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Vegetable Oils as a Source of Wax Ester: Extraction and Transesterification

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Abstract

Waxes are long chain esters that are derived from fatty acids and alcohols with chain lengths of 12 carbons or more. Synthesis of wax esters by chemical-catalyzed method has many disadvantages, such as corrosive acids required, hazards in handling, high energy consumption and degradation of synthesized esters. Hence, attention has been diverted to synthesis of wax esters from natural products which are reported to be environment friendly. Natural wax ester can be extracted from animals and plant materials such as beeswax, sperm whale, jojoba seeds, sheep wool, seafowl feathers etc. The present study was undertaken to examine the oil content in naturally available plant seeds, i.e. Annona reticulate, Parkia timoriana and Citrus reticulata and synthesize wax esters through transesterification reaction.

Keywords: Wax esters, vegetable oil seeds, Transesterification.

Introduction

Waxes is a general term used to refer to the mixture of long-chain apolar lipids forming a protective coating (cutin in the cuticle) on plant leaves, fruits and also in animals (wax of honeybee, cuticular lipids of insects, spermaceti of the sperm whale, skin lipids, uropygial glands of birds, depot fat of planktonic crustacea), algae, fungi and bacteria. Some waxes are of mineral origin. Wax usually refers to a substance which is solid at ambient temperature and, on being subjected to slightly higher temperatures, becomes a low viscosity liquid. These are long chain esters that are derived from fatty acids and alcohols with chain lengths of 12 carbons or more[1]. Fatty acid profiles, its composition and physico-chemical properties from *Citrullus vulgaris* seed oils that have been evaluated by Garba and his co-workers[2]. Seed oils from *Lophira lanceolata* and *Carapa procera* have been utilized in the analysis of fatty acids, sterols, tocopherols and trocotrienols[3]. A lot of works have been done in the production of biodiesel from seed oils in order to preserve the day by day diminishing fossil fuels. However, no specific efforts have been made towards the production of synthetic wax ester from naturally available seed oils.

Natural wax ester can be extracted from animals and plant materials such as beeswax, sperm whale, jojoba seeds, sheep wool, seafowl feathers etc. Out of these, spermaceti oil and jojoba oil are of great industrial importance but sperm whale is listed as an endangered species in 1970. Jojoba oil is similar to spermaceti oil in some properties and can be used as a substitute, but the growth of jojoba plants is restricted to desert environment and its production cost is quite high. As a result, the natural unsaturated wax esters are too scarce and expensive for commercial use.

Keeping in view of the aforesaid aspects, synthesis of synthetic wax esters is in demand with the primary goal to use them as a substitute for natural ones. Chemical-catalyzed method has many disadvantages, such as corrosive acids required, hazards in handling, high energy consumption and degradation of synthesized esters. Lipase-catalyzed processes have attracted attention because of their mild reaction condition and eco-friendly nature[4,5]. Apart from lipase, Lipozyme RMIM and Novozym 435 were also used in the synthesis of wax ester[6].

Plant oils are widely available and renewable sources for the production of wax ester. Apart from the production of wax esters, plant oils (Jatropha oil)[7] were widely used by many research workers for the synthesis of medicinal soaps. Further, attempts were also made to improve the quality of traditional soaps by analyzing the ashes obtained from vegetables[8]. As far as the environmental considerations are concerned, unlike hydrocarbon-based fuels, the sulphur content of vegetable oils is close to zero and hence, the environmental damage caused by sulphuric acid is reduced. Keeping this in view, research works were carried out for producing hydrocarbon liquids from plant oils (Castor oil)[9]. Moreover, plant oils take away more carbon dioxide from the atmosphere during their production than is added to it by their later combustion. Therefore, it alleviates the increasing carbon dioxide content of the atmosphere.

In terms of chemical composition, plant oils are basically triglycerides which are esters of three fatty acids and one glycerol. The fatty acids vary in their carbon chain length and in the number of double bonds. Commonly found fatty acids in vegetable oils are stearic, palmitic, oleic, linoleic and linolenic. However, plant oils can't be directly used; certain modifications have to be done through chemical reactions. In this context, the use of plant oil, for the production of wax ester- a necessary component in industrial sectors is concerned, proving to be

viable alternatives for conventional sources.

Chemical interpretation of wax ester production: Transesterification reaction: Transesterification is the general term used to describe the important class of organic reactions where an ester is transformed into another through interchange of the alkoxy moiety (Scheme-1). In the production of biodiesel, oils and fats are transesterified with methanol in presence of acid, base or enzyme catalyst to afford fatty acid methyl esters and glycerol as side product. In case of wax ester, long chain alcohol with chain length of 12 carbons or more are used instead of methanol (Scheme-2).

Transesterification is a reversible reaction which requires 1 mol of a triglyceride and 3 mol of the alcohol. An excess amount of alcohol is used so as to increase the yields of the alkyl esters and to allow its phase separation from the glycerol formed. In fact, transesterification consists of a number of consecutive and reversible reactions. The first step is the conversion of triglycerides to diglycerides, followed by the conversion of diglycerides to monoglycerides, and of monoglycerides to glycerol, yielding one alkyl ester molecule per mole of glyceride at each step (Scheme-3).

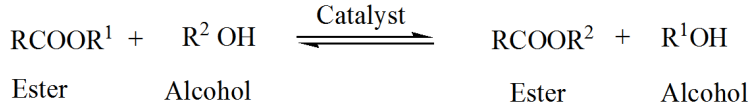
The present work was undertaken to examine the oil content in naturally available plant seeds, i.e. *Annona reticulata, Parkia timoriana and Citrus reticulata* and synthesize wax esters through transesterification reaction.

Materials and Methods

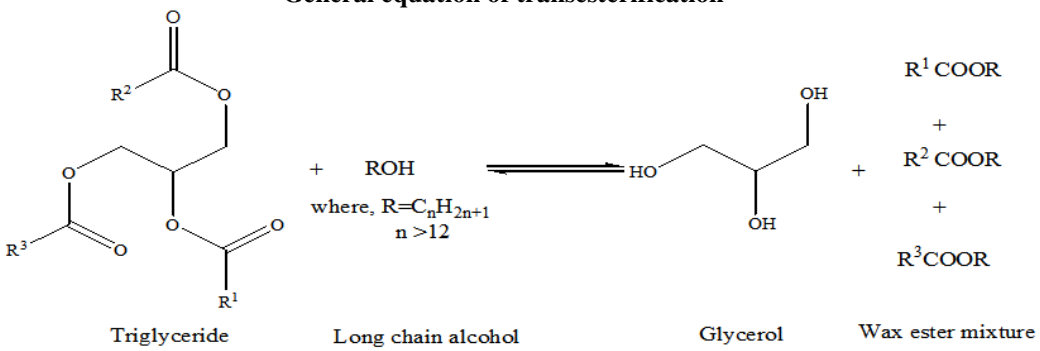
The seeds under investigation were crushed using a grinder. To extract oil and to carry out the reaction, a magnetic stirrer was used. For evaporation of solvents, Buchi rotavapour R-200 was used. NMR spectrometer: Bruker 300 MHz- the ^{1}H NMR analysis in $CDCl_3$ of the product is performed with 300 MHz and that of ^{13}C analysis in $CDCl_3$ with 600 MHz NMR-spectrometer. Further, IR-Spectrometer, Perkin-Elmer FT-IR was used for recording IR spectra.

A total of three seeds were collected for extraction of oil from the north-eastern region of India. Two were collected from Nagaon and Guwahati, located in Assam. These include Annona reticulata and Citrus reticulata. The remaining one was Parkia timoriana, a variety of seed from Manipur. Cetylalcohol, purchased from Himedia, was used for transesterification. The catalyst used for this purpose was ash from trunk of *Musa balbisiana*.

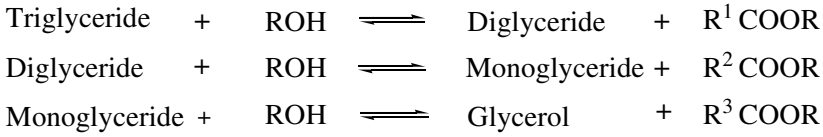
Preparation of catalyst: The trunk of the banana plant was sliced into thin pieces and dried under sun for several days. Dry material was ignited, and allowed to burn and cool down to ambient temperature in its own. Time required for burning and natural cooling down process depends on the quantity of material taken. Generally, burning is expected to complete within half an hour, but cooling down process takes time. The catalyst acts as a heterogeneous manner and is the means of value addition to the post-harvest plant.



Scheme-1
General equation of transesterification



Scheme-2
Transesterification of a trigyceride to give wax ester



Scheme-3

Transesterification reaction of vegetable oil with alcohol to esters and glycerol

Composition of the catalyst: The chemical composition of the catalyst was estimated by chemical analysis, atomic absorption spectroscopy and flame photometry[10]. Major components present are K^+, Na^+, CO_3^{2-} and Cl^- along with eleven other metals viz. Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb which are present only in trace amounts (ppm level). Metals are present as their carbonates, chlorides or oxides. Finely divided carbon particles are also present[11]. (Figure-1).

General Procedure for Extraction of oil: The procedure followed for oil extraction was the solvent extraction process. First, the seeds were sun-dried and then crushed with a grinder. These crushed seeds were stirred magnetically using magnetic stirrer by mixing with petroleum ether (40-60^{o}C, 10 mL/g) at room temperature for about 4 hours. The solid residue was removed by filtration with Buchner funnel and the solvent from the filtrate was removed using a rotary vacuum evaporator to yield the crude oil. The process was repeated again with the seed cake using fresh solvent in order to extract most of the oil. Finally, the extracted oil was purified by column chromatography over silica gel (60-120 mesh) using a mixture of light petrol (40-60^{o}C) and ethyl acetate (2%) as the eluent.

Experimental details: Seed no. 1: *Annona reticulate,* **Local name**: Seetaphal, Weight of seeds taken= 10 g, Oil content after column = 28 %. **Seed no. 2***: Parkia timoriana,* **Local name**: Yougchak, Weight of seeds taken= 12 g, Oil content after column = 22 %. **Seed no. 3**: *Citrus reticulata*, **Local name**: Mandarin orange, Weight of seeds taken= 10g, Oil content after column = 22%.

Results and Discussion

In search of a suitable non-conventional source of oil, three different seeds shown in Table-1 were investigated. These seeds were collected mainly from the north-eastern region of India.

Table-1
Name of the plants investigated along with oil content

Common name	Scientific name	Oil content before purification (%)	Oil content after purification (%)
Seetaphal	Annona reticulate	33%	28%
Yougchak	Parkia timoriana	35%	22%
Mandarin orange	Citrus reticulate	30%	22%

Transesterification of oils to long chain wax esters: Optimising the synthesis of wax ester with soyabean oil under various reaction condition: With different solvent: Weight of soya oil taken = 872.33 mg (1 mmol equivalent), Weight of cetyl alcohol = 726 mg (3 mmol equivalent), Weight of catalyst = 175 mg (20% of oil). Thus, the ratio of oil to cetyl alcohol was maintained in 1:3 ratio under all reaction conditions.

Figure-1
Preparation of catalyst

Table-2
Optimising the synthesis of wax ester with soyabean oil under various reaction condition with different solvent

Reactant A	Reactant B	Solvent	Temperature	Time	Yield
Soyabean oil	Cetyl alcohol	Petroleum ether	110^{0}C	3.5hours	95%
Soyabean oil	Cetyl alcohol	Acetone	110^{0}C	5.5 hours	96%
Soyabean oil	Cetyl alcohol	Chloroform	110^{0}C	5hours	95%
Soyabean oil	Cetyl alcohol	Toluene	110^{0}C	4 hours	94%
Soyabean oil	Cetyl alcohol	Tetrahydrofuran	110^{0}C	4.5hours	96%
Soyabean oil	Cetyl alcohol	Solvent-free	110^{0}C	7hours	96%

Soyabean oil	Cetyl alcohol	Acetone	Room temperature	24hours	5%

Thus it was found that the best optimised condition for the synthesis of wax ester is oil/alcohol ratio (1:3), catalyst (20% of oil), reaction temperature 110^0C with petroleum ether as solvent.

Wax esters were synthesized using cetyl alcohol in presence acetone as solvent using the catalyst derived from the trunk of *Musa balbisiana* at temperatre 110^oC.

Table-3
Transesterification with cetyl alcohol

Name of plant	Reaction time	Yield of wax ester before purification (%)	Yield of wax ester after Purification (%)
Annona reticulata	3	98	95
Parkia timoriana	3.5	97	94
Citrus reticulata	3	98	96

Conclusion

In the present investigation, oil contents available in seeds collected from different regions of North East India were evaluated and analyzed following proper available laboratory methodology. All the three plant seeds, evaluated in the investigation viz., *Annona reticulata, Parkia timoriana* and *Citrus reticulata* has been found to contain a good amount of oil content (28%, 22% and 22% respectively). Most importantly the best optimized condition for wax ester preparation has been evaluated and thereby wax esters were synthesized with the oil content of the seeds under investigation. Further, from the spectral analysis of oil and wax ester, the characteristic peak were identified.

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