

LIPASE CATALYSED INTERESTERIFICATION FOR THE PRODUCTION OF OLEOCHEMICALS FROM NON TRADITIONAL OILS

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ABSTRACT

Non-traditional oils which are not used for edible purpose are to be converted into value added products. Thus interesterification of such oils catalyzed by lipase to produce oleo chemicals is carried out in present work. Conventional chemical method of interesterification has got several disadvantages which can be overcome by proposed work using enzyme catalyst. Enzyme catalyzed process is eco-friendly and less expensive. Besides these, it has one more advantage of selective action of enzyme to produce desired product. Here, interesterification is carried out by using L-asparagine monohydrate lipase as a catalyst on nontraditional oils like Neem, Karanja and Rice bran oil to produce oleo chemicals having entirely different properties.

Keywords: Lipase, Interesterification, Oleo chemicals, Non-traditional oils.

INTRODUCTION

Intesterification has been the method most commonly employed for modification of oils and fats¹. Interesterified fats having improved physicochemical properties can be obtained by varying the degree of unsaturation of the native FA residues. Recently, enzymatic interesterification between a fat and oil has been considered as an alternative method for improving the physical properties of fats. The enzymatic interesterification reactions between a solid fat and a liquid oil permit one to produce semi-solid fats that contain no trans FA residues²⁻³. Enzymatic processes based on the use of either sn-1, 3-specific or nonspecific lipases are advantageous because of their selectivity (generation of fewer undesired byproducts), mild reaction conditions, and ease of product recovery⁴.

Intesterification reactions between a fat and an oil involve exchange of FA residues between the precursor acylglycerols and are accompanied by a concomitant change in the properties of the original material. The properties of the product are in large measure governed by the convenient choice of the precursor reagents, their respective proportions in the starting mixture, and the time that the reactants are in contact with the biocatalyst⁵.

Most vegetable oils in their native state have only limited applications due to their specific chemical compositions. To widen their use, vegetable oils are modified chemically by hydrogenation or interesterification⁶. Interesterification is one of the most important processes for modifying the physicochemical characteristics of oils and fats⁷. During interesterification, fatty acids (FAs) are exchanged within and among triacylglycerols (TAGs) until a thermodynamic equilibrium is reached⁸. Interesterification of vegetable oils can produce a fat blend with optimum characteristics. Rearrangement or randomization of acyl residues in TAGs has provided fats or oils with new physical properties⁹. In most oils and fats, unsaturated FAs preferentially occupy the 2-positions of the TAG molecules. The degree of unsaturation or isomeric state of the FA does not change¹⁰.

Microbial lipases are used as catalyst for enzymatic interesterification of oils and fats. The natural function of lipase is to catalyze the hydrolysis of the acylglycerols & other fatty acid esters. But these

reactions are easily reversible and consequently the enzymes are also effective catalyst for various interesterification reactions¹¹. Lipase catalyzed interesterification of mixtures of triglycerols or triacylglycerols plus fatty acids can be used to modify the oils & fats.

The immobilized lipases are used for their easier separation, recovery and reuse¹². But these reactions are easily reversible and consequently the enzymes are also effective catalyst for various interesterification reactions. By using the selective lipases for high value specialty oils and fats, a good commercial process for production of the oleo chemicals.

EXPERIMENTAL

Materials

Enzyme: L-aspergine monohydrate lipase from species of *Aspergillus niger* was purchased from Himedia Laboratories, Mumbai.

Non Traditional oils: The Neem oil was purchased from Viprama Ind., Malakapur, Karanja from Nagpur, Rice bran oil from Bhandara.

Celite and other reagents: Celite 545 LR, Buffer Solution having pH 6.7 are used to maintain the pH of the reaction. All the chemicals used were of AR grade.

Immobilization of lipase

Immobilization of lipase was done by taking 50 gm of neem oil, 5 gm of Celite, 0.5 gm ion exchanged water and 0.1 gm of L-aspergine monohydrate lipase which were stirred together at 27 °C in closed vessel for 12 hrs, to carry out reaction i.e. immobilization. After completion of reaction, an insoluble matter was separated by filtration. It was washed with 5 ml n-Hexane three times for complete removal of oil. The product was then dried at 20-30°C for 1 hr.

Interesterification

Known quantities of oil blends were heated under vacuum to remove moisture and air. The blends were mixed in a conical flask with 1.25 % (w/w) of immobilized lipase, 2.5 % of Celite and 0.35 % of pH 6.7 buffer solution. This flask was kept in incubator shaker at 40°C temperature, at 160 rpm for 72 hrs, with constant agitation. After 72 hrs the flask were removed from incubator shaker. The reaction mixture was then separated with the help of high speed centrifuge machine, in which two layers were obtained. The precipitated layer was washed with 80% volume of petroleum ether. These washings were added to the oil layer. The petroleum ether was recovered by distillation. Vacuum evaporator was used to remove the moisture content to get the final product is after drying.

Analysis of oils and interesterified products

Neem, Karanja and Rice bran oil which were analyzed before and after Interesterification as per IS: 548 (Part I)-1964.

Gas chromatography

The esterified oil samples were analyzed before and after esterification on Gas Chromatograph using Flame Ionization Detector. The packed column (SS OV-210) is used. Nitrogen is used as the carrier gas at constant flow rate of 25 ml/min. The column oven temp. is programmed from 150-250°C with injector and detector temperatures at 170°C and 230°C respectively.

Thin layer chromatography

The formation of mono, diglycerides and triglycerides of non traditional oils in the reaction mixture was also analyzed by thin layer chromatography with silica gel- G. 15 µL of interesterified sample is dissolved in 1ml hexane and 15 µL of the prepared sample is spotted on TLC plate treated with Petroleum ether: Diethyl ether: Acetic acid (70:30:1) mobile phase. Lipid profiles on the TLC plate are examined for the presence of neutral lipids comprising TAG, DAG, MAG. The spots were detected in the iodine chamber and distinct separation is observed on the plate.

RESULTS AND DISCUSSION

The fatty acid composition of Rice bran oil, Neem Oil and Karanja oil and their blends were examined before and after interesterification as depicted in Table-1. Karanja oil contains comparatively high content of saturated fatty acids (SFA,40.69) and Rice bran oil contains high amount of unsaturated fatty acids(USFA,83.14) in which the major fatty acid are oleic(C18:1,,42.50) and linoleic (C18:2,39.61).After

interesterification by lipase catalyst, the fatty acid composition was changed, based on different blending ratios. This has also resulted in an increase in melting point.

Table-1: Fatty acid composition (wt %) of nontraditional oils and interesterified fats

Fatty Acids	RBO	Neem oil	Karanja oil	Intesterified fats			
				RBO and Karanja (75:25)	RBO and Neem (25:75)	RBO and Neem (50:50)	Neem and Karanja (50:50)
C 16:0	15.20	19.34	19.42	16.25	18.30	17.27	19.38
C 18:0	1.66	12.82	21.27	6.57	10.02	7.24	17.04
C 18:1	42.50	52.15	42.83	42.57	49.73	47.32	47.48
C 18:2	39.61	15.17	15.49	33.57	21.27	27.38	15.32
C 18:3	1.03	0.52	1.00	1.02	0.64	0.77	0.76

Table-2 shows the physical and chemical characteristics, before and after interesterification of nontraditional oils by using L-aspergine monohydrate as a lipase. The figures in brackets depict the values before interesterification of oils. The main purpose of this analysis is to study the changes in various properties, before and after the interesterification of reaction. The acid values of these batches after interesterification of oils are lower as compared to the values before interesterification. The acid value is going down which is an indication of conversion of a few fatty acids. The saponification value of these batches decreases in batches Ist to IVth. Iodine value is a valuable characteristic in oil and fat analysis, which measures the degree of unsaturation. The Iodine value of interesterified blends is slightly decreasing because of the proportion of hard stocks of blends. .

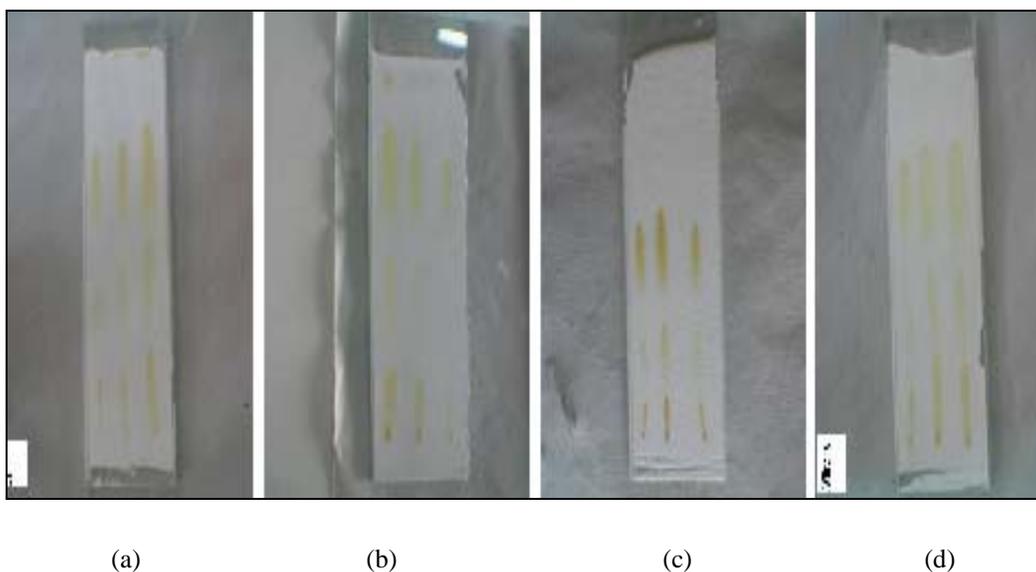
Table-2: Physical and Chemical characteristics of nontraditional oils before & after interesterification by using L-aspergine monohydrate lipase

Sr. No & Batches(w/w)	Acid Value	Sap Value	Iodine Value	Ester Value	R. I.	SMP (°C)	Peroxide Value
I RBO and Karanja (75:25)	20.58 (23.17)	182.69 (188.75)	98.26 (101.75)	162.11 (165.58)	1.3828 (1.3660)	16 (12)	10.51 (24.81)
II RBO and Neem oil(25:75)	15.45 (20.81)	179.89 (186.25)	83.89 (88.5)	164.44 (165.44)	1.3825 (1.3664)	15 (12)	11.24 (34.35)
III RBO and Neem oil(50:50)	24.45 (27.89)	183.65 (187.35)	82.76 (89.54)	159.20 (159.46)	1.3830 (1.3675)	14 (11)	3.03 (17.05)
IV Neem and Karanja oil(50:50)	8.47 (11)	170.89 (185.11)	85.14 (87.29)	162.42 (174.11)	1.3720 (1.3725)	18 (14)	12.13 (30.32)

*Figures in bracket indicate the values before interesterification of nontraditional oils

The refractive index for all batches remains almost same before and after interesterification. There is no significant change. The peroxide value decreases after interesterification of oil in all these batches, confirming that no unwanted side reactions are taking place. The melting point of these batches is increasing after the reaction which means that the triglyceride composition is changing after the interesterification. For rice bran oil and karanja oil (75: 25) and For neem oil and karanja oil (50:50) there is a notable increase in the slip melting point which depicts that the triglyceride composition of these fat blends are changing.

The TLC profiles of various interesterified samples are shown in Fig.-1. All the four batches, exhibited formation of distinct spot of ester, confirming the interesterification of oil. The presence of monoglycerides and diglycerides is also indicated by distinct spots. The profile and retention front separation of various neutral lipids component such as Triacyl glyceride (TAG), Diacyl glyceride(DAG), and Mono acyl glycerides (MAG) profiles are deduced by comparing with the standards. The lipid components are separated based on relative front, polarity and hydrophobicity. The significance of the presence of DAG is monitored in all TLC sheets and the thickest band represents strong presence of DAG band.



(a)Batch 1, RBO and Karanja oil
(c)Batch-3, RBO and Neem oil

(b) Batch- 2 , RBO and Neem oil
(d) Batch-4, Neem and Karanja oil

Fig.1: TLC Analysis after interesterification reaction of nontraditional oils by using L-aspergine monohydrate lipase

CONCLUSION

Enzymatic interesterification of nontraditional oils and fats provides a safe, easy and cost-efficient alternative to chemical interesterification of oil and fats. The process gives a more natural product, free of trans fatty acids. The process can easily be implemented in existing factories for continuous operation and application in various products. Since the operating conditions are mild, the process development presented is an ecofriendly approach to the utilization of relatively low value oils. After interesterification reaction their intrinsic properties will add value to the products.

The slip melting point show major changes after interesterification, in all the four batches. This significant increase means the interesterification of oils has resulted in a change in triglyceride composition.

The TLC analysis further confirms that optimum reaction conditions were established for lipase-catalyzed interesterification of oils. The experimentally explored route thus could be expected to result in reduction in capital investment in production of oleochemicals as compared to chemical interesterification of oils.

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Erratum

A typographical error in the name of Affiliation of the corresponding author has been published in the research paper entitled, “**Synthesis of Novel Thiazolo-.....Agents**”, **RJC, Vol.2, No.3, 753-759 (2009) by T. Panneer Selvam et al.**

Readers, please read the Affiliation of P.Selvam (Corresponding author) as D.C.R.M. Pharmacy College, Inkollu (A.P.) India in place of D.C.R.M. Charmacy College, Inkollu (A.P.) India.
Thank You.

-Editor