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DETERMINATION OF FFA's AND PHYSIOCHEMICAL PROPERTIES OF COOKING OIL WASTE TRAETED WITH CRUDE LIPASE FROM CARP FISH

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ABSTRACT

The aimed of this study was to used the partial purified Lipase from the intestinal treat of Carp Fish in treating cooking oil waste in order to reduce averment pollution from it, and to produced fatty acid and glycerol. Cooking oil waste samples were collected from local restaurants in the Kut City, Iraq. The sample treated with the partially purified enzyme at different concentration (0.25, 0.5, 1) % with an enzymatic activity about 100 unit \ gm for (2, 4, 6) hrs. The concentration of fatty acids for untreated and treated cooking oil with Lipase were determined by high pressure liquid chromatography (HPLC). The physical and chemical properties of cooking oil were studied before lipase treatment Acidity number, density, refractive index, surface tension, impurity percent and viscosity. The result showed highly enzyme activity to hydrolysis ester bond at ph 7 and 40°C for 4 hr for all enzyme concentration and all test.

Key words: Lipase enzyme, Glycerol, Acidity, Fat waste.

تحديد FFAS، والخصائص الفيزيائية والكيميائية لنفايات زيت الطهي المعالجة باللايبيز الخام من أسماك الكارب

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الخلاصة

هدفت هذه الدراسة إلى استخدام الليبيز المنقى جزئيا من المعالجة المعوية لأسماك الكارب في معالجة مخلفات زيت الطبخ لتقليل التلوث الناتج عنها وإنتاج الأحماض الدهنية و الكليسيرول, تم جمع عينات مخلفات زيت الطبخ من المطاعم المحلية في مدينة الكوت بالعراق، عولجت العينة بالانزيم المنقى جزئيا بتراكيز مختلفة (0.25 , 0.5 , 1) % و بنشاط انزيمي حوالي 100 وحدة/ غم لمدة (2 , 4 , 6) ساعات , تم تحديد تركيز الأحماض الدهنية لزيت الطبخ غير المعالج أو المعالج باللايبيز بواسطة تحليل كروماتوجرافيا السائل عالي الضغط (HPLC) , تم دراسة الخواص الفيزيائية والكيميائية لزيت الطبخ قبل معالجة اللايبيز والتي تشمل رقم الحموضة ، الكثافة ، معامل الانكسار، التوتر السطحي، نسبة الشوائب واللزوجة , أظهرت النتيجة نشاط إنزيم عالي لرابطة إستر التحلل المائي عند الرقم الهيدروجيني 7 و 40 درجة مئوية لمدة 4 ساعات لجميع تراكيز الإنزيم ولجميع الاختبارات.

الكلمات المفتاحية: إنزيم اللايبيز، الكيسيرول، رقم الحموضة، المخلفات الدهنية

[*]**This article is taken from the first researcher's master's thesis.**

INTRODUCTION

Waste cooking fat is one of the hazardous wastes because improper disposal of waste cooking fat can cause major environmental problems such as blockage of drains and sewers as well as soil or water pollution **(Foo,*et al* .,2021).** One Liter of fat can contaminate 1 million liters of water despite its small size **(Kumar &Negi, 2015).** Waste is increasing rapidly with the increase in population. It is estimated that global waste generation will nearly double by 2050 and triple by 2100 compared to 2016 **(Firdaus, *et al*., 2021).** For example, 2.5 kg of waste is produced per person per day in the United States, Norway and Italy. On the other hand, 89 thousand tons of waste is produced in urban areas alone **(da Silva César, *et al*., 2017).**

It is estimated that the food industry, restaurants and households around the world produce approximately 200 million tons of waste fried oil annually, with a continuing increasing trend expected. If not handled properly, not only will the environment be polluted but a variety of safety related crises can occur **(Cheng, *et al*., 2024).** Many research interestsare being gained in the use of waste in general and fried oils in particular to produce many useful substances such as glycerol, fatty acids, soap, energy and surfactants **(Hind, &Shakir, 2023),** Waste cooking fat is characterized by the presence of high molecular weight hydrocarbons and polymerized ester derivatives, which are thermal and oxidative pollutants that negatively effecton environment **(Kumar &Negi, 2015).** There are great varieties of impurities present in waste cooking fat.The main components still correspond to triglycerides, partial glycerides, and fatty acids. This makes waste cooking fat a suitable second generation raw material for the manufacture of oleochemicals **(Hussien, 2019).**

The use of enzymes is important because they are biological catalysts **(Rincón, *et al*., 2019)**. They are also specialized catalytic proteins with exceptional catalytic power and also have a distinctive specificity **(Awda, &Fayyadh, 2018)** They also give great biotechnology **(Mohamad, & Sedrah,2023)** and they are industrially important **(Al-Haidari, *et al*.,2021).** They are necessary for all forms of life by catalyzing various reactions. Chemicals in cells (**Sedrah, *et al,* 2021**) and also used in the diseasestreatment **(Abbood &Awda, 2020).**Lipases (EC 3.1.1.3, triacylglycerol acylhydrolases) are lipid hydrolysis enzymes **(Mohamad, &Sedrah, 2023).**It breaks the ester bonds between the carboxylic groups, producing mono and fats, free fatty acids, and glycerol **(Odeh &Khalifa, 2019).**It has ability to benefit from a wide range of reactants and its high stability towards pH, temperatures and organic solvents. Most industrial processes take place at high temperatures. It is one of the enzymes with high stability **(Ali, *et al*., 2022).**

Lipases are a large group of industrial enzymes. Due to their wide applications that can be produced in large quantities, they are an important group of enzymes in terms of biotechnology **(Sood,*et al*.,2023).** They are used in various industries such as food, beverages, cosmetics, pharmaceuticals, biofuels, detergents, and paper. Lipases are produced by many living organisms such as bacteria, yeast, fungi, plants and animals **(Ali *et al.,* 2021; Auda &Khalifa, 2019)** Fats and carbohydrates are the main waste components generated by the food

industry. In addition, it causes damage to the environment due to the formation of oily layers on water surfaces. It hinders the diffusion of oxygen, and blockages resulting mainly from emulsification with organic materials, and methane of fats, which leads to aggravation of the phenomenon of global warming **(Okino, *et al*., 2017; Al-Daraghi, *et al*., 2019).** Converting the fatty acid and glycerol content of waste fried oils into a useful product is a better option for managing these wastes **(Pathania& Jana, 2020).** The substance that can be extracted from waste cooking fat is glycerol, especially from waste cooking fat exposed to the lipaseenzyme. Glycerin is a high value commercial chemical with thousands of uses, providing great opportunities for new applications such as in the manufacture of artificial uses **(Maegala, *et al*., 2020)**. Many industries use glycerin as raw materials, including the paint, food, soap, and pharmaceutical. It is also used in feeding cows. It enhances growth, increases particle weight, and improves blood characteristics by increasing glucose in blood **(Khalid, & Al-Anbari, 2024).** Also, it increases the rate of milk production when used in cows" diets **(Khalid, & Al-Anbari, 2023).** Therefore, this study aimed to use the lipase enzyme that extracted from the intestinal tract of common carp **(Karim, *et al*., 2022; Yousif, & Hassan, 2023)**, in treating waste cooking fat, studying some of its physical and chemical properties, and conducting an HPLC examination.

MATERIALS AND MATHODS

Applications of lipase enzyme on cooking oil

Collection of samples:

The samples were collected from restaurants and fast-food stores. Samples were filtered with Whitman filter paper grade 4 to reduce the solids. They were filled in an opaque glass bottle and placed in the refrigerator to avoid the possibility of sample deterioration.It is necessary to equalize the pH using 0.1 M sodium phosphate buffer solution and a temperature of 40°C for all treatments.

Enzyme prepared:

The enzyme was extracted from carp fish and prepared according to the method (**Ali *et al*. 2022)**. The intestinal tract of the carp was cleaned with cold distilled water to remove fats and unwanted substances. A 0.2 m of potassium phosphate solution, pH 7, was added at a mixing ratio of 1 from the intestinal tract of the carp to 4 from a 0.2m potassium phosphate solution, mixed with an electric mixer for 2 mins, and filtered with whiteman filter paper No. 1, after which the extract was centrifuged at 5000xg for 30 mins at 4°C. The supernatant was classified as the crude enzyme. A lyophilized enzyme with an enzymatic activity of 100 U\g was used.

Acidity number determination

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method from **Hasan *et al.* (2016).** It was by weighing 5 g of waste cooking oil, added to 50 ml of 95% ethyl alcohol, then adding 2-3 drops of phenolphthalein concentration indicator. Then, the titration is done directly with 0.1 M of

potassium hydroxide until the pink color appears and remains stable for 30 seconds. The Blank prepared in the same way, which is free of the sample, and the acid number is calculated according to the equation below: -

$$\text{Acidity Number} = \frac{The\ volume\ of\ used\ base\ of sample - Blank \times N \times 56.1}{\text{Weghit of oil sample}}$$

Immobilization of lipase enzyme:

Preparation of Lipase solution with sodium alginate

It was done by add 6.6 ml of distilled water to 1g of sodium alginate, with the addition of 0.5 g of enzyme solution (lyophilized enzyme with 5 ml of 0.2M potassium phosphate buffer) and mixed well.

Preparation of calcium chloride

Prepared by dissolving 2.94 g in an amount of distilled water and bring the volume to 100 ml of distilled water.

Enzyme immobilization

The restriction process was applied according to **Priyanka *et al*. (2019).** It was taking the enzyme solution with the alginate in a sterile syringe and adding it slowly as adrops to the calcium chloride solution in a 100 ml beaker, leaving it for 10 mins to solidify, then placing it in a strainer and washing it with distilled water. Then, put the balls in the separating funnel and incubate with the base material for 3 hrs.

Determination of free fatty acids after treatment with lipase enzyme

Preparing the emulsion

The emulsion was prepared according to **Priyanka *et al*. (2019)** by mixing 5 g of arabic gum with 100 ml of the 0.2 M phosphate buffer and adding it to 100 g of waste cooking oil. Then, add three concentrations of Lipase powder extracted from the intestinal tract of common carp. The concentrations were (0.25, 0.5, 1) g/ 200 ml of emulsion, and the mixture was placed on a magnetic stirrer at a temperature at 40°C. The mixture was incubated for 2, 4 and 6 hrs, flush with 0.1M potassium hydroxide.

High-pressure liquid chromatography analysis

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method decreed from **Majnooni *et al*. (2016)**. The HPLC system used in this study consists of two pumps from the German SYKAM Solvent Delivery System, a fluorescence spectrophotometry system with the detector operating at excitation and emission wavelengths of 265 and 315 nm, respectively. The analytical column was Shim-Pack C18-ODS (250) mm*4.6 mm. The acetonitrile-water gradient conditions were as follows: A: 85-15% from 0 to 4 minS, B: 87-13% from 5 to 8 mins, and C: 97-3% from 9 to 14 min. The column oven temperature was set at 50°C and the mobile phase was filtered, evacuated, and pumped at a flow rate of 1.5 ml/min.

The analysis was applied using 250 µL of 9-fluorenylmethyl chloroformate solution to 1 mL of oil after adding 25 µL of sodium phosphate buffer (0.05M, pH 9.3) and mixing briefly.

The samples were kept at 40°C for 10 minutes. Then, a volume of 100 µl was then injected into the HPLC systems**.**

Extraction and determination of glycerol

Glycerol was prepared according to method **Eghbili *et al*. (2020).**

Density measurement

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude lipase , according to the method decreed from **Kumar *et al* .(2018)**.Density was measured using a density bottle **.** The density bottle was taken after washing with distilled water and placed in an oven at a temperature of 121°C for 30 mins for the purpose of sterilization. Then, weigh the empty bottle and record the weight. After that, fill the bottle with waste cooking oil at room temperature until the bottle is filled and close it. Then, weigh the full bottle and calculate the density according to the following asbelow:

$$\text{Density}= \frac{weight of full\ bottle - weight of empty\ bottle}{\text{Volum sample}}$$

Viscosity measurement

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method decreed from **Mudawi *et al*.(2014)**.The viscosity of samples of waste cooking oil was recorded using an Ostwald-U tube viscometer according to**.**

Measuring the refractive index

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method decreed from **Godswill *et al*. (2018).** The refractive index (RI) was determined using an Abbe 60 Refractometer.

Surface tension measurement

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method decreed from **Adhikari *et al.* (2023).**

Measuring the percentage of impurities

This test was done for palm oil before and after frying, and for cooking oil waste treated with crude Lipase, according to the method decreed from **Varona *et al*. (2021).** It was by placing the filter paper in a petri dish and placing it in an oven at a temperature of 103°C for 20 mins. Place it in the dispenser for 10 mins, then weigh the petri dish with the filter paper. It takes 20 g of the sample and add 200 ml of N-hexane with shaking, wait 30 mins, put the filter paper in a funnel, filter the sample and add hexane to wash the remainder in the beaker and the filter paper. Then, takes the filter paper and put it in the petri dish and put them in the oven at a temperature of 103 °C for a period of 20 mins. Then weigh the dish and extract the amount of impurities according to the following equation:

Impurities ratio % = $\frac{After\ the\ weight before the. - before\ the\ weight after the.}{\text{sample weight}}$

RESULTS AND DISCUSSION

Statistical analysis

The statistical program Statistical Analysis System **SAS (2018)** was used to analyze the data to study the effect of different parameters on the studied traits, according to a complete random design (CRD), and the significant differences between the means were compared with the Least Significant Difference (LSD) test.

Enzyme immobilization

The results showed **Priyanka *et al*. (2019),** that immobilization of lipase with sodium alginate was not effective. It was observed that there was no hydrolytic activity of the immobilized enzyme. There was no decrease in the pH of the samples treated with the enzyme when incubated in the shaking incubator for 3 h. At a temperature of 37°C. This could be due to the high molecular weight of the lipase (25 – 65 KD) which was not absorbed by the alginate network. It led to its leaching with the calcium chloride solution. Based on what was stated above, the free enzyme was approved in the treatment of waste cooking oil.

Chemical properties

Acidity number

The value of the acid number of waste cooking oil before the frying was estimated to be 0.24, estimated as oleic acid, and after frying many times for 20 h at a temperature of 180–200 °C, it was 0.3, estimated as oleic acid.

The results noted an increase in free fatty acids by 30–32%. This result miscued by thermal hydrolysis This is similar to what was reported by **Paunović *et al*. (2020),** as the increase in the acid number was 26% for a sample of palm fat treated at 165°C for 40 minutes over 7 d (Table 1).

It is also noted that the value of the estimated acidity number, such as oleic acid increases with the increase in the concentration of the enzyme in the sample. This is because the lipase enzyme liberates the fatty acids by breaking the ester bond. Thus, the acidity number increases with increasing time. However, a decrease in these values is observed at 6 h. of interaction between the enzyme and the substrate because the optimal time for the enzyme reaction was 3 h **(Saranya, *et al,* 2019).** In addition, the effectiveness of the lipase enzyme is affected by the availability of water for the purpose of continuing the reaction, otherwise the reaction is reversed. This explains the decrease in the acidity number after 6 h of the reaction. It is possible that the enzyme becomes saturated in the interface area between the oil and water phases **(Serri, *et al*,2008).**

Table (1): The Acid number of samples of waste cooking oil untreated and treated with the lipase enzyme.

enzyme concentration (g/100ml emulsion)	incubation time (h)	Acidity no.%
Untreated cooking oil	-	0.322
0.25	2	5.208
	4	6.732
	6	5.835
0.5	2	4.936
	4	7.180
	6	5.610
1	2	8.527
	4	8.976
	6	4.936
LSD : 0.05	--	1.894 *
		* (P≤0.05).

Determination of fatty asids produced by the enzyme lipase by HPLC.

The results show the decrease in fatty acids after being exposed to heat for a long time. The sample was exposed to the heat of frying for 20 hrs. in frying potatoes and falafel at a temperature ranging from 180-200 °C (Table 2). This result was similar with **Nizori, & Mishra (2018),**as the index of boiled oil for furring potato chips decreased significantly with increasing frying time and temperature. Moreover, unsaturated fatty acids (oleic and linoleic acid) decreased after a long period of frying compared to untreated oil. It is concentration that the enzymatic treatment for 4 hrs. is the highest in terms of high concentration of fatty acids in the samples **(Morselli, *et al*, 2017).** Confirmed that the enzymatic process with 3 h. of reaction time provides more selectivity and lower acyl migration in terms of enzymatic esterification. In a study applied to high-oleic sunflower oil and fully hydrogenated soybean oil, **(Saranya, *et al*, 2019)** showed that the optimal time for the lipase enzyme reaction is 3 h. at a temperature of 50°C and with a pH of 7.

Table (2): Concentration of some fatty acids in cooking oil before and after the frying process using HPLC.

enzyme concentration (g/100ml emulsion)	incubation time (hrs.)	Palmitic acid (C16)	Oleic acid (C18:1)	Stearic acid (C18)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Arachidonic acid (C20)	Myristic acid (C14)
Untreated cooking oil	-	14.6	11.5	0.58	4.6	0.15	0.25	1.00
0.25	2	18.9	16.9	1.00	5.8	0.17	0.28	1.08
	4	33.6	30.5	2.60	9.2	0.32	0.42	1.42
	6	27.4	24.9	1.90	7.9	0.22	0.34	1.25
0.5	2	21.5	19.5	1.19	6.5	0.19	0.30	1.16
	4	36.5	32.6	2.85	9.8	0.33	0.44	1.50
	6	30.9	26.5	2.05	8.4	0.26	0.36	1.31
1	2	24.6	22.1	1.58	7.1	0.20	0.32	1.20
	4	38.9	34.9	2.99	10.2	0.35	0.45	1.70
	6	31.5	28.7	2.33	8.9	0.30	0.39	1.36
LSD : 0.05	-	7.93 *	6.88 *	0.769 *	2.738 *	0.216 *	0.209 *	0.541 *
	*P≤0.05.							

The results indicates that the enzyme dealt with all fatty acids according to their concentrations present in the sample, which gives the idea that the enzyme is random in the way it deals with the sample, so the selectivity of the lipase (Table 2). It is defined as the ability to direct the reaction in a preferential direction over other reactions. On this basis, reactions catalyzed by non-specific and specific lipases (1,3) **(Albayati, *et al*, 2020).** Analysis of the sample enzymatically shows a gradual increase in the ability to analyze the used palm olein fat. The increase in enzyme concentration leads to promising results in terms of dealing with waste cooking oil. The observed decrease in fatty acids treated with frying heat, which leads to the difficulty of chemical esterification. This condition can be treated gradually by relying on enzymatic stimulation (Table 2). Treating the samples enzymatically led to the observation of the possibility of esterification and clearly high levels of fatty acids **(Prakash, *et al*, 2010).**

The enzymatic treatment at a rate of 0.25 g to the sample shows an increase in the percentages of fatty acids with an increase in the incubation period in oleic acid. After treatment with the enzyme for 2 hrs, the percentage increased from 7.55% to 8.50% as a result of the enzymatic activity in breaking the existing ester bonds. An increase in the incubation time for 4 h, the percentage was 11.44%, and this percentage represented the highest result for a concentration of 0.25 g of enzyme per 100 g of oleic acid sample.

It confirms that the best time for incubation, as mentioned previously, but a decrease in the percentages of fatty acids is observed as the incubation period continues to 6 h, because the optimal time for the enzyme reaction was 3 hrs. It is also possible that the effectiveness of the

lipase enzyme may be affected by the availability of water for the purpose of continuing the reaction. Otherwise, the reaction is reversed, and this explains the decrease in the acid number 6 h. after the reaction. It is possible that the effect of saturation of the enzyme in the interface area between the aqueous phases is the reason for this decrease **(Saranya, *et al,*2019).**

The results of the enzyme concentration of 0.5 g per 100 g of sample also show a noticeable increase in the percentages of fatty acids, as the Fatty acid linoleic acid increased from 2.4% to 3.2% with the reaction time increasing from 2 to 4 h **(Serri, *et al,*2008).**The treating sample with the lipase enzyme at a ratio of 1/100 weight/weight of the sample for a period of 4 h have reprded the best result of all treatments, with an increase in palmitic acid by 37.4% of the sample treated with frying oil and oleic acid. It was 26.5%, as well as oleanolic acid was 32.15%. As for linoleic acid, the increase was 45.5%, myristic acid was 12.8%, archidonic acid was 37.04%, and linolenic acid was 34%.

Extraction and determination of glycerol

The results recorded that the concentration of glycerol obtained from waste cooking oil treated with concentrations of (0.25, 0.5, 1) g of enzyme per 100 g of sample, as the amount obtained from regular fat was 2.5 ml (Table 3). This is similar to what was found **(Eghbili, *et al* 2020),** that the use of the lipase enzyme provides many advantages over the traditional lipid splitting process, especially the low temperature and high substrate specificity, which leads to products with high selectivity and low byproducts **(Zaharudin, *et al,*2018)**. Therefore, it notes that the percentage of glycerol extraction increases to 90% when using an enzyme at a rate of 0.25 g per 100 g of sample with an incubation period of 4 h, which is the best among the treatments (2 , 6 h).The higher enzyme concentration of 0.5 g is used for every 100 g of sample, while the conditions for all reactions are fixed at a temperature of 40°C for incubation and with a pH of 7. The increase in the amount of glycerol over the original sample appears by 101%. However, when using 1/100 the weights of the enzyme to the sample, results notice an increase of 100% over the original sample. The addition of the lipase enzyme is to improve the results of extracting glycerol from waste cooking oil **(Ferreira, *et al,* 2019).** The extraction method is a hybrid method that combines the traditional chemical method with an enzymatic treatment that precedes it, to maximize practical results that are promising in making use of waste cooking oil.

One of the major barriers to achieving higher glycerin conversions is the presence of water, which is byproduct of esterification. The conversion of smaller esters is delayed in the presence of polar compounds.Polar compounds will interfere in the reaction by competing for hydrogen ions, hindering the ability of these ions to catalyze, leading to a decrease in the reaction rate **(Saifuddin, 2009).**The glycerol was examined sensory, as the resulting liquid was colorless, had a distinctive odor, and did not contain the phenomenon of phase separation.

Table (3): Extraction of glycerol from cooking oil before and after frying using the hybrid method.

enzyme concentration (g/100ml emulsion)	incubation time (hrs.)	Raw glycerol ratio (v\v)	Purified glycerol (ml)
Untreated cooking oil	-	45	2
0.25	2	48	3
	4	54	3.8
	6	50	3
0.5	2	50	4.6
	4	58	5.2
	6	55	4.8
1	2	50	5
	4	65	6.5
	6	52	5.5
LSD : 0.05	-	6.351 *	1.866 *
*P≤0.05.			

Physical characteristics

According to the Iraqi Standard Specification IQS 2023\1904m, the refractive index of palm olein fat was 1.449-1.455 at a temperature of 50°C, and one of the points that must be taken into consideration is the refractive index of the heat-treated sample, which was 1.4722 at a temperature of 40°C.

It is noted from the results that increase in the refractive index as a result of the heat treatment. The results were agreed with **(Baig, *et al*, 2022),** which found that the activity of the lipase enzyme and the increase in free fatty acids, which led to a change in the refractive values (Table 4).

The viscosity of palm olein fat was 54.2556 n.s\m^2. After heat treatment for 20 hrs at a temperature of 180-200°C, it was 61.6057. The increase in viscosity is due to the loss of moisture during the heat treatment and oxidation of vegetable oils, as well as the polymerization of unsaturated oils, and fats can also be produced. It was agreed with **(Wiege, *et al*, 2020)** as the study showed an increase in the viscosity of palm oil with an increase in the duration of exposure to the frying process with a slight increase in the percentage of impurities.

Table (4): the physical tests conducted on samples treated with fish lipase enzyme.

enzyme concentration (g/100ml emulsion)	incubation time (hrs.)	Refractive index	Density Gm/cm^3	Viscosity $n.s\backslash m^2$	Impurity percentage %	Surface tension d\cm
Unused Palm oil	0	1.4671	0.9965	54,2556	0.0705	21.2
Untreated cooking oil	0	1.4660	0.9975	61,6057	0.1072	12.20
0.25	2	1.4658	0.9965	20.25	0.1066	21.4
	4	1.4652	0.9921	16.33	0.1087	21.8
	6	1.4656	0.9886	20.75	0.1030	21.4
0.5	2	1.4655	0.9950	13.33	0.1045	17.06
	4	1.4650	0.9870	8,41	0.1035	24.12
	6	1.4652	0.9927	11.83	0.1036	21.88
1	2	1.4656	0.9954	12.08	0.1027	26.83
	4	1.4642	0.9845	12.06	0.0953	28.49
	6	1.4646	0.9847	12.16	0. 1017	26.42
LSD : 0.05	-	0.0163 NS	0.0184 NS	7.802 *	0.0218 *	5.279 *
*P≤0.05.						

CONCLUSIONS

The study concludes that waste cooking oil is one of the wastes that cause many environmental challenges and problems. It increases proportionally with the increase in population. It has become a focus of interest for researchers in order to find useful and effective solutions for the purpose of reducing the environmental impact for the purpose of obtaining complementary raw materials for other industries. The enzyme lipase extracted from the intestinal tract of common carp fish was used for the purpose of reducing environmental pollution and benefiting from the fatty acids and glycerol that can be extracted from them. It has gave promising indications for the use of the enzyme in the biological treatment of palm olein fat waste, as well as the possibility of obtaining glycerol in higher percentages than using the esterification process only.

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