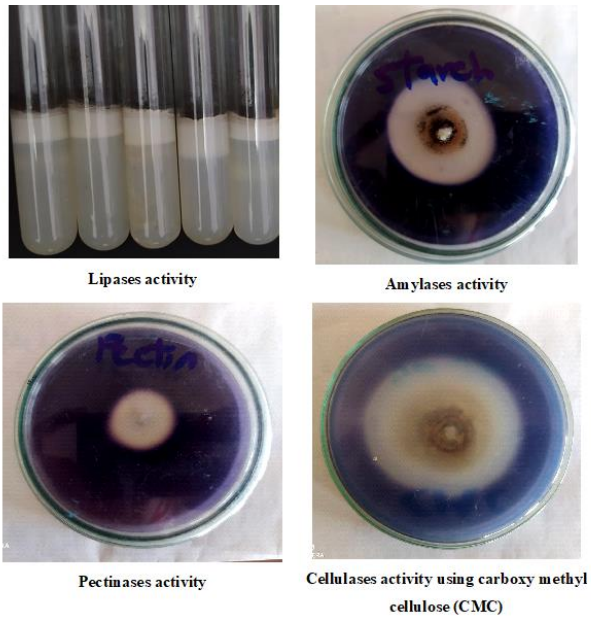


Figure 1: Enzymatic activities of *Aspergillus niger* (ASU49) represented the white precipitate (lipase activity) and by the clear zone around the fungal growth (amylase, cellulases, and pectinases activity).

Amylases activity was examined by using the method of Bridge (1985) on 0.5% starch medium and incubated at

28°C for 7 days. After growth, cultures were flooded with iodine solution (I_2, 1 g; KI, 3g and distilled water, 200 ml), and clear zone around the colonies indicates hydrolysis of starch (Mahmoud *et al.*, 2019). Cellulases activity was examined by using the method of Eggins and Pugh (1962) on 0.5% carboxy methyl cellulose medium (CMC). After growth at 28°C for 7 days, cultures were flooded with zinc iodide (1%) solution and clear zone around colonies indicates hydrolysis of carboxy methyl cellulose (Ibrahim and Mahmoud, 2019). On a 0.5 percent pectin medium, pectinases activity was measured using the method of (Hankin *et al.*, 1971). After growth at 28°C for 7 days, cultures were flooded with iodine solution, and clear zone around colonies indicates hydrolysis of pectin as cleared in Figure 1.

3. Optimization of lipases production from oil wastes using solid state fermentation

3.1. Oil wastes

Simmondsia chinensis L. (Jojoba), and Cocos nucifera L. (coconut) wastes were punctured from the oil extraction and analytical unit, Assiut University, Egypt. They were chemically analysis for the oil quantities in the central laboratory of chemical analysis. Jojoba seeds and coconut have 44%, and 52%, while their wastes after the oil extraction have 10.08%, and 31.76%, respectively**.**

3.2. Experimental design

Box–Behnken statistical experimental design was utilized to optimize and evaluate the interaction effects, main effects, and the quadratic effects of the tested variables on the production of lipase enzyme by *Aspergillus niger* (ASU49) in solid state fermentation using two oil waste. A 3-parameter, three-level statistical design with 13-runs was established exploring the quadratic responses and the second order polynomial models. Table 1 was used to expain the design's independent and dependent parameters. Oil waste (g/l) (A), Waste cooking oil (%) (B), and Temperature (C°) (C) were analyzed by three levels (-1, 0, +1). Each a 250-ml Erlenmeyer conical flask filled with the specific contents according to the experimental design were supplemented with 20 ml mineral solution contains $NaNO_3$, 2.0; KCl, 0.5; K_2HPO_4, 1.0; $MgSO_4$. $7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0,01 and $CuSO_4$, 0.005 before autoclaving. Then inoculated with 2 ml of spore suspension obtained from 3-day-old culture of *Aspergillus niger* (ASU49). The non-linear quadratic statistical model was generated by the quadratic equation (1) as cleared by Liu *et al.* (2013):

$$Y= \beta_0+ \sum\beta_i x_i +\sum\beta_{ii} x_i^{\,2}+ \sum\beta_{ij}x_{ij} \quad (1)$$

Y are the predicted values of lipase enzyme of the tested fungus, β_0 is the intercept, β_i is the linear impact, β_{ii} is the squared impact, β_{ij} is the interaction impact, x_{ij} is the independent levels of variables. Response surface (3D) plots were drawn, comparison curves between the actual and predicted values were sets, also the statistical efficiency of the design and generated data were analyzed (Mahmoud and Bashandy, 2021). To confirm the optimized selected parameters Derringer's desired equations were applied for the validity of the tested models (Yan *et al.,* 2014).

Table 1. Ranges of the operational parameters of lipase enzyme (U/g SSF) production for the experimental design through Box-Behnken design

Symbol	Parameters	Level of Parameters		
		-1	0	+1
A	Oilseeds waste (g/l)	10	20	30
B	Waste cooking oil (%)	0	1	3
C	Temperature (C°)	30	35	40

Table 2. Box-Behnken design with five variables; Oil wastes (g/l) (A), Waste cooking oil (%) (B), and Temperature (C°) (C), with actual and/or predicted responses of lipase enzyme (U/gds).

Trial	Oil wastes (g/l)	Waste cooking oil (%)	Temperature (C°)	Jojoba oil waste		Coconut oil waste	
				Actual values of lipase enzyme (U/gds)	Predicted values of lipase enzyme (U/gds)	Actual values of lipase enzyme (U/gds)	Predicted values of lipase enzyme (U/gds)
1	-1(1)	-1(0)	0(35)	1.17	1.15	3.29	3.32
2	-1(1)	1(3)	0(35)	1.17	1.19	1.17	1.14
3	0(2)	-1(0)	-1(30)	2.33	2.36	4.32	4.29
4	1(3)	0(1)	-1(30)	2.24	2.24	2.85	2.85
5	0(2)	0(1)	0(35)	1.75	1.75	1.46	1.46
6	-1(1)	0(1)	1(40)	1.10	1.10	0.9917	0.9898
7	1(3)	1(3)	0(35)	1.63	1.65	1.28	1.25
8	0(2)	-1(0)	1(40)	1.47	1.48	0.1167	0.0835
9	0(2)	1(3)	-1(30)	2.10	2.08	0.2917	0.3248
10	1(3)	0(1)	1(40)	1.85	1.85	1.46	1.47
11	1(3)	-1(0)	0(35)	1.75	1.73	2.74	2.76
12	-1(1)	0(1)	-1(30)	1.94	1.94	3.79	3.78
13	0(2)	1(3)	1(40)	1.75	1.73	0.3333	0.3581

Table 3. ANOVA results for Box-Behnken quadratic model of lipase enzyme (U/gds) from oil wastes by *Aspergillus niger* (ASU49)

Source	Sum of Squares	Mean Square	*F*-value	*p*-value	Sum of Squares	Mean Square	*F*-value	*p*-value
Model	1.93	0.2140	187.50	0.0006	23.27	2.59	1086.26	< 0.0001
A-Oil wastes (g/l)	0.5521	0.5521	483.83	0.0002	0.1016	0.1016	42.70	0.0073
B-Cooking oil (%)	0.0006	0.0006	0.5240	0.5215	6.82	6.82	2864.57	< 0.0001
C-Temperature(°C)	0.7498	0.7498	657.06	0.0001	8.72	8.72	3662.11	< 0.0001
AB	0.0034	0.0034	2.98	0.1827	0.1089	0.1089	45.76	0.0066
AC	0.0510	0.0510	44.69	0.0068	0.4959	0.4959	208.35	0.0007
BC	0.0674	0.0674	59.05	0.0046	4.50	4.50	1890.00	< 0.0001
A^2	0.1156	0.1156	101.31	0.0021	1.59	1.59	668.97	0.0001
B^2	0.0210	0.0210	18.44	0.0232	0.0690	0.0690	28.99	0.0125
C^2	0.1519	0.1519	133.13	0.0014	0.0009	0.0009	0.3842	0.5793

Table 4. Fatty acid methyl esters (FAMEs) produced from *Aspergillus niger* lipase esterification process from maize oil. Values are % of total FAMEs using GC/MS

Methyl esters	% of total FAMEs of crude lipases	% of total FAMEs of lyophilized lipases
hexadecanoic acid, (palmitic acid)	21.63	-
9,12-octadecadienoic acid (E,E)-	33.5	12.37
10-octadecenoic acid	13.09	7.31
octadecanoic acid, 9,10-dihydroxy-	-	7.28
cyclopropanedodecanoic acid, 2-octyl-	1.5	14.13
[1,1'-bicyclopropyl]-2-octanoic acid,2'-hexyl-	11.38	7.77
oxiraneundecanoic acid, 3-pentyl-, trans-	0.85	7.28
Total FAMEs	81.95	56.14

Table 5. Free fatty acids produced from *Aspergillus niger* lipase esterification process from maize oil. Values are % of total FFA using GC/MS

Free fatty acids (FFA)	Crude	Lyophilized
oleic Acid	45.15	55.64
9,12-octadecadienoic acid (Z,Z)	38.55	-
9-hexadecenoic acid	8.91	44.36
17-octadecynoic acid	5.82	-
trans-13-octadecenoic acid	1.57	-
Total FFA	100	100

3.3. Statistical analysis

All data was statistically analyzed with multiple regression statistical analysis utilizing Design Expert 7.0.0 statistical software from the United States. One way ANOVA with probability 0.05 was used to examine the quadratic regression and variable interaction.

3.4. Extraction and assay of Aspergillus niger (ASU49) lipases

Following incubation, the contents of each flask were thoroughly mixed, and 2 g of fungal growth were collected and homogenised separately in 20 ml of 1% NaCl solution, 1% NaCl + 1% tween 80, or phosphate buffer (pH 7.0). The cell-free supernatant was obtained after filtering the mixture using filter paper No. 1 and centrifugation at 15,000 xg for fifteen minutes. For the lipase assay, two millilitres of supernatant were combined with two millilitres of tween 80 and six millilitres of phosphate buffer (pH 8.0), and the reaction mixture was incubated in a water bath at 35 °C for three hours. By adding 25 mL of 95 percent ethanol, the reaction was brought to a halt. Two drops of 0.2 percent phenolphthaleine solution (ethanol/water ratio of 1:1) were added, and the flask content was titrated against 50 mM sodium hydroxide solution until a purple colour was observed, then calculated as mg/g oil waste (Borkar *et al.*, 2009).

3.5. Partial purification of lipase enzyme

Ten 250-ml Erlenmeyer conical flasks each containing 150 ml 1% tween 80 Czapek's broth medium utilized. Flasks inoculated individually with 2 ml spore suspension of the tested isolate (*Aspergillus niger* (ASU49)). The inoculated flasks were then incubated at 28±1 ºC in shaking condition of 120-150 rpm for 7 days. Following the incubation time, Filtration with filter paper No. 1 and centrifugation at 15,000 xg for fifteen minutes yielded the cell-free supernatant.

Precipitation using ammonium sulphate; ammonium sulphate was slowly added while gently stirring to 800 ml of crude enzyme in supernatant resulted from liquid fermentation (Kalyani *et al.*, 2015). Ammonium sulphate was added gradually under cooling and then kept overnight at 4˚C for complete precipitation, then centrifuged at 15,000 xg for fifteen minutes and the pellet was dissolved in buffer and kept for dialysis.

Dialysis; after precipitation, the crude enzyme may contain a high salt buffer, so dialysis was carried out by placing the product in cellulose dialysis tube (29.6 mm ×

45 mm). Centrifugation at 15,000 xg for fifteen minutes and filtration with filter paper No. 1. Filtrate putting in dialysis bag, dialysis bag placed in conical containing 2 L phosphate-citrate buffer in magnet stirrer for 2 hr, then dialysis bag placed in conical containing another 2 L phosphate-citrate buffer on magnet stirrer for 2 hr, then dialysis bag placed in conical containing another 2 L phosphate-citrate buffer in cold room for 24 hr. Proteins (enzymes) remain in the bag, whereas the ions and other low molecular weight component diffuse out of the bag. The precipitated protein was lyophilized for removing other salts.

Enzyme lypholization; the obtained crude protein was dried at the Assiut University Mycological Center (AUMC) using lypholization (Virtis, Model 6KBTES-55, USA).

3.6. Efficiency of Aspergillus niger (ASU49) crude and lyophilized lipases to synthesize fatty acid methyl esters via chemical transesterfication of maize oil (biodiesel production)

Esterification reaction mixture was carried out in 5 ml stopper vials contained 1 ml crude fungal enzyme or 1 ml lyophilized fungal enzyme, 1 ml maize oil, 2 ml methanol and 1 ml tris HCl buffer (50 mM). Then tubes were shaken at 40 ˚C at 100 rpm for 3 d (Yoo *et al.*, 2011). The produced fatty acid methyl esters (biodiesel) were extracted by n-hexane and analyzed using GC/MS, Agilent Model 6890N/5975B [Column DB 5ms, Agilent from (30, 0.25 mm, 0.25 mm)] at the Analytical Chemistry Unit, Chemistry Department, Faculty of Science, Assiut University.

4. Results

Aspergillus niger (ASU49) that was recovered from soybean seeds (*Glycine max* L.) on 1% tween 80 Czapek's Oil agar medium (CzOA) showed ability to produce several enzymes other than lipase enzyme like amylases, cellulases and pectinases enzymes which help it to grow in complex organic matters and serve its life as cleared in Figure 1. Two oil wastes of Simmondsia chinensis L. (jojoba) and Cocos nucifera L. (coconut) contains 10.08%, and 31.76%, residual oils respectively were utilized in lipase enzyme production. Box–Behnken statistical experimental design was utilized to optimize and evaluate the interaction effects, main effects, and the quadratic effects of the tested variables on the production of lipase enzyme by *Aspergillus niger* (ASU49) in solid state fermentation using two oil wastes. A 3-parameter (Oil waste (1, 2, 3g/l) (A), Waste cooking oil (0, 1, 3%) (B), and Temperature (30, 35, 40C°) (C)), three-level (-1, 0, +1) statistical design with 13-runs was established as mentioned in Table 1.

The predicted values of the performed runs were calculated by applying the multiple regression analysis via the second-order polynomial equation (1); lipase enzyme (U/gds) predicted values calculated by the equation 2 (for jojoba wastes) and 3 (for coconut wastes) as following:

$$\text{Lipase activity (U/gds)} = 1.75 + (0.262)\ \text{Jojoba oil waste (g/l)} + (-0.009)\ \text{Cooking oil (\%)} + (-0.306)\ \text{Temperature (°C)} + ((-0.029)\ \text{Jojoba oil waste (g/l)} * \text{Cooking oil (\%)}) + ((0.113)\ \text{Jojoba oil waste (g/l)} * \text{Temperature (°C)}) + ((0.129)\ \text{Cooking oil (\%)} * \text{Temperature (°C)}) + (-0.225)\ \text{Jojoba oil waste (g/l)}^2 + (-0.096)\ \text{Cooking oil (\%)}^2 + (0.258)\ \text{Temperature (°C)}^2 \quad (2)$$

$$\text{Lipase activity (U/gds)} = 1.46 + (-0.113)\ \text{Coconut oil waste (g/l)} + -0.923)\ \text{Cooking oil (\%)} + (-1.04)\ \text{Temperature (°C)} + ((0.165)\ \text{Coconut oil waste (g/l)} * \text{Cooking oil (\%)}) + ((0.352)\ \text{Coconut oil waste (g/l)} * \text{Temperature (°C)}) + ((1.06)\ \text{Cooking oil (\%)} * \text{Temperature (°C)}) + (0.835)\ \text{Coconut oil waste (g/l)}^2 + (-0.174)\ \text{Cooking oil (\%)}^2 + (-0.02)\ \text{Temperature (°C)}^2 \quad (3)$$

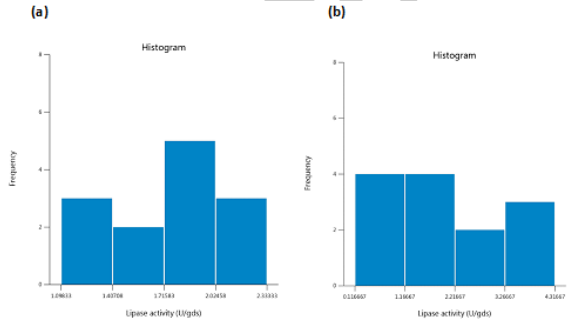


Figure 2: Lipase activity (U/gds) data histogram using jojoba oil waste (a) and coconut oil waste (b) by *Aspergillus niger* (ASU49).

Maximum experimental values of lipase enzyme from jojoba wastes was 2.33 U/gds; whereas the corresponding predicted values was 2.36 U/gds obtained in run number (3) using Oil waste (2, (0) g/l) (A), Waste cooking oil (0, (-1) %) (B), and Temperature (30, (-1) C°) (C); however the lowest lipase activity cleared was 1.1 U/gds; whereas the corresponding predicted values was 1.1 U/gds obtained in run number (6) using Oil waste (1, (-1) g/l) (A), Waste cooking oil (1, (0) %) (B), and Temperature (40, (1) C°) (C). From coconut oil waste the maximum experimental values of lipase enzyme was 4.32 U/gds; whereas the corresponding predicted values was 4.29 U/gds obtained also in run number (3) using Oil waste (2, (0) g/l) (A), Waste cooking oil (0, (-1) %) (B), and Temperature (30, (-1) C°) (C); however the lowest lipase activity cleared was 0.118 U/gds; whereas the corresponding predicted values was 0.084 U/gds obtained in run number (8) using Oil waste (2, (0) g/l) (A), Waste cooking oil (0, (-1) %) (B), and Temperature (40, (1) C°) (C) as cleared in Table 2 and the overall lipase activity histogram cleared in Figure 2.

The predicted values of lipase activity (U/gds) were observed in closely to the response model experimental values for both wastes that reflect the accuracy of the established model as cleared in Table 2 and Figure 3. The quadric polynomial equations (2 & 3) were further tested for the confirmation of the statistical model suitability, accuracy, and significance via statistical analysis using one way analysis of variance (ANOVA) as established in Table

3. The *F* and *P*- values of the tested model of lipase activity (U/gds) for jojoba wastes (*F;* 187.50, and *P*; 0.0006) and coconut wastes (*F;* 1086.26, and *P*; 0.0001) cleared obtaining significant results from the parameter (probability ≤0.05). Also for evaluation the model goodness, accuracy, and fitting, evaluating statistical parameter coefficient (R^2) was calculated; R^2 values of lipase activity was 0.998 and adjusted R^2 value 0.993 for jojoba wastes and 0.999 and adjusted R^2 value 0.998 for coconut wastes which indicated that the whole variations were explained highly by the statistical model.

The significance and effects of each individual variable and/ or the interactions were set in Table 3 as one way ANOVA results. For jojoba wastes; individual variables of (A) Oil waste (0.0002), and (C) Temperature (0.0001) has significant effects on lipase production, while (B) Waste cooking oil (0.5215) reflects insignificant effect. For jojoba wastes; the interaction between different variables AC (Oil waste (g/l) * Temperature (C°)), and BC (Waste cooking oil (%) * Temperature (C°)) have significant effects on lipase production ($p<0.005$), while AB (Oil waste (g/l) * Waste cooking oil (%)) was not significant. For coconut wastes; individual variables of (A) Oil waste (0.0073), (B) Waste cooking oil (0.0001) and (C) Temperature (0.0001) has significant effects on lipase production. For coconut wastes the interaction between different variables AB (Oil waste (g/l) * Waste cooking oil (%)), AC (Oil waste (g/l) * Temperature (C°)), and BC (Waste cooking oil (%) * Temperature (C°)) have significant effects on lipase production ($p<0.005$).

Response surface plots and the contour plots draw for the 3D visualization of the cleared interaction between pair-wise of the two factors when the other factor constant as showed in Figures 4 and 5 explaining the effect of (Oil waste (A), Waste cooking oil (B), and Temperature (C), on lipase production and reflect the interaction between AB (Oil waste (g/l) * Waste cooking oil (%)), AC (Oil waste (g/l) * Temperature (C°)), and BC (Waste cooking oil (%) * Temperature (C°)) on lipase production using jojoba and coconut wastes by *Aspergillus niger* (ASU49).

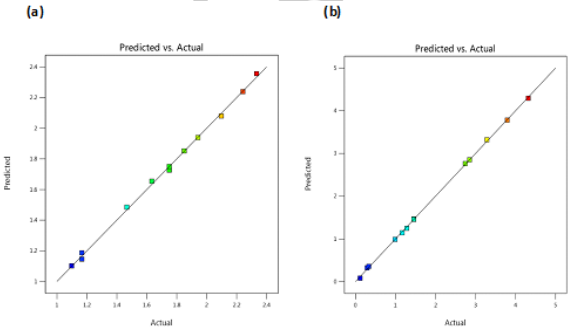


Figure 3: Comparison between the actual and predicted values of lipase activity (U/gds) using jojoba oil waste (a) and coconut oil waste (b) by *Aspergillus niger* (ASU49).

The previous results showed the effects of three variables (Oil waste (A), Waste cooking oil (B), and Temperature (C)) on lipase production (U/gds) through 13 different runs; However, from the three tested values, the most ideal variable concentrations must be calculated. An optima variables concentration for high lipase production was estimated from Derringer's desirability function. By applying the function, the obtained optimum levels were Oil waste (2, (0) g/l) (A), Waste cooking oil (0, (-1) %) (B), and Temperature (30, (-1) C°) (C), which gives desirability equal to 1.000 for both wastes as cleared at Figure 6. Also, for model validation, five experiments of the optimum parameters were performed and the mean values were confronted with predicted values. The actual values were in harmony with the predicted once by application the desirability functions which reflect the sufficiency of the quadratic model developed for enhancing the production.

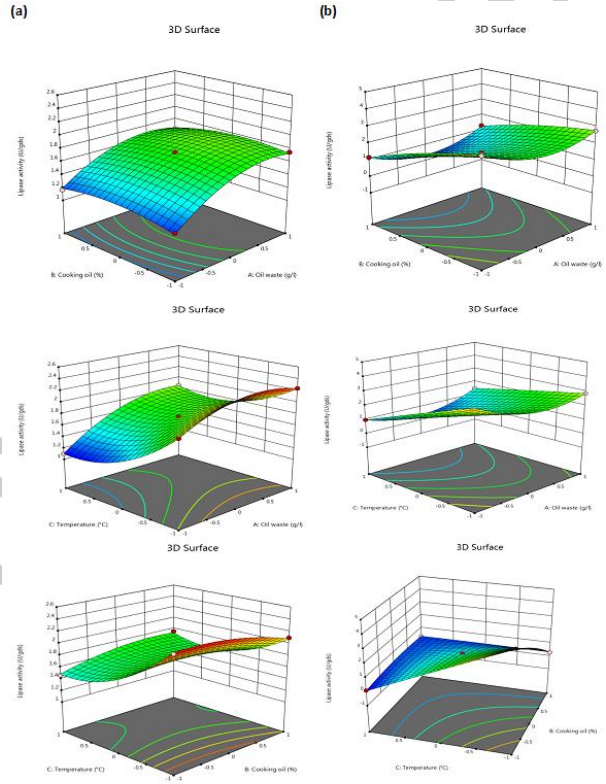


Figure 4: 3D surface plots and contor plots of Box–Behnken statistical experimental design explaining the interactions between AB (Oil wastes (g/l) * Waste cooking oil (%)), AC (Oil wastes (g/l) * Temperature (C°)), and BC (Waste cooking oil (%) * Temperature (C°)) on lipase production using jojoba oil waste (a) and coconut oil waste (b) by *Aspergillus niger* (ASU49).

4.1. Efficiency of Aspergillus niger (ASU49) crude and lyophilized lipases to synthesize fatty acid methyl esters via chemical transesterfication of maize oil (biodiesel production)

The direct acid transesterfication was performed to test the efficiency of *Aspergillus niger* (ASU49) lipases as crude and lyophilized enzymes to hydrolyze and synthesize biodiesel from maize oil. The produced fatty acid methyl esters (FAME) were analyzed using GC/MS in order to ascertain and compare their potential as biodiesel feedstock (Tables 4 & 5).

Data in Table 4 showed the composition of fatty acid methyl esters (biodiesel) produced from crude lipase enzyme esterification process of maize oils. Whereas,

Methyl esters of 9,12-octadecadienoic acid (E,E), hexadecanoic acid, (palmitic acid) were represented as major components, accounting for 33.5 % 21.63 %, of total FAMEs, respectively. In addition, 10-octadecenoic acid, [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, cyclopropanedodecanoic acid, 2-octyl- and oxiraneundecanoic acid, 3-pentyl-, trans were represented minor components of biodiesel produced, accounting for 13.09%, 11.38%, 1.5 % and 0.85 % of total FAMEs, respectively. As well as, the biodiesel yield produced from crude lipase esterification process of maize oils were 81.95 %. For lyophilized lipases, methyl esters of cyclopropanedodecanoic acid, 2-octyl- (14.13%) and 9,12-octadecadienoic acid (E,E) (12.37%) were represented as major components, produced from lyophilized lipase enzyme esterification process of maize oils. In addition, [1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl-, 10-octadecenoic acid, oxiraneundecanoic acid, 3-pentyl-, trans and octadecanoic acid, 9,10 dihydroxy- were represented the minor components of biodiesel produced, accounting for 7.77 %, 7.31 %, 7.28 % and 7.28 % of total FAMEs, respectively. As well as, the biodiesel yield produced from lyophilized lipase esterification process of maize oils were 56.14 %.

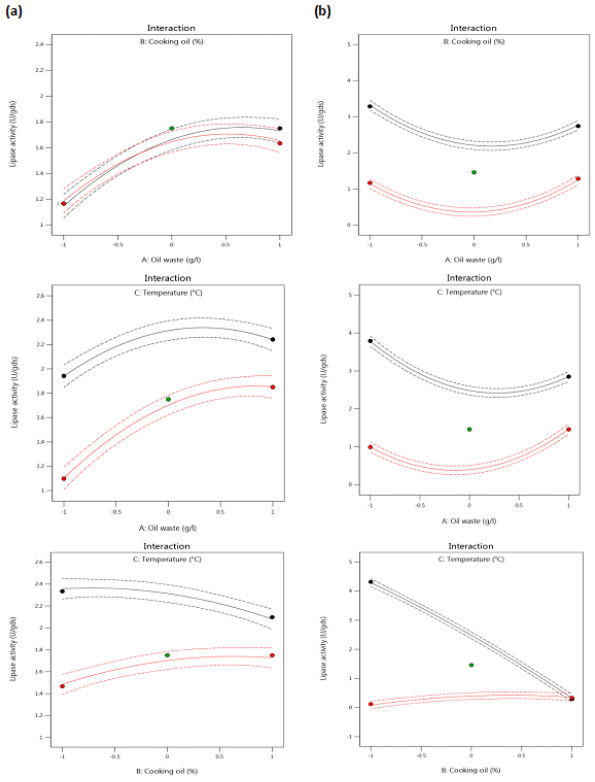


Figure 5: The interaction plots of Box–Behnken statistical experimental design explaining the interactions effects of AB (Oil wastes (g/l) * Waste cooking oil (%)), AC (Oil wastes (g/l) * Temperature (C°)), and BC (Waste cooking oil (%) * Temperature (C°)) on lipase production using jojoba oil waste (a) and coconut oil waste (b) by *Aspergillus niger* (ASU49).

Furthermore, free fatty acids of oleic Acid and 9,12-octadecadienoic acid (Z,Z) were represented as major components, accounting for 45.15%, and 38.55% of total FFA, respectively. In addition,9-hexadecenoic acid, 17-octadecynoic acid and trans-13-octadecenoic acid represented minor components of free fatty acids produced, accounting for 8.91%, 5.82% and 1.57% of total FFA, respectively (Table 5).While in lyophilized lipases, free fatty acids of oleic Acid represented major component, accounting for 55.64%, of total FFA. In addition, 9-hexadecenoic acid represented moderate component of free fatty acids produced, accounting for 44.36 % of total FFA (Table 5).

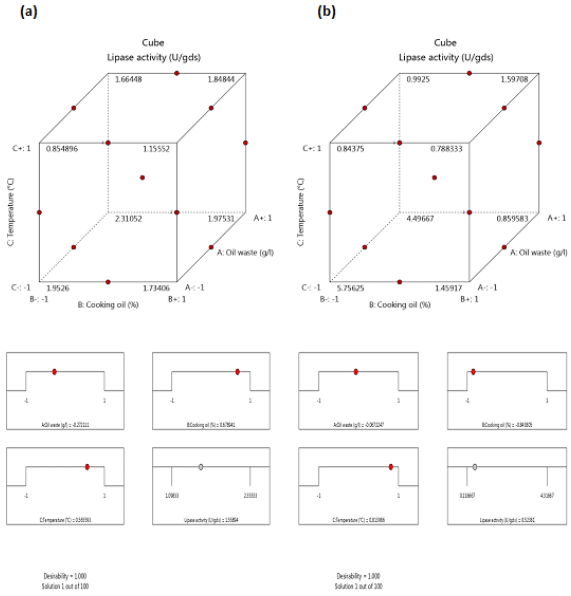


Figure 6: The statistical optimization desirability ramp plot for lipase production using jojoba oil waste (a) and coconut oil waste (b) by *Aspergillus niger* (ASU49).

5. Discussion

Industrial oil wastes are valuable sources for microbial growth and the synthesis of metabolites. It employs several fermentation processes to reduce production costs, materials, and energy consumption, as well as represent an environmentally acceptable method of waste recycling. Jojoba (*Simmondsia chinensis* L.) seeds are a viable oil crop for economic growth, particularly in dry and semiarid areas **(**Larraburu *et al.*, 2016). Miklaszewska and Bana (2016) found that it contains up to 65 percent of a light golden, odourless high-viscosity liquid-oil that is unlike any other plant oil. Coconut and its wastes are high in lipids and nutrients, with around 90% saturated fat, carbohydrates, amino acids, vitamin B, pantothenic acid B5, biotin, riboflavin B2, folic acid, thiamine B1, and pyridoxine B6. (Yong *et al.,* 2009; Wallace, 2019). *Aspergillus niger* (ASU49) is promising isolate produced several hydrolytic enzymes like lipases, amylases, cellulases and pectinases which qualified it to grow on complex organic matter like wastes. Two oil wastes of jojoba and coconut contains 10.08%, and 31.76%, residual oils were utilized efficiently for lipase production by *Aspergillus niger* (ASU49), both gives high production when they used in concentration (2 g/l) at 30C°. Fungal lipases have advantages over bacterial lipases since modern technology favors low-cost extraction techniques. *Aspergillus*, *Penicillium*, *Mucor*,

Beauveria, *Rhizomucor*, *Fusarium*, *Alternaria*, and *Eurotium* are some of the most important filamentous fungal genera (Singh and Mukhopadhyay, 2012).

Maximum experimental values of lipase enzyme from both wastes were 4.32, 2.33 U/gds of jojoba and coconut. In agreement with our data; Pimente *et al.* (1997) examined lipase production through SSF by *Penicillium citrinumgiving* and obtained 1.585 U/ml lipases. Ferrer *et al.* (2000) obtained 0.15 U/mglipase by *P. chrysogenum* using groundnut as substrate. Maia *et al.* (2001) produced 0.45 U/ml of lipase from *Fusarium solani FS1* using sesame as substrate. De Luccio *et al.* (2004) obtained 21 U/ml lipase by *Penicillium simplicissimum* using soy cake as substrate. Kaushik *et al.* (2006) found that through solid-state fermentation *Aspergillus carneu* utilized sunflower and gives 12.7 U/ml of lipase. Dazhang and Liming (2008) stated that 0.85 U/mL of lipase was obtained by *Penicillium expansum* using groundnut as substrate. Shukla *et al.* (2011) optimize the lipase production from different oil cakes by *Rhizopus oryzaeKG-10* using jatropha, teesi, mustard, and groundnut oil cakes (80, 60, 170, and 60 IU of lipases). Amin and Bhatti (2014) optimize the lipase production from peanut shells by *Penicillium fellutanum* and obtained 150 U/g lipase. Fleuri *et al.* (2014) obtained 2.55, 6.6 U/mL of lipase using Soybean bran as substrate by *Fusarium sp.* and *Penicillium* sp. Oliviera *et al.* (2017) optimize the lipase production from different oil cakes by *Aspergillus ibericus* MUM 3.49 using andiroba, cupuassu, canola, macauba, palm kernel, crambe, green coffee, and sesame oil cakes as substrates (1, 11, 47, 1, 127, 44, 4, 78 U/g of lipase). Dobrev *et al.* (2018) obtained 4.2 U/glipase by *Rhizopus arrhizus* using sunflower as substrate. El Aal *et al.* (2019) optimize the lipase production from different oil cakes by *Aspergillus niger NRRL-599* giving 80, 60 and 90 U/mL of lipase through SSF of wheat germ, jojoba and almond oil cakes as substrate, respectively.

Lipases are widely utilized in a variety of industries, including food, pharmaceutics, biofuels, oleochemical, textile, agro-chemical, paper production, cosmetics, and many more. Lipases can be employed in the food business as flavour modifiers via synthesis of short chain fatty acid esters and alcohols, as well as to generate goods with higher nutritional value by altering the triacylglycerol structure for inter- or transesterification (Verma *et al.,* 2012). The direct acid transesterfication was performed to test the efficiency of *Aspergillus niger* (ASU49) lipases as crude and lyophilized enzymes to hydrolyze and synthesize biodiesel from maize oil. The produced fatty acid methyl esters (FAME) were analysed using GC/MS in order to ascertain and compare their potential as biodiesel feedstock. Crude enzyme was most efficient (81.95% of total FAMEs) for biodiesel production that lyophilized one (56.14% of total FAMEs). Interestingly, Xie and Wang (2014) stated that biodiesel yield from soybean oil using immobilized lipase reported that 86% was attained at a reaction temperature of 35 °C for 24 h with a biodiesel yield of 92.8% was recorded using a three-step methanol addition at 40 ^{0}C using *Candida rugosa* lipase bound on the Fe_3O_4 magnetic nanocomposite (Xie and Huang, 2020). Furthermore, Cervero´ *et al.* (2014) reached 48% biodiesel by use of the one-step methanol addition method during the lipase esterification process, whereas 90% of the biodiesel production was obtained through the use of the three-step addition method.

6. Conclusion

Solid state fermentation of oil industry wastes represents sheep, energy serving, time serving, eco-friendly, and easy way for the enzyme production. The using of good microbial producer could utilize different wastes through enzymes and the propriety statistical optimizing design will maximize the production in low experiments number and low time. Also, utilizing lipases in energy production (biodiesel) essentially needed these days especially was we suffer from energy crisis.

Author contributions

GAM; participate in the experiment design, data analysis, and manuscript writing, RA; supervision and manuscript revision, MA; supervision and manuscript revision, AG; participate in the practical work and manuscript writing. The final manuscript approved by all authors.

Data availability:

All data generated or analyzed during this study are included in this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study does not contain any experiments with human participants or animals.

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