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Ultrasound assisted enzymatic conversion of non edible oil to methyl esters

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ABSTRACT

Conventional and ultrasound-assisted hydrolysis and subsequent esterification of Nagchampa oil under mild operating conditions have been investigated with an objective of intensification of methyl esters production using a sustainable approach. The effect of ratio of reactants, temperature, enzyme loading, pretreatment of enzyme (using ultrasonic irradiations) on the hydrolysis and esterification reaction has been studied. Optimum conditions for hydrolysis were observed to be 1:1 weight ratio of oil: water for Lip Z and 1:3 for Lip 2 enzymes, enzyme loading of 400 units for Lip Z and 800 mg for Lip 2 enzymes and reaction time of 6 h. In the case of esterification reaction, optimum conditions obtained were oil to methanol molar ratio of 1:2, enzyme loading of 1000 mg and reaction time of 20 h. Use of pretreated enzyme (using ultrasonic irradiations) was found to increase the extent of esterification reaction from 75% to 92.5%. It was observed that use of ultrasound in the reaction significantly intensified the esterification reaction with time requirement reducing from 20 h for conventional stirring based approach to only about 7.5 h in the presence of ultrasound. The extent of esterification obtained with sonicated enzyme also increased to 96% from 75% with unsonicated enzyme.

1. Introduction

With increasing demand of fragrances in cosmetic industry, it has become a necessity to design and develop fragrance chemicals which originate from sustainable sources. Some of the suggested sources include renewable liquids coming from biological raw material which can prove to be good substitutes in the cosmetics sector. Nagchampa oil is an essential oil used in fragrance industry, but owing to the high acid value, it has limited stability. The stability of such essential oils can be significantly improved by converting it to its methyl ester. Methyl ester can be produced from oils through stepwise hydrolysis-esterification reactions or transesterification reactions. A large variety of plants that produce non-edible oils can be considered for methyl esters production [1]. Non-edible oils from sources such as neem, mahua, karanja, babassu, *Jatropha*, microalgae, *Camelina sativa*, Nagchampa etc. are easily available in many parts of the world, and are very cheap compared to edible oils. Also, for growing non-edible oil crops, less fertilizer, herbicides and insecticides are required compared to edible oil crops [2]. Methyl ester production can be carried out with acid catalyst or by using biocatalysts. The main advantages of employment of lipases as catalyst for methyl ester production are mild reaction conditions and easy recovery of glycerol without purification. Additionally, free fatty acid content in the oil can be entirely converted to methyl esters with no soap formation, thereby increasing the methyl ester yields [3]. These features of the lipase enzymes allows the direct usage of materials with high free fatty acids (FFA) or high water content such as non-edible oils, waste cooking oils and industrial waste oil for methyl ester synthesis. Lipase enzyme has been widely used for hydrolysis of fats [4], esterification and transesterification [5]. In spite of numerous advantages, enzymatic processes have drawbacks such as low reaction rate, high enzyme cost in comparison to acid and alkali catalyst and low enzyme stability in the presence of excess methanol [6,7].

Besides the enzyme related problems, process of production of methyl esters faces various problems related to the immiscible nature of the reactants causing poor mass transfer rate. This problem is responsible for longer reaction time and low reaction rate leading to an energy intensive process. Ultrasound can eliminate the problem of poor immiscibility between reactants as ultrasonic energy can emulsify the reactants offering much higher interfacial area for reaction also possibly reducing the catalyst requirement, reaction time and reaction temperature. Ultrasound action in methyl ester production is primarily based on the emulsification of the immiscible liquid reactants by microturbulence generated by cavitation bubbles [8]. Gole and Gogate [9] performed experiments on methyl ester formation from non-edible Nagchampa oil and found that temperature and reaction time required for esterification, as well as the transesterification stages, are substantially

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lower in the case of sonochemical reactors, compared to the conventional heating method. Liu et al. [10] used ultrasound cavitation for lipase catalyzed hydrolysis and reported that around 2-fold intensification was obtained. The present work reports the ultrasound assisted synthesis of methyl esters from high-acid-value Nagchampa oil using the two-stage approach of hydrolysis followed by esterification. The objective of first stage was to achieve hydrolysis to considerable extent in order to get acids in sufficient quantity for esterification to methyl esters in the next step of esterification. The effect of different operating parameters such as reaction temperature, ratio of reactants, catalyst concentration and sonication of enzyme has been investigated. Two types of approaches for ultrasound based synthesis were followed in the work viz. enzyme was initially treated with ultrasound and then used in the reaction whereas in the second approach ultrasound was used throughout the reaction period.

2. Materials and methods

2.1. Materials

Candida antarctica Lipase Enzyme standard was received as a gift sample from Novozyme, Denmark. Tributyrin, sodium dihydrogen phosphate and disodium hydrogen phosphate were procured from Himedia laboratories, Mumbai. Ultrasonic horn used in the experiments was procured from Dakshin, Mumbai having variable power output up to 220 W, and frequency of 20 kHz.

The raw Nagchampa oil used for hydrolysis was procured from M/s Sanjay Shirsat Oil Mill (Vengurla, Dist: Sindhudurg, Maharashtra, India). Oil was initially filtered to remove traces of particles and mud. Table 1 gives the typical composition of oil consisting of 25% saturated acid (stearic and palmitic) and 72.7% unsaturated acid (oleic, linoleic, and linolenic). The initial saponification value of the oil was found to be 156 mg KOH/g of oil. Methanol, potassium hydroxide and oxalic acid (required for standardization and titration) were procured from S.D. Fine chemicals Pvt. Ltd., Mumbai. Distilled water was used as a reactant for hydrolysis and was prepared from the laboratory scale distillation unit. All the chemicals were used as received from the supplier, unless otherwise specified.

2.2. Experimental procedure

2.2.1. Sonication of enzyme

Sonication was done in direct mode with 100 ml volume of 3 mg/ml enzyme solution subjected to ultrasonic irradiations using a ultrasound probe. The ultrasound probe was dipped in solution to 0.5 cm height and initial parameters were chosen as duty cycle of 66% and frequency of 20 kHz and power of 50 W. The setup used for enzyme sonication studies has been shown in Fig. 1.

2.2.2. Hydrolysis

The hydrolysis reaction was carried out using lipase from two different sources namely *Candida rugosa* and *C. antarctica*. The objective was to achieve hydrolysis to considerable extent so as to get acids in sufficient quantity for esterification to methyl esters in the next step. During experiments four important factors were considered and optimized in order to get maximum hydrolysis. The effect of ratio of oil to water, time of hydrolysis, temperature of hydrolysis and enzyme loading has been investigated. Initially reaction time was optimized by taking readings every one hour interval up to 8 h and acid values of the samples were analysed using titration. The temperature was optimized by checking the acid value at different temperatures in the range of 25–55 °C for optimized reaction time. Effect of reactant ratio was then investigated over the range 1:1–1:4 at optimized time and temperature. Catalyst concentration was optimized between 100 and 500 activity units for both *C. rugosa* (Lip Z) and *C. antarctica* (CALB Lip 2) lipase. All the reactions were carried out for 30 ml volume. After optimization of the entire range of parameters, final run was done to get maximum hydrolysis at larger scale of 200 ml to check the scale up aspects.

Table 1
Fatty acid composition of Nagchampa oil.

Fatty acid	Composition (%)
Palmitic, C16:0	12
Stearic, C18:0	13
Oleic, C18:1	34.1
Linoleic, C18:2	38.3
Linolenic, C18:3	0.3

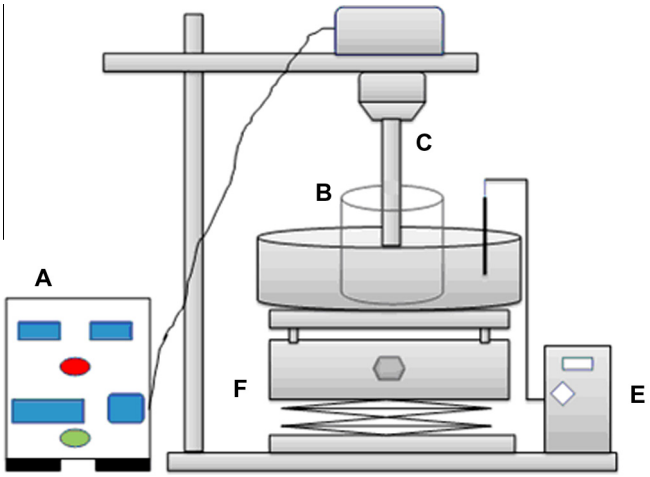

Fig. 1. Experimental setup for enzyme sonication A: Generator, B: Beaker introduced in ice bath, C: Ultrasound Probe, E: Temperature Indicator, F: Stand.

2.2.3. Esterification

Next step of methyl ester synthesis after hydrolysis is esterification. This step converts free fatty acids into methyl esters using methanol. In order to prevent the progress of reaction in reverse direction i.e. hydrolysis which results in lowering the ester yield, molecular sieves were added in the reaction mixture in order to remove the water produced in the reaction. Parameters optimized were same as described for the hydrolysis reaction. First the reaction time was optimized by taking samples after every 4 h for a total reaction time as 28 h. The samples were analyzed to get the optimum time for minimum acid value which signifies maximum methyl ester yield. The molar ratio of oil to methanol was also optimized. This is a critical step in the process as excess methanol kills the enzyme and less methanol being a limiting reactant does not drive the reaction in the forward direction in an efficient way. Molar ratio was optimized over the ratio of 1:1–1:4. Finally the catalyst loading was also optimized. As the enzyme (CALB Lip 2) used for this step was an immobilized enzyme, the loading was taken in terms of weight of dried immobilized beads. The total volume of the mixture was made to 40 ml in each experiment using hexane. All the samples were analyzed for the determination of acid value.

2.2.4. Hydrolysis and esterification with sonication

Hydrolysis and esterification both were done with sonication at 50 W with same reaction mixture in order to check the effect of