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Research Article

Mycodiesel Production from some Isolates of Oleaginous Fungi Isolated from Oil-Rich Soils in Basrah

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ABSTRACT

The present study was aimed to determine of mycodiesel production from oleaginous fungal isolates that included *Aspergillus niger*, *A.flavus*, *A.ochraceus*, and *Penicillium chrysogenum*, three different fermentation media were used to cultivate fungal isolates that included nitrogen limiting medium NLM, chees why medium CWM, and waste molasses dates medium WMDM, the accumulation lipids of drying biomass were extracted by using 1:2 methanol: chloroform. The result of biomass and lipids production were showed that NLM and CWM represented the best media for accumulation of lipids and yield high biomass, while *A. niger* and *P.chrysogenum* were accumulated high percentage of lipids by 36% and 38% respectively. Furthermore, the analysis of fatty acid methyl ester FAMEs was used by transesterification reactions of extracted lipids. Subsequently, the FAMEs were analyzed by using GC/MS then Cetane Number (CN) of mycodiesel was calculated, therefore, the results were showed that the percentage of the composition of FAME ranged between 66.06 - 92.29 % , while the results of CN was 52.6 - 57. On the other hand the results of residual carbon and sulfur content of mycodiesel product were 0.04 and 0.0069 wt% respectively.

Keywords: Mycodiesel , Oleaginous fungi , Oil-rich soils

INTRODUCTİON

Physical, chemical and biological techniques in the ecosystem attempt to maintain the ecological balance. However, the large volume of pollutants has prevented the ability of these technologies to treat all pollutants and to reach the state of environmental balance in particular when there are many pollutants are difficult to decompose. As a result, in order to treat all types of contaminants, various biological, chemical and physical methods have been developed, but biological treatments are the most widely used where, Advances in genetics, biotechnology, process chemistry, and engineering are leading to a new manufacturing concept for converting renewable biomass to valuable fuels and products [1,2, 3]

The most important of these is the use of sustainable energy technology. The production of biomass-based biofuels, such as vegetable oils, animal fats and microorganism's lipids are pivotal approach to rising energy prices and the potential depletion of crude oil reservoirs, reducing greenhouse gas emissions and p,kromoting a sustainable economy [4, 5, 6].

Fungi can be a valuable alternative to raw materials for the production of biodiesel, and a potential solution for oil decrease in the world [7], where microorganisms that store lipids more than (20%) of their cell dry weight are called oleaginous microorganisms. Oleaginous fungi have the ability to accumulate lipids in their biomass, which can be a promising alternative to the production of biodiesel, as these fungi are preferred to vegetable oils due to many advantages, including the accumulation of high amounts of lipids in their cellular mass, does not require arable land, not competing for food resources as well as their potential for growth on multiple sources. Many species of yeast and filamentous fungi are oil-rich because they have the ability to synthesis and accumulate high amounts of lipids such as triglycerides inside their cells, which contain high amounts of long chain fatty acids (C16:1, C18:1, C16:0, C18:3, C18:2, C18, and C16) which very similar to vegetable oils [8, 9, 11, 12]

These lipids have the same structure as well as energy value as vegetable oils and animal fats, but their production does not compete for food resources, especially if they are based on inexpensive carbon sources. Moreover, oleaginous fungi have a short life cycle, also production is not affected by weather changes, does not require light to grow, does not need CO2 and has the ability to analyze compounds with high organic content.

Biodiesel offers many advantages over petroleum-derived fuels because biodiesel is environmentally friendly and significantly reduces carbon dioxide, carbon monoxide, sulfur dioxide. Biodiesel can be used pure or mixed with fossil diesel for the operation of diesel engines, the cost of production is less than the cost of production of fossil diesel, the possibility of production in any country without exception, and it is less harmful to the environment [1,12,13,14].

MATERIALS AND METHODS

Source of Isolates

Four oleaginous fungi isolated previously from oil-rich soils by [15] were provided from the laboratories of the Biology Department, Science College, Basrah University and used in this study. They included *Aspergillus niger, A.flavus, A.ochraceus*, and *Penicillium chrysogenum*.

Biomass Production

In order to select the highest biodiesel production among purified fungal isolates they were activated on PDA for 5-7 days while three different fermentation media were used to cultivate all fungal isolates including, Nitrogen limited medium (NLM) [16], while cheese whey medium (CWM) and waste molasses dates medium (WMDM) were used as natural media. NLM was composed of (g/l) KH_2PO,7; $(NH_4)2SO_4$,2; Mg $SO_4.7H_2O$,1.5; NaH_2PO_4, 2; Yeast extract, 1; Glucose 40 and Distilled Water,1L). CWM was prepared by filtering 1L of cheese whey through two layers of medical gauze as well as WMDM was prepared as follows: Two kilos of palm dates were soaked for 6-8 hours. The mixture was mashed and filtered through gauze. Each 300g of solid waste residue was add to 500ml of D.W and boiled for 30 minutes. After cooling, the solution was filtered and the volume measured 1 L of D.W.

The above media were sterilized in autoclave (121℃ and pressure15 pounds/inch^2 for 20 minute), as well as, the pH was adjusted to 6.5. The process was carried out by cutting 5 plugs of fungal growth for each isolate which inoculated in 500 mL Erlenmeyer flask containing 250 ml of fermentation medium then incubated in shaking incubator at 30ºC at 120 rpm for 8 days.

Determination of Dry Weight and Extraction of Lipids from Fungi

The dry weight was determined and estimated according to [17] with some modifications. After incubation period, the biomass was centrifuged at 3000 rpm then filtered by using Whatmman No.2 filter paper. After that, the product was washed three times with D.W. and dried at 60 ºC for 24h. Finally, the dry weight was determined and estimated As the lipids were extracted using methanol, chloroform 1:2 methanol chloroform according to [14].

Transesterification Reactions and Analysis of Gas Chromatography Mass Spectrometry (GC/MS)

The analysis of Fatty Acid Methyl Esters (FAMEs) was used by transesterification reactions to determine the fatty acid (FA) content of extracted lipids from fungal biomass according to [18]. FA composition of the crude lipid, which was produced by oleaginous fungal isolates were determined by analysis of (FAMEs). The sulfuric acid as a catalyst with methanol (2.5%V/V) was added to the crude lipids. The reaction continued at 90 ºC (water bath) for 45 min as well, 2mL of n-hexane and 1mL of H2O were added. The FAMEs dissolved into the n-hexane and then the solution was centrifuged for 15min at 2000rpm to separate the hexane phase which contained FAMEs from the water. The hexane phase was transferred into new a tube using Pasteur pipettes. Then, FAMEs were analyzed using (GC/MS, 7890 B / USA). These tests were conducted in the Research and Quality Control Department for the Ministry of Oil /Iraq- Basrah.

Calculation of Cetane Number (CN)

CN was calculated based on compositions of produced FAME as in the following formula according to [19]: CN = 61.1 + 0.088x2 + 0.133x3 + 0.152x4 – 0.101x5 – 0.039x6 - 0.243x7 - 0:395x8. Where x2 Myristic Acid, x3 Palmitic Acid, x4 Stearic Acid, x5 Palmitoleic Acid, x6 Oleic Acid, x7 Linoleic Acid, and x8 Linolenic Acid.

Calculation of Residual Carbon and Sulfur Content

Residual carbon and sulfur content were calculated in the laboratories of the Research and Quality Control Department of the Iraqi Ministry of Oil by Standard Methods ASTM-D4530 IP-13 and ASTM-D4294. The weighted percentage (wt. %) of carbon residual (CR) and sulfur content (SC) was determined based on the method of calculating the values of the product mixture mycodiesel with hexane and calculating the value of only the solvent (hexane) independently using the following formula:

Net wt % (CR, SC) of mycodiesel = wt% of CR or SC in the mixture - wt. % of hexane phase.

Statistical analysis

Using SPSS statistical package for social sciences for data analysis by using ANOVA at significant level $p \leq 0.05$.

RESULTS AND DİSCUSSİON

Biomass Production

The determination and estimation of biomass for oleaginous fungi showed a significant difference at $p \leq 0.05$ of biomass production for fungal isolates when grown in different fermentation media. The isolates that were cultivated in NLM. All oleaginous isolates were given different values of biomass (Table 1). Where, *A. ochraceus* yielded 20.8g/l as the highest dry weight, while the lowest dry weight was yielded by *A.niger* (14.70 g/l). Furthermore, fungal isolates were grown on natural media in addition to synthetic media, so that, fungal isolates also gave good biomass values. *A. ochraceus* and *P. chrysogenum* yielded the highest biomass values (15.6g/l and 12.4g/l) respectively when cultivated in CWM, while the lowest dry weight (8 g/l) was yielded by *A. flavus*. In addition, the same isolates were given different products of biomass when using WMDM media as an alternative source, where the highest value of biomass was recorded for *A.flavus* (5.67 g/l) when grown in WMDM.

Table 1. Biomass production of oleaginous fungi after eight days of incubation in different fermentation media.

Fungal Isolates	Biomass Production in Fermentation Media (g/l)		
	NLM	CWM	WMDM
Aspergillus flavus	17	8	5.67
A. niger	14.7	8.8	3.70
A. ochraceus	20.8	15.6	4
Penicillium chrysogenum	16.5	12.4	4.2

Biomass production was influenced by different factors, like appropriate temperature, pH, aeration and type of carbon source. In the present study, the temperature of 30°C, a pH of 6.5, and the carbon source of glucose played an important role in the production of this biomass. This is in accordance with studies [20, 21]. On the other hand, the use of alternative media that are inexpensive and readily available, to produce large biomass is very necessary to produce large amounts of lipids [12, 16]. The result of the present study revealed that CWM and WMDM appeared as good media when used for fungal growth and produced biomass consistent with [22]. CWM is considered very inexpensive and is readily available as it is produced from the waste in the manufacture of cheese. The amount of biomass produced may be due to the rich content of proteins, sugar and minerals, as cheese whey contains 10-20% protein and 60-70 lactose [23,24]. Reducing production costs that associated with the fermentation process is still of essential importance to increase economic viability therefore, the present study agrees on this point.

Lipids Extracted

The four selected isolates showed varying values for lipid accumulation in its biomass when growing on different fermentation media with significant differences at $p \leq 0.05$ among fungal isolates in the same medium and different media. When NLM was used to cultivate fungal isolates with glucose as a source of carbon, the highest lipid yield appeared for *A. ochraceus* (4.76g / l, 22.9%), while the lowest lipid yield appeared for *A. niger* (3.234g / l.22%) (Fig.1). Moreover, oleaginous isolates showed variation of lipid accumulation when cultured in CWM, where the lipid content of *P.chrysogenum* was measured as 4.71g/l, about 38% from total biomass, followed by *A. niger* about 36% with lipid weight 3.16g/l (Fig.1). On the other hand, the ratio for lipid accumulation differed when fungal isolates were cultivated in WMDM, where *A. ochraceus* yielded 1.07g/l, 20% in WMDM as the highest lipid yield of the four isolates (Fig.1).

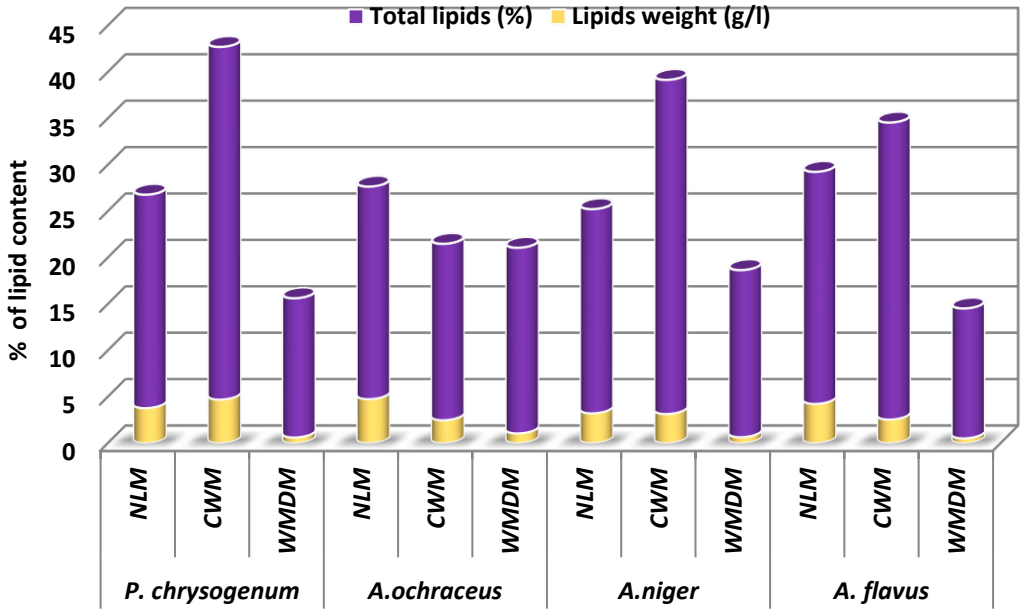

Fig.1: The lipids yields of oleaginous fungi after incubation in fermentation media.

The present study showed that lipid accumulation of oleaginous fungi differed among isolates; these percentages vary depending on the fermentation medium which was used for cultivation. NLM induced all fungal isolates to accumulate good amounts of lipids, so that the accumulation of lipids reached more than 20% and this is consistent with the other studies [25, 14]. The accumulation rate of lipids for *A.niger* was more than 22% when grown in NLM, therefore, this finding differs with [12], this difference may be due to the environment, where the isolate of *A. niger* has been isolated from oil-polluted areas that form a harsh environment with little organic matter. Furthermore, using organic waste to produce large quantities of lipids is of great economic feasibility, and this is in accordance with the results of the present study. Therefore, CWM and WMDM were used as fermentation media it was noted that it constitutes the highest accumulation of lipids from fungi reached to 38%. This is economically feasible and in accordance with [26]. This achieves the economic objective of the study which aimed to produce biodiesel from very inexpensive and readily available, environmentally friendly substrates.

Characteristics of Mycodiesel Production

The results of the analysis of GC/MS of FAs after the esterification process showed that fungal isolates formed stores for the synthesis of FAs, such as saturated and unsaturated, which is the main component of biodiesel. The results revealed that the percentage of the composition of FAME important for biodiesel ranged between 66.62 - 92. 29% (Table 2). This ratio appeared in Hexadecanoic Acid, Octadecanoic Acid, Hexadecenoic Acid, Octadecenoic Acid, and Octadecadienoic Acid. As well, the current study showed that the Cetane Number of the FAME compositions for the four isolates ranged from 52.6% for *A. niger* to 57.9% for *A. Flavus*. These numbers are in conformity with international and Iraqi specifications., Furthermore, the results of CR and SC were 0.04 and 0.0069 wt% respectively of produced mycodiesel, where values appeared very low in comparison with petroleum diesel (Table 3).

Table 2. The most important FAME produced and CN of oleaginous fungi.

Isolates	CN	% Compositions of FAME					
		16:0	16:1	18:0	18:1	18:2	Total
A. flavus	57.9	21.92	-	-	41.96	18.09	81.97
A. niger	52.6	15.29	-	-	40.36	36.64	92.29
A. ochraceus	57.1	17.30	-	-	27.90	21.42	66.62
P. chrysogenum	56.8	19.68	0.80	1.21	19.94	25.60	67.23

Table 3. Carbon residue and Sulfur content of mycodiesel production.

Characteristics	Standard Methods	Unit	Standard of Fossil Diesel Fuel (max)	Result of Mycodiesel
Carbon Residue	ASTM-D4530 IP-13	Wt.%	1.5	0.04
Sulfur Content	ASTM-D4294	Wt.%	2.5	0.0069

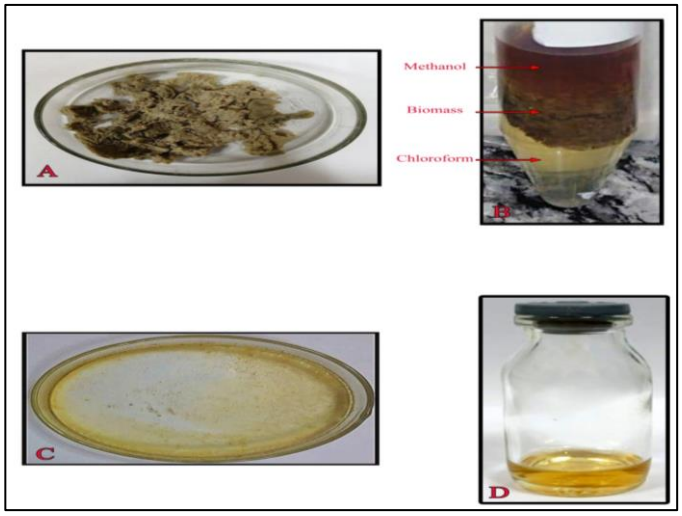

Fig.2: The most important FAME produced and CN of oleaginous fungi.Steps of producing mycodiesel from oleaginous fungi (A, biomass; B, biomass with solvent; C, extracted lipids; D, mycodiesel product).

In plants, the structures of FAs are important and essential for determining biofuel efficiency, as well as, unsaturated FAs are among the best fats to improve biodiesel efficiency [27]. In plant, animals and fungi the FAs such as Palmitic Acid, Palmitoleic, Stearic Acid, Oleic Acid, Linoleic Acid, and Linolenic Acid are associated with triglycerol structure. To separate these FAs and produce FAME, it must go thourgh a process called transesterification by using a catalyst and methanol [28]. However, in the present study the produced FAME that appeared in the results of GC/MS was a clear indicator for the ability of that catalyst to complete the process of esterification in accordance with [18,12, 29]. In the current study, Oleic Acid and Linoleic Acid formed about 77% from total fatty acids for *A. niger*, as well, most of the FA extracted from oleaginous fungi represented by (C16; C16:1; C18; C18:1 and C18:2) is similar to the FA extracted from vegetable oil and consider the best a better, stock and available for biodiesel production [17] and that result corresponds to [30]. The characteristics of diesel fuel greatly affect the diesel engine. One of the most important of these specifications is the Cetane Number (CN) which indicates the quality of diesel fuel (the combustion efficiency) where CN represents the fuel model as a quality factor for diesel fuel [30]. The present study showed that the CN of FA conformity with international and Iraqi specifications where the

specifications of Iraq Cetane Number reached 57min, as well, according to international and Iraqi standard specifications, the CR and SC of produced mycodiesel were very small compared to fossil diesel, which reduces the pollution from the combustion of fuel in engines very significantly [31, 32, 33].

CONCLUSİONS

The current study shows the ability of mycodiesel production from oleaginous fungal isolates isolated from oily soils is possible, inexpensive, they are readily available, and the raw materials are environmentally friendly. There is no need to employ plants as sources of biodesel which compete for sources of food for the community.

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REFERENCES

1. Giampietro, M., and Mayumi, K., 2009. The biofuel delusion: The fallacy of large scale agro-biofuels production.
2. Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., and Mielenz, J.R., 2006. The path forward for biofuels and biomaterials. *Science*, 311(5760), 484-489.
3. Aljasani,N., 2007. Global warming and its effects in Iraq. *Journal of Geographic Research*, 2, 91-102.
4. Kumari, A., Mahapatra, P., Garlapati, V., and Banerjee, R., 2009. Enzymatic transesterification of jatropha oil. *Biotechnology for Biofuels*, 2, 1-7.
5. Azócar, L., Ciudad, G., Heipieper, H., and Navia, R., 2010. Biotechnological processes for biodiesel production using alternative oils. *Applied Microbiology and Biotechnology*, 88(3), 621-636.
6. Zinoviev, S., Müller Langer, F., Das, P., Bertero, N., Fornasiero, P., Kaltschmitt, M., Centi, G., and Miertus, S., 2010. Next-generation biofuels: Survey of emerging technologies and sustainability issues. *Chemistry and sustainability, energy and materials*, 3(10), 1106-1133.
7. Liang, M.H., and Jian, J.C., 2013. Advancing oleaginous microorganisms to produce lipids via metabolic engineering technology. *Progress in Lipid Research*, 52, 395-408.
8. Zhu, M., Zhou, P., P., and Yu, L., J., 2002. Extraction of lipids from Mortierella alpina and enrichment of arachidonic acid from the fungal lipids. *Bioresource technology*, 84(1), 93-95.
9. Beopoulos, A., Cescut, J., Haddouche, R., Uribelarrea, J., L., Molina-Jouve, C., and Nicaud, J.M., 2009. Yarrowia lipolytica as a model for bio-oil production. *Progress in Lipid Research*, 48(6), 375-387.
10. Khan, S.A., Hussain, M.Z., Prasad, S., and Banerjee, U., C., 2009. Prospects of biodiesel production from microalgae in India. *Renewable and Sustainable Energy Reviews*, 13(9), 2361-2372.
11. Ratledge, C., 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie, 86(11), 807–815.
12. Shafiqe, S.A., and Ali, R.H., 2017. Myco-diesel Production by Oleaginous Fungi. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 8(2), 1252.
13. Magdum, S., Minde, G., Adhyapak, U., and Kalyanraman, V., 2015. Competence evaluation of mycodiesel production by oleaginous fungal strains: Mucor circinelloides and Gliocladium roseum. *International Journal of Energy and Environment*, 6(4), 377-382.
14. Kamoun, O., Ayadi, I., Guerfali, M., Belghith, H., Gargouri, A., and Trigui-Lahiani, H., 2018. *Fusarium verticillioides* as a single-cell oil source for biodiesel production and dietary supplements. *Process Safety and Environmental Protection*, 118, 68S-78S.
15. Kadhim, K. and Alrubayae, I., 2019. Study of Lipase Production and Lipids Accumulation of Oleaginous Fungi Isolated from Oil-rich Soil in Basrah. *Scientific Journal of Medical Research*, 3, 123-127.
16. Neema, P., and Kumari, M., 2013. Isolation of Lipid Producing Yeast and Fungi from Secondary Sewage Sludge and Soil. *Australian Journal of Basic and Applied Sciences*, 7(9), 283-288.
17. Li, S., L., Feng, S., L., Li, Z., T., Xu, H., Yu, Y., P., Qiao, D., R., and Cao, Y., 2011. Isolation, identification and characterization of oleaginous fungi from the soil of Qinghai Plateau that utilize D-xylose. *African Journal of Microbiology Research*, 5(15), 2075-2081.
18. Shin, D.Y., Cho, H., U., Utomo, J., C., Choi, Y., N., Xu, X., and Park, J.M., 2015. Biodiesel Production from *Scenedesmus bijuga* Grown in Anaerobically Digested Food Wastewater Effluent. *Bioresource Technolog*, 184, 215–221.
19. Bamgboye, A., and Hansen, A.C., 2008. Prediction of cetane number of biodiesel fuel from the fatty acid methyl ester (FAME) composition. *International Agrophysics*, 22(1), 21.
20. Van't, R., and Tramper, K.J., 1991. Basic bioreactor design. New York: Marcel Dekker, INC ,p116.
21. Velayutham, P., and Nithya Devi, M., 2011. Biodiesel Production from Fungi. *Indian Journal of Natural Sciences*, 11(5), 275-281.

22. Vamvakaki, A., N., Kandarakis, I., Kaminarides, S., Komaitis, M., and Papanikolaou, S., 2010. Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes. *Engineering in Life Sciences,* 10(4) 348-360.
23. Gonza´lez, M., I., 1996. The biotechnological utilization of cheese whey: a review. Bioresource Technology, 57, 1–11.
24. Koutinas, A., A., Papapostolou, H., Dimitrellou, D., Kopsahelis, N., Katechaki, E., Bekatorou, A., and Bosnea, L., A., 2009. Whey valorisation: A complete and novel technology development for dairy industry starter culture production. *Bioresource Technology*, 100(15), 3734-3739.
25. Wynn, J.P., Hamid, A.A., Li, Y., and Ratledge, C., 2001 . Biochemical events leading to the diversion of carbon into storage lipids in the oleaginous fungi *Mucor circinelloides* and *Mortierella alpina*. *Microbiology*. 147(10), 2857-2864.
26. Shafiqe, S., A., 2017. Biodiesel production by oleaginous fungi before and after exposing of U.V. light. *International Journal of ChemTech Research*, 10(12), 357-363 .
27. Abu Naga, H., 2011. Biofuels (Production, Characteristics , Environmental and Developmental Risks and Impacts). Qahra Academic Library, First Edition, p90. In Arabic
28. Lin, Y.S., and Lin, H., P., 2011. Spray Characteristics of Emulsified Castor Biodiesel on Engine Emissions and Deposit Formation. *Renewable Energy*, 36, 3507-3516.
29. Dennis, Y.C., Leung, X., W., and Leung, M.K.H., 2010. A review on biodiesel production using catalyzed transesterification. *Journal of Applied energy*, 87, 1083-1095.
30. Arous, F., Azabou, S., Triantaphyllidou, I., Aggelis, G., Jaouani, A., Nasri, M., and Mechichi, T., 2017. Newly isolated yeasts from Tunisian microhabitats: lipid accumulation and fatty acid composition. *Engineering in Life Sciences*, 17(3), 226-236.
31. DMSIOP (Directory of marketing specifications for Iraqi oil products), 2000.
32. Teama, L., T., 2008. Improving diesel oil specifications using solvent extraction method. *Journal of Basrah Research Scientific*, 34(1B), 49-59.
33. Moser, B., R., Knothe, G., Vaughn, S., F., and Isbell, T., A., 2009. Production and evaluation of biodiesel from field pennycress (*Thlaspi arvense* L.) oil. *Energy and Fuels*, 23(8), 4149-4155.