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Research Article
Reduction of Free Fatty Acid Content of Crude Sardine Oil by Enzymatic Esterification at Laboratory Scale

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Abstract

Background and Objective: In oil refining process, lipase-catalyzed de-acidification is a promising alternative to conventional neutralization. The enzymatic esterification of free fatty acids in crude sardine oil was carried out by immobilized lipase novozymes 435. The aim of this study was to upgrade the sardine oil processed in Morocco by using enzymatic solutions and to investigate the effect of the various parameters involved in enzymatic deacidification of fish oil: Pressure, ratio of oil/novozymes 435, ratio of oil/glycerol, temperature. **Materials and Methods:** The enzymatic deacidification optimization was performed with novozymes 435, by using two oils with different initial acidity. The results obtained in the present study were analyzed through two-way ANOVA test. **Results:** The greater deacidification yields were obtained with: A temperature of 70°C, a ratio oil/glycerol: 2%, a ratio oil/novozyme 435: 1%, a pressure: 15-25 mbar. **Conclusion:** From an industrial point of view, this eco-friend enzymatic solution may allow Moroccan fish oil producers to achieve acid number values that meet the standard.

Key words: Deacidification, esterfication, novozymes 435, sardine oil, free fatty acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Morocco is the first African fish oil producer. Fish oils domestic exports in terms of volume have reached 14.940 t in 2013[1]. Recently, there has been an emerging oil refining industry in Morocco. Normally natural fish oil contains low level of EPA and DHA but have substantial amounts of other undesirable fatty acids and cholesterol[2]. The fish oil acidity affects the commercial value of the oil and the subsequent uses of final product. In the oils processing, alkali refining aims to reduce the acidity by eliminating non-triglyceride impurities mainly free fatty acids[3]. The deacidification step impacts significantly the production of fish oil. Any inefficiency during this step may be detrimental to subsequent operations. The removal of free fatty acids from the crude oil is the most delicate and the most difficult unit operation of the whole refining cycle.

Chemical deacidification is able to produce neutral oil of good quality from any crude oil by reducing oil acidity up to 0.03%, but this process leads to several disadvantages:

Excessive loss of neutral oil binding to the low value soap: This chemical deacidification operation is performed with the addition of a caustic solution precipitating the generated soap which is separated from neutral oils by centrifugation. Soap can retain up to 50% of its weight of neutral oil which severely penalizes the performance of the entire process[4]. It is important to underline that the loss of neutral oil is big if the oil is very acidic. Alkaline deacidification by NaOH requires high energy consumption since it is performed at temperatures between 180 and 200°C. It also requires a large amount of water for washing in order to free oil from sodium hydroxide. Finally this chemical process generates pollutants flows due to sodium hydroxide and the washing waters.

The implementing enzymatic processes in place of conventional processes generally leads to reduced contributions to global warming and also reduced contributions to acidification, eutrophication, photochemical ozone formation and energy consumption to the extent that this has been investigated [5]. Chemical neutralization has been widely applied in industry for removing free fatty acid (FFA) in fish and vegetal oils. This result in a loss which is increased by the binding of oil to the soap formed. By reacting the free fatty acid with glycerol or partial glycerides the free fatty acid can be recovered by enzymatic esterification. The reaction utilizes the ability of specific esterases to couple free fatty acid to glycerol or partial glycerides under reduced pressure where the generated water can be removed. In an excess of water a lipase will hydrolyze fats to glycerol and fatty acid. Enzymatic fatty acid esterification was shown to be a technically viable process[6]. Different immobilized lipases were screened for their ability to esterify free fatty acids such us a lipase from Rhizomucor miehei[4,7].

The unique properties of enzymes such as high specificity, fast action and biodegradability allow enzyme-assisted processes in industry to run at milder reaction conditions, which improved yields and reduced waste generation. In presence of alcohols acting as acyl acceptor, lipase acylglycerol hydrolase are able to catalyze the esterification of free fatty acid and thus the neutralization of crude oils.

In biotechnological processes, synthesis to achieve maximum yields may be carried out either as an equilibrium controlled or kinetically controlled reaction. Only in the latter case, is the yield of condensation product influenced by the properties of the enzyme that act as a transferase in this reaction. With the same amount of enzyme, the maximum yield is also obtained much more rapidly than the equilibrium controlled process[8]. The mechanisms of enzyme catalyzed condensations allow rational analysis of how yield controlling factors (pH, temperature, ionic strength, enzyme and substrate properties) may be changed to obtain optimum yields[9]. Ravn *et al.*[10] have examined enzymatic conversion of fish oil free fatty acids or fatty acid ethyl esters into glycerides via esterification or transesterification catalyzed by Lipozyme™ 435. Influence on conversion yields of fatty acid chain length, saturation degree, temperature, enzyme dosage, molar ratio glycerol:fatty acids, acyl source composition (w/w ratio FFA:FAE) and reaction time was evaluated collectively by multiple linear regression. All reaction variables influenced the conversion into glycerides. The same trend is reported by Medina *et al.*[11] on the synthesis of triglycerides by enzymatic esterification of polyunsaturated fatty acids (PUFA) with glycerol in fish and microalgae oils. The main factors influence the degree of esterification and triglyceride yield were the amount of enzyme, water content, temperature and glycerol/fatty acid ratio. Gofferje *et al.*[4] have investigated the kinetics of enzymatic esterification in crude jatropha oil of glycerol and free fatty acids by immobilized lipase from Rhizomucor miehei. The reaction seems to follow a multisubstrate ping pong mechanism with competitive inhibition by the acyl acceptors (mono, diacylglycerides and glycerol). Free fatty acid content did not affect lipase activity within the ranges investigated. Beside those factors, relevance of the biocatalyst is one of parameters that define the yields of enzymatic reaction. Dos Santos *et al.*[12] discuss how the properties of the enzymatic support and the reactive groups placed on its surface may determine its suitability to use in enzyme immobilization or purification, mainly at an industrial level. The use of ultrasonic system increased the biocatalyst

activity. The study of Ma *et al.*[13] demonstrated that the use of ultrasonic systems increased the immobilized biocatalyst activity by 30.05%.

Against chemical refining which can leave residues, new acidification approaches of vegetal oils and fish oils have emerged. The use of immobilized enzymes as biocatalysts can substitute chemical solutions[14-16]. The enzymatic deacidification frees the refining process of a washing step and releases oil from residue soap which is detrimental for the oil price.

The global aim of this study was to upgrade the sardine oil processed in Morocco by using enzymatic solutions and the specific objective was to optimize the various parameters involved in enzymatic deacidification of sardine oil processed in Morocco: Pressure, ratio of oil/novozymes 435, ratio of oil/glycerol and temperature.

MATERIALS AND METHODS

Materials: The crude sardine oil used in this study comes from factories in the South of Morocco and was produced in July, 2013. This study was conducted at 2014. The oil was kindly supplied by ANAFAP, National Association of fish Meal and Fish oil Producers. The immobilized enzyme (novozyme 435) was provided by novozymes A/S (Bagsvaerd, Denmark). The alcohol used was purified glycerol (SDFCL-Mumbai). The analysis of the acid number was carried out with absolute ethanol (99.8%), Diethyl ether (99.5%), potassium hydroxide (KOH) and phenolphthalein of analytical grade.

Optimization of oil deacidification de by direct esterification: A sample of 300 g of crude fish oil has been reacting in the presence of novozyme 435 with different ratios (oil/enzyme): 0.1, 1 and 2% for 24 h. The quantity of glycerol added varies between 2 and 4%. The sample size has been stirring at 250 rpm in a Rotavapor (R BÜCHI-205) at a temperature ranging from 60-75°C and at low pressure (15-25 mbar) and atmospheric pressure. A nitrogen injection was performed into the reaction medium to eliminate water generated by reaction. The sampling is carried out every 1, 2, 4, 6, 8 and 24 h to achieve the analysis of the acid number. The following figure shows the system of enzymatic deacidification used in this study at laboratory scale (Fig. 1).

Analysis of acid value NF Iso 660 1990: Ten grams of crude oil was mixed with 150 mL of ethanol and diethyl ether mixture. After homogenization and complete solubilization of the oil, the free fatty acids of the oil were neutralized by 0.05N ethanolic potassium hydroxide and phenolphthalein until the indicator changed to pink.

The acid value is equal to M (KOH)×V×C/m. The data reported in this study are mean values from replicate analyses of two separate samples.

Validation of enzymatic deacidification optimization: To validate the operating parameters selected before (pressure, ratio oil/enzyme, ratio oil/glycerol and temperature), was performed enzymatic deacidification with the optimized parameters by using two oils with different initial acidity (42.1 and 86.1%).

Statistical analysis: All experiments were performed in triplicate. Statistical analysis was performed using the software Minitab 16.0. The results obtained in the present study were analyzed through two-way ANOVA test following Zar[17]. The $p<0.05$ was considered as statistically significant.

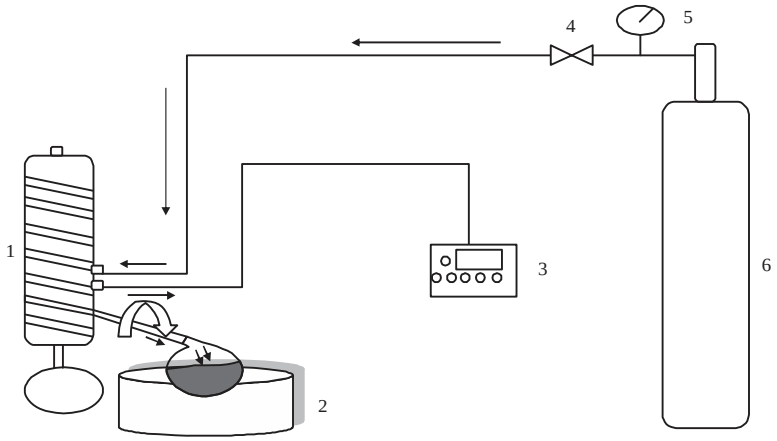

Fig. 1: Enzymatic deacidification system at laboratory scale
1: Nitrogen injection system, 2: Warm water, 3: Pump, 4: Control valves, 5: Pressure gauge, 6: Bottle nitrogen and 7: Raw fish oil with enzyme and glycerol

RESULTS AND DISCUSSION

Optimization of enzymatic deacidification

Effect of pressure: The acid value evolution of the fish oil samples esterified at 1013 mbar and at low pressure 15-25 mbar with a ratio of 1% (W%) of oil/enzyme as represented by Fig. 2. The reaction mixture contains 300 g of crude oil (initial acidity, 42%), 6 g of glycerol and 3 g of enzyme. Esterification was performed at 70°C and stirred at 250 rpm for 24 h.

In the beginning of the de-acidification at 1013 mbar, a strong increase of the acid value was observed. Therefore, hydrolysis instead of the desired esterification was the prevailing reaction. Operating under vacuum allows removal of generated water, increases reaction rate and moves the equilibrium to free fatty acid removal. After 1-2 h of reaction, when the water in the reaction system was completely stripped out via N_2, the reaction equilibrium shifted towards esterification, leading to a strong decrease of the acid number. After further 8 h the reaction remained static and no further change in free fatty acid content was observed, indicating that the chemical equilibrium was achieved. The analysis of variance (two-way) revealed that the acid value due to the pressure was statistically significant ($F = 8.97$, $p<0.05$). But acid value evolution due to the variation in time was not statistically significant ($F = 0.39$, $p>0.05$).

The same trend was reported by Makasci *et al.*[18], Fadiloglu *et al.*[19] and Gofferje *et al.*[4].

The deacidification reaction should be conducted at low pressure to remove water in the medium. In further experiments, the deacidification will be performed under low pressure.

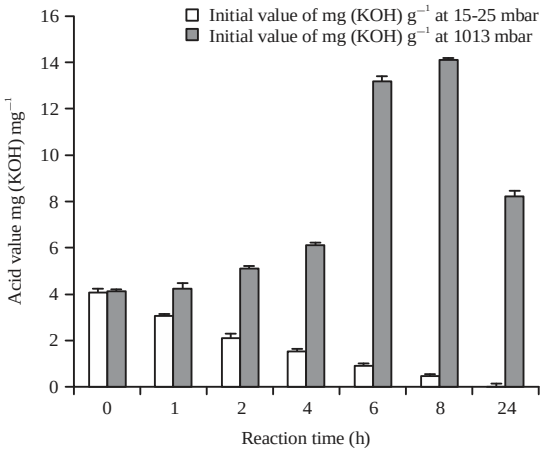

Fig. 2 : Effect of pressure on the enzymatic deacidification of sardine oil
All the values were represented as Mean±SD

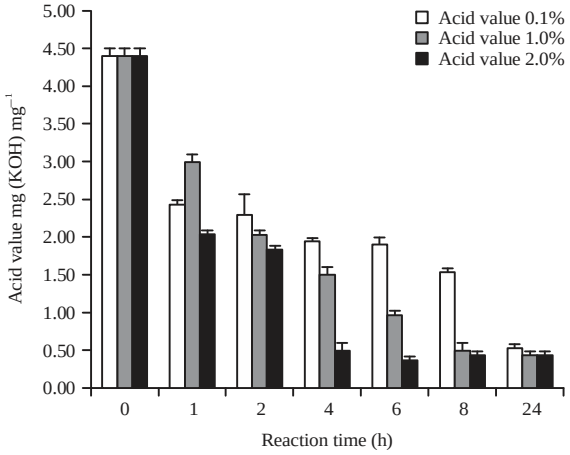

Fig. 3: Effect of ratio oil/novozymes 435 on the enzymatic deacidification of sardine oil
All the values were represented as Mean±SD

Effect of ratio of oil/novozymes 435: The results of acid value analysis of the fish oil samples esterified at low pressure 15-25 mbar with a ratio of 0.1, 1 and 2% of oil/enzyme are represented in Fig. 3. The reaction mixture contains 300 g of crude oil (initial acidity 42%), 6 g of glycerol and 0.3, 3 and 6 g of enzyme. Esterification was performed at 70°C and stirred at 250 rpm for 24 h.

The three ratios of enzyme/oil have reduced the acidity of crude fish oil with returns of 85.4% for deacidification at 0.1% enzyme, 91% for the one conducted at 1% enzyme and finally a 90.5% yield for the experiment conducted with 2% enzyme. The increment of enzyme dosage could accelerate the reaction as shown in Fig. 3. However, no significant differences were observed between the enzyme load of 1 and 2%. In fact, the further addition of enzyme in the reaction medium does not necessarily reduce more oil acidity. A state of reaction equilibrium was probably reached. The same finding was reported by author who has worked on pomace olive oil[18].

Deacidification of sardine oil performed in this study with 1% of novozymes 435 leads to a maximal performance (91%) of our deacidification system. These results are comparable to other biorefinery researches on various substrates of fish oil and vegetable oils, tuna oil with a yield of 94%[20], palm oil with a yield of 94%[11] and rice bran oil with yield of 94% 20, a very acidic olive oil with a yield of 88.43%[17]. Furthermore it also revealed that the influence of reaction time on acid value was more than the influence exerts by variation in ratio enzyme/oil. Statistical analysis by two way ANOVA on acid value revealed that the variation due to ratio of oil/novozymes 435 and reaction time were statistically significant ($F = 6.71$, $p<0.05$ and $F = 14.23$, $p<0.05$).

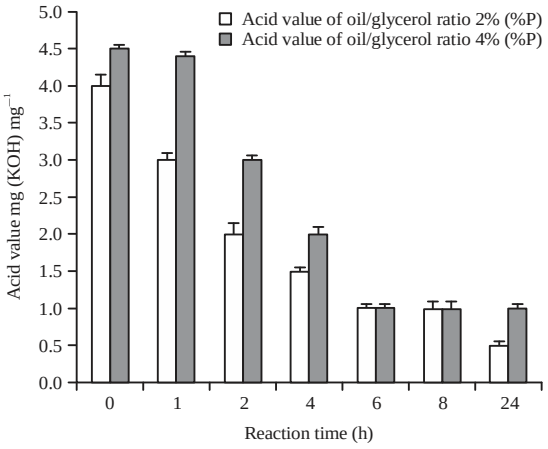

Fig. 4: Effect of ratio oil/glycerol on the enzymatic deacidification of sardine oil
All the values were represented as Mean±SD

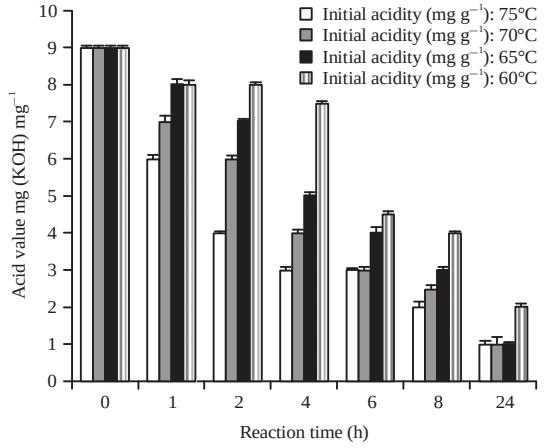

Fig. 5: Effect of temperature on the enzymatic deacidification of sardine oil
All the values were represented as Mean±SD

In this study, globally 91% is a satisfactory deacidification rate. This good performance could be related to the fact that biocatalysis was conducted by novozymes 435 which is a non-specific enzyme. This kind of catalysts compared to specific enzymes reduce the content of free fatty acids in the oil more rapidly due to its ability to attach a free fatty acid at any position on the glycerol[21,22]. Considering the economic status of the reaction and the deacidification yield, the enzyme load of 1% was determined.

Effect of oil/glycerol ratio: The results of acid value analysis of the fish oil samples esterified at low pressure 15-25 mbar with a ratio of 1% (W%) of oil/enzyme as shown in Fig. 4. The reaction mixture contains 300 g of crude oil (initial acidity 42%), 6 and 12 g of glycerol respectively biocatalyzed by 3 g of enzyme. Esterification was performed at 70°C and stirred at 250 rpm for 24 h.

Deacidification which was conducted with a ratio oil/glycerol 2% has the best performance after 24 h compared to the one conducted with an oil/glycerol ratio 4%. Acid value decreases rapidly during the first 6 h and slightly after. This can be explained by the consumption of glycerol and subsequent hydrolysis. In addition to this, there is probably an increase of viscosity induced by glycerol. The addition of glycerol with 2% increases the yield of this deacidification reaction by providing an optimal amount of additional condensation sites. The statistical test carried out by two-way ANOVA revealed that deacidification which was conducted with different ratio oil/glycerol was statistically less significant ($F = 8.48$, $p<0.05$) than the variation by time ($F = 30.44$, $p<0.05$).

This result is consistent with that of Noureddini and Harmeier[23], who has worked on the enzymatic glycerolysis of soybean oil by testing three molar ratios of oil/glycerol [1:1, 1:2, 1:3]. The glycerids formation increases with the molar ratio of [1:2] and slightly decreases beyond this point and tends to stabilize. Makasci *et al.*[18] which investigated the enzymatic de-acidification of a very acid olive oil, adding twice the amount of glycerol does not affect the efficiency of deacidification reaction. However, the ethanolic deacidification of highly acidic fish oils studied by Wang *et al.*[20] have required ratio oil to ethanol 1:1. While comparing performance of our enzymatic deacidification system with the substrate molar ratio [1:1] and [1:2] and considering the negative effect on the enzyme that may caused by excess glycerol, the molar ratio of fish oil to glycerol [1:1] was selected.

Effect of temperature: Figure 5 shows the results of acid value analysis of the fish oil samples esterified at low pressure 15-25 mbar with a ratio of 1% (W%) of oil/enzyme. The enzymatic deacidification in this study seems to be more effective at 70°C with a final yield of 91%. Decreasing oil acidity is much less effective at 65 and 60°C with a similar yield of 74%. Temperature is a key factor that has a direct influence on the reaction efficiency. A high temperature improves the movement of molecules and thus increases the chances of having a good collision between different reagents. It also improves the miscibility of the reaction medium which is quite complex. Increasing the temperature also decreases the viscosity of the medium.

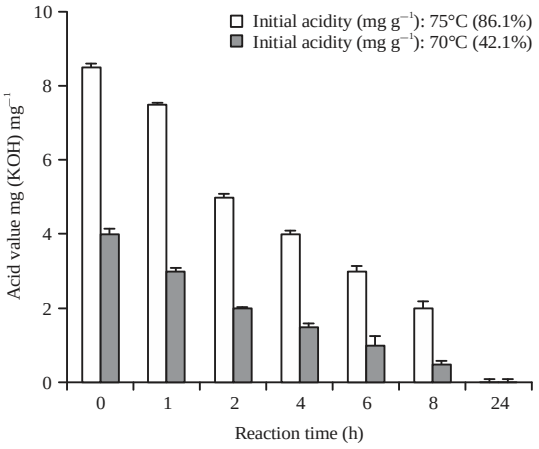

Fig. 6 : Effect of initial acidity on the performance of enzymatic deacidification of sardine oil
All the values were represented as Mean±SD

On the other hand, the temperature of 75°C increases the rate of deacidification; however, after 24 h of reaction, the final yield is slightly lower than that obtained with a temperature of 70°C. Indeed, the use of high temperatures has a negative impact on biocatalyst operational stability. The two-way ANOVA) revealed that the acid value due to different reaction time and due to various temperatures were statistically more significant ($F = 53.25$, $p<0.05$ and $F = 10.87$, $p<0.05$).

This result is similar to that of Makasci *et al.*[18], who have demonstrated that the enzymatic glycerolysis of a very acidic olive oil foresaw the best performance deacidification at 70°C. The same tendency was reported by Noureddini and Harmeier[23] following the formation of the glycerides after soybean oil glycerolysis based on the evaluation of the reaction products instead of substrate as in this case. In another study performed by Fadiloglu *et al.*[19] on free fatty acids reduction in the pomace olive oil olive, it was demonstrated that the free fatty acids decrease is important with the three temperature 40, 50 and 60°C but its importance is still significant for a temperature of 60°C. On similar substrates to our case, fish oil, Ravn *et al.*[10] showed that 70°C was the most efficient operating temperature which generates the most consistent synthesis of glycerides after 24 h of reaction.

Considering the economic factor of the reaction and taking into account the risk of enzyme instability, the temperature 70°C seems perfectly adequate to carry out enzymatic deacidification.

Validation of the enzymatic deacidification optimization: Figure 6 shows the acid value evolution of the fish oil samples esterified at low pressure 15-25 mbar with a ratio of 1% (W%) of oil/enzyme. The reaction mixture contains 300g of crude oil (initial acidity 42.1 and 86.1 %), 6g of glycerol biocatalyzed by 3 g of enzyme. Esterification was performed at 70°C. The mixture was stirred at 250 rpm for 24 h.

According to the obtained results (Fig. 6), the deacidification is practically conducted in the same way for both types of oils; the final deacidification yield of the first oil (42.1%) is 91% while that of the second is 90.5%. These are two very similar performances, no significant difference was found between deacidifications yields of those two kinds of oil ($p>0.05$). These results show that regardless of oil acidity, enzymatic deacidification conducted with the optimized parameters has excellent performance and does not limit in any way the reaction efficiency From an industrial point of view, this is a competitive an advantage to be taken into account. These results prove the performance of enzymatic deacidification system that was designed, developed and optimized.

CONCLUSION

This study makes an effort to reduce the acidity of crude sardine oil processed in Morocco which usually contains high content of free fatty acids by using enzymatic esterification with glycerol and investigate the effect of various parameters involved in the esterification reaction. The optimum conditions to perform the decrease of free fatty acids were found to be: A pressure of 15-25 mbar, a ratio oil/novozyme 435: 1%, a ratio Oil/Glycerol: 2%, a temperature of 70°C. Those findings generates knowledge of the reaction system especially with regard to operating factors necessary for any design of a large scale processes and allow a rational analysis of how yield controlling factors may be changed to obtain optimum yields. The acidity reduction of acid sardine oils by biorefining approach with immobilized lipase could be an attractive application for a more sustainable edible oil production by fish oil industry in Morocco using soda refining.

SIGNIFICANCE STATEMENTS

This study discovers the potential of enzymatic esterification of crude sardine oil processed in Morocco with regard to operating factors that can be beneficial for further design of large scale fish oil biorefining processes. This study will help the researcher to uncover the critical areas of deacidification process optimization that many researchers were not able to explore. Thus this alternative approach to reduce acidity oil may give yield that can compete favorably with the existing chemical processes.

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REFERENCES

1. Wiefels, R., 2014. Report on International Markets for Moroccan Sea Products. 3rd Edn., International Trade Center, Geneva, Switzerland.
2. Wanasundara, U.N. and F. Shahidi, 1999. Concentration of omega 3-polyunsaturated fatty acids of seal blubber oil by urea complexation: Optimization of reaction conditions. Food Chem., 65: 41-49.
3. Young, F.V.K., C. Poot., E. Biernoth, N. Krog, N.G.J. Davidson and F.D. Gunstone, 1994. Processing of Fats and Oils. In: The Lipid Handbook, Gunstone, F.D., J.L. Harwood and F.B. Padley (Eds.). 2nd Edn., Chapman and Hall, London, UK., ISBN: 9780412433207, pp: 249-276.
4. Gofferje, G., A. Stabler, T. Herfellner, U. Schweiggert-Weisz and E. Floter, 2014. Kinetics of enzymatic esterification of glycerol and free fatty acids in crude Jatropha oil by immobilized lipase from *Rhizomucor miehei*. J. Mol. Catal. B: Enzymatic, 107: 1-7.
5. Jegannathan, K.R. and P.H. Nielsen, 2013. Environmental assessment of enzyme use in industrial production-a literature review. J. Cleaner Prod., 42: 228-240.
6. Facioli, N.L. and A. Barrera-Arellano, 2001. Optimisation of enzymatic esterification of soybean oil deodoriser distillate. J. Sci. Food Agric., 81: 1193-1198.
7. Von der Haar, D., A. Stabler, R. Wichmann and U. Schweiggert-Weisz, 2015. Enzymatic esterification of free fatty acids in vegetable oils utilizing different immobilized lipases. Biotechnol. Lett., 37: 169-174.
8. Kasche, V., 1986. Mechanism and yields in enzyme catalysed equilibrium and kinetically controlled synthesis of β-lactam antibiotics, peptides and other condensation products. Enzyme Microbial Technol., 8: 4-16.
9. Kasche, V., U. Haufler and L. Riechmann, 1987. Equilibrium and kinetically controlled synthesis with enzymes: Semisynthesis of penicillins and peptides. Methods Enzymol., 136: 280-292.
10. Ravn, H.C., M.L. Damstrup and A.S. Meyer, 2012. Optimization of reaction parameters for enzymatic glyceride synthesis from fish oil: Ethyl esters versus free fatty acids. Biocatal. Agric. Biotechnol., 1: 273-279.
11. Medina, A.R., L.E. Cerdan, A.G. Gimenez, B.C. Paez, M.J.I. Gonzalez and E.M. Grima, 1999. Lipase-catalyzed esterification of glycerol and polyunsaturated fatty acids from fish and microalgae oils. J. Biotechnol., 70: 379-391.
12. Dos Santos, J.C.S., O. Barbosa, C. Ortiz, A. Berenguer Murcia, R.C. Rodrigues and R. Fernandez Lafuente, 2015. Importance of the support properties for immobilization or purification of enzymes. ChemCatChem., 7: 2413-2432.
13. Ma, X., D. Wang, M. Yin, J. Lucente and W. Wang *et al*., 2017. Characteristics of pectinase treated with ultrasound both during and after the immobilization process. Ultrasonics Sonochem., 36: 1-10.
14. Cowan, D., 2010. Concentration of omega-3 fatty acids using enzymes. Agro Food Ind. Hi Tech., 21: 30-33.
15. Rubio-Rodriguez, N., S. Beltran, I. Jaime, S.M. de Diego, M.T. Sanz and J.R. Carballido, 2010. Production of omega-3 polyunsaturated fatty acid concentrates: A review. Innov. Food Sci. Emerging Technol., 11: 1-12.
16. Baeza-Jimenez, R., K. Miranda, H.S. Garcia and C. Otero, 2013. Lipase-catalyzed glycerolysis of fish oil to obtain diacylglycerols. Grasas Aceites, 64: 237-242.
17. Zar, J.H., 1974. Biostatistical Analysis. 1st Edn., Prentice Hall, New Jersey, pp: 620.
18. Makasci, A., K. Arisoy and A. Telefoncu, 1996. Deacidification of high acid olive oil by immobilized lipase. Turk. J. Chem., 20: 258-264.
19. Fadiloglu, S., O.N. Ciftci and F. Gogus, 2003. Reduction of free fatty acid content of olive-pomace oil by enzymatic glycerolysis. Food Sci. Technol. Int., 9: 11-15.
20. Wang, W., T. Li, Z. Ning, Y. Wang, B. Yang, Y. Ma and X. Yang, 2012. A process for the synthesis of PUFA-enriched triglycerides from high-acid crude fish oil. J. Food Eng., 109: 366-371.
21. Misra, G. and S. Nandi, 2014. Enzymatic deacidification of rice bran oil containing high free fatty acids with recycling. Chem. Sci. Rev. Lett., 2: 376-381.
22. Cowan, D., H.C. Holm and H.S. Yee, 2012. Reduction in free fatty acids in crude palm oil by enzymatic remediation. J. Oil Palm Res., 24: 1492-1496.
23. Noureddini, H. and S.E. Harmeier, 1998. Enzymatic glycerolysis of soybean oil. J. Am. Oil Chem. Soc., 75: 1359-1365.