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Proceeding Paper

Enzymatic Synthesis of Phenylethyl Fatty Esters from Fixed Oil Extracted from *Syagrus coronata* (Mart.) Becc. †

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Abstract

Fatty esters have wide industrial importance due to their aroma and versatile applications. Enzymatic esterification emerges as a sustainable method, offering high selectivity, fewer byproducts, and mild conditions, which align with the principles of green chemistry. This study focused on synthesizing phenylethyl esters from licuri oil (*Syagrus coronata*), 2-phenylethanol, and *Pseudomonas* lipase. The oil composition was characterized by GC-MS, while GC-FID and FTIR confirmed the conversion of the esters formed. The disappearance of the hydroxyl band (3327 cm^{-1}) indicated alcohol consumption, and the carbonyl shift (1743 to 1736 cm^{-1}) revealed new esters. Additional signals at 1240–1150 cm^{-1} and 1607 cm^{-1} confirmed the presence of ester groups and the aromatic ring, demonstrating the viability of this enzymatic route.

Keywords: enzymatic esterification; *Syagrus coronata*; 2-phenylethanol

1. Introduction

Fatty esters represent one of the most important classes of organic compounds due to their industrial applicability, their unique properties, and characteristic aroma. Therefore, the development of methodologies for ester synthesis has gained momentum. In this context, one of the routes for obtaining esters is the reaction between a carboxylic acid and an alcohol, with the elimination of water and in the presence of a catalyst, called esterification [1–5].

In this scenario, biotechnological processes emerge as an excellent alternative for the fatty acid esterification process due to their high catalytic efficiency, mild operating conditions, selectivity, and the synthesis of products classified as natural. Thus, enzyme-catalyzed esterification reactions emerge, which have numerous applications [6,7].

Enzyme-catalyzed esterification offers other advantages, in addition to those already mentioned, such as lower energy consumption, selectivity, specificity, stability under adverse conditions, and reduced waste generation. Among the enzymes capable of performing this reaction, lipases, belonging to the serine hydrolase family, stand out. These enzymes act on the ester bonds of numerous organic compounds. This class of enzymes offers further advantages, such as mild process conditions, short reaction time, catalyst reuse, and the ability to perform the reaction in solvent-free systems [6–10].

In the esterification process, selecting a source rich in fatty acids is essential. Vegetable oils, being natural, renewable, available, and low-cost, represent sustainable alternatives for the synthesis of esters with applications in the chemical, pharmaceutical, food,

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and biofuel industries [11]. In this context, Syagrus coronata (licuri) stands out, a palm tree of the Arecaceae family, widely distributed in the Brazilian semiarid region, especially in the dry areas of the Caatinga biome. Its fruits have high oil and protein contents, conferring socioeconomic relevance to local communities and potential for applications in various industrial and cultural sectors. Furthermore, because it is rich in fatty acids, this species constitutes a promising source of raw material for the synthesis of esters [12–16].

When choosing a substrate for esterification reactions, it is essential to consider compounds with biological, pharmacological, and industrial potential to generate products with promising applications in different sectors. In this context, 2-phenylethanol stands out because it exhibits antimicrobial and antibacterial activity, as reported in the literature. Furthermore, its characteristic floral rose aroma lends it great interest for use in perfumery, cosmetics, and food, significantly expanding its industrial and biotechnological value [17,18].

Thus, the present work aims at the enzymatic synthesis of phenylethyl esters, through the esterification reaction between licuri oil and 2-phenylethanol, catalyzed by Pseudomonas lipase in a solvent-free system.

2. Materials and Methods

2.1. Chemical Composition of Licuri Fixed Oil

The first step consisted of determining the chemical composition of licuri fixed oil, following the method described by Lima et al. (2013) [19]. This method involves the following steps:

Transesterification—150 μL of licuri oil were esterified with 2 mL of 0.5 M NaOH in methanol and heated in a water bath at 100 °C for 5 min. Then, 2 mL of 12% BF_3 in methanol (1.3 M) were added under inert atmosphere and refluxed for 30 min. The mixture was cooled, transferred to a separatory funnel with 20 mL of heptane, shaken vigorously for 1 min, and allowed to separate. The organic phase was collected, dried over anhydrous sodium sulfate, and filtered. The resulting fraction was purified by column chromatography using a gradient of eluents (heptane, heptane 1:1 dichloromethane, and dichloromethane; 25/30/15 mL). Finally, the volume of each fraction was reduced under a nitrogen stream at room temperature.

Chromatographic Analysis—Quantitative analysis of fatty acid methyl esters (FAMEs) was performed by gas chromatography–mass spectrometry (GC-MS). Samples (1 μL) were injected directly into the column using hydrogen as the carrier gas at 1 mL/min (splitless). The oven temperature was held at 40 °C for 1 min, then ramped to 150 °C at 55 °C/min and to 220 °C at 1.7 °C/min for 15 min. FAMEs were identified by retention times and coinjection with Mix C4–C24 standards (Sigma-Aldrich).

2.2. Enzymatic Synthesis of Phenethyl Esters

For the enzymatic synthesis, 100 μL of licuri fixed oil, 300 μL of 2-phenylethanol, and 50 mg of Pseudomonas lipase (Sigma-Aldrich) were added to a 5 mL vial and stirred at 50 °C for 24 h. After the reaction, 2 mL of hexane was added, and the enzyme-free supernatant was transferred to a clean vial for purification. Fatty acid esters were purified by column chromatography using an ethyl acetate/hexane gradient (1:4), monitored by thin-layer chromatography (TLC). The isolated esters were analyzed by GC-FID and FTIR to confirm purity. Finally, the aroma of the obtained esters was evaluated organoleptically.

3. Results

3.1. Chemical Composition of Licuri Fixed Oil

Using the previously described methodology, the chemical composition of the oil was identified. After injecting the sample into the GC-MS, the chromatogram of the methyl esters was obtained, as shown in the figure below.

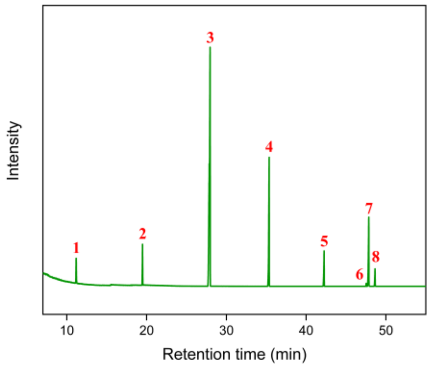


Figure 1. Chromatogram obtained from methyl esters (FAMEs).

The results obtained allowed, through the comparison of retention times with the Mix C4-C24 standards, the complete identification of the fatty acids present in the fixed licuri oil, as presented in the following table.

Table 1. Fatty acids present in licuri fixed oil.

Fatty Acids	Peak Number	Retention Time (min)
Caprylic acid	1	11,317
Capric acid	2	19,739
Lauric acid	3	28,155
Myristic acid	4	35,569
Palmitic acid	5	42,476
Linoleic acid	6	47,792
Oleic Acid	7	48,059
Stearic Acid	8	48,679

3.2. Obtaining and Purifying Esters Obtained by Enzymatic Esterification

Before purification, the phenylethyl ester sample was analyzed by GC-FID. The chromatogram (Figure 2) showed peaks corresponding to the esters, along with hexane (solvent) and 2-phenylethanol (substrate). This preliminary analysis is essential for assessing purity and detecting potential residual impurities.

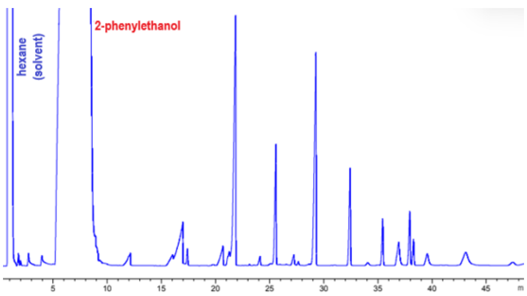


Figure 2. Sample injected after 24 h in GC-FID.

After purification, GC-FID analysis was repeated, and the chromatogram (Figure 3) confirmed the formation and isolation of the fatty esters from 2-phenylethanol. The peak at the beginning corresponds to hexane, used as a dilution solvent. These results demonstrate the effectiveness of the purification process and ensure accurate subsequent analyses.

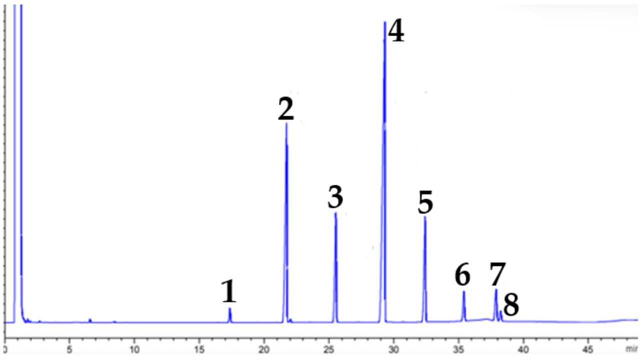


Figure 3. Separated and purified phenylethyl esters.

Moreover, it was possible to establish a correlation between the peaks and the retention times with the phenylethyl esters formed, as presented in Table 2.

The esterification reaction was confirmed by Fourier transform infrared spectroscopy (FTIR), as illustrated in Figure 4. For this analysis, the spectra of 2-phenylethanol, *Syagrus coronata* oil and the product (phenylethyl esters) were compared, enabling the identification of the main structural modifications resulting from the reaction.

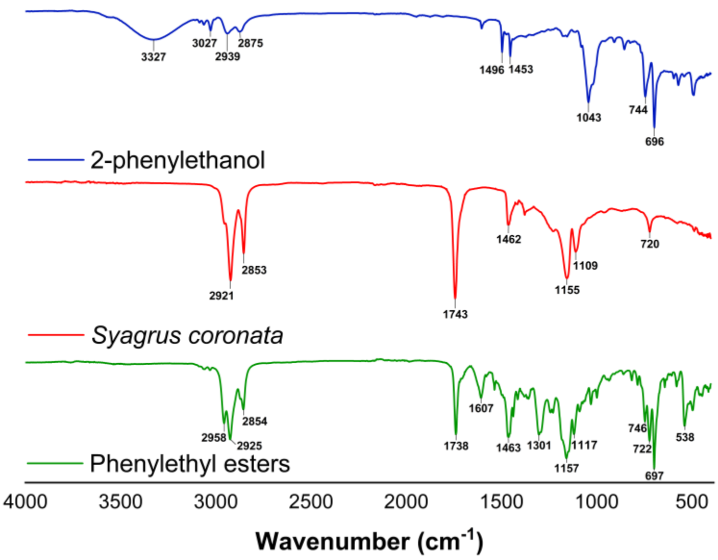
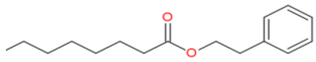
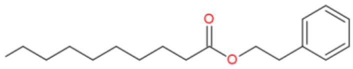
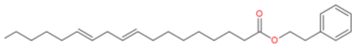
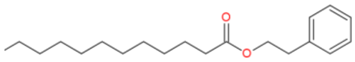
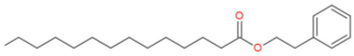
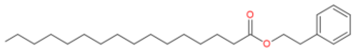
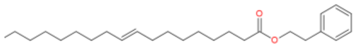
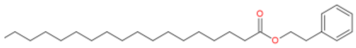


Figure 4. FTIR spectra of 2-phenylethanol (blue), fixed oil of *Syagrus coronata* (red) and the obtained phenylethyl esters (green).

Table 2. Fatty esters obtained.

Fatty Ester Obtained	Peak Number	Chemical Structure
2-Phenethyl octanoate	1	
2-Phenylethyl decanoate	2	
Phenylethyl linoleate	3	
2-Phenylethyl dodecanoate	4	
2-Phenylethyl tetradecanoate	5	
2-Phenylethyl hexadecanoate	6	
Phenylethyl oleate	7	
Phenylethyl stearate	8	

4. Discussion

4.1. Chemical Composition of Licuri Fixed Oil

As described in the literature, licuri oil is rich in fatty acids, mainly lauric (C12:0) and myristic (C14:0), which correspond to approximately 50% of its composition. While saturated acids caprylic, capric, palmitic and stearic, and unsaturated acids (linoleic and oleic) make up the remainder of the oil. Saturated fatty acids correspond to approximately 85% of the oil composition, while 15% correspond to unsaturated fatty acids [14,15,19].

Thus, it can be stated that, in addition to the identification of the fatty esters present in the fixed licuri oil based on the standards used, the chemical composition of the oil was consistent with the data previously reported in the literature, demonstrating the conformity of our results with existing studies.

4.2. Obtaining and Purifying Esters Obtained by Enzymatic Esterification

4.2.1. Analysis in GC-FID

GC-FID chromatographic analysis confirmed the effective separation and purification of the ester mixture obtained through enzymatic esterification. Furthermore, as shown in Figure 3 and Table 2, it was possible to identify the different fatty esters formed, with 2-phenylethyl dodecanoate being the most concentrated constituent. This predominance can be attributed to the fact that this ester is derived from the major fatty acid present in licuri oil, lauric acid, thus reflecting the initial composition of the raw material.

Finally, GC-FID analysis confirmed the efficiency of the purification process, since no signals corresponding to residual 2-phenylethanol or remaining fatty acids were detected in the chromatogram, demonstrating that a properly purified material had been obtained.

4.2.2. FTIR Analysis

The spectrum of 2-phenylethanol (blue line) exhibits a broad band at 3327 cm^{-1}, attributed to the axial vibration of the hydroxyl group. The disappearance of this band in the product spectrum (green line) indicates the consumption of alcohol in the esterification reaction. From this perspective, it is noteworthy that no bands corresponding to hydroxyl groups (3200–3600 cm^{-1}) were observed in the FTIR spectrum of *Syagrus coronata*, indicating the absence of free fatty acids and confirming that they are predominantly present in the form of triacylglycerides.

In the spectrum of *Syagrus coronata* oil (red line), an intense band is observed at 1743 cm^{-1}, corresponding to the carbonyl of the esters present in the triglycerides. This band is maintained in the spectrum of phenylethyl esters, with a slight shift to 1736 cm^{-1}, suggesting the formation of new esters from the reaction between the oil's fatty acids and 2-phenylethanol.

Furthermore, bands in the region of 1240–1150 cm^{-1}, associated with the C–O stretching, corroborate the formation of the ester group. The presence of the aromatic ring of 2-phenylethanol in the product is confirmed by the bands at 1607 cm^{-1} and 746 cm^{-1}, consistent with the characteristic vibrations of the benzene ring (1604 cm^{-1}), indicating its incorporation into the structure of the ester formed.

Furthermore, the fatty esters obtained exhibited a characteristic rose-like aroma, attributed to 2-phenylethanol, which was confirmed by organoleptic analysis. This aspect gives the product broad application potential, considering the importance of aromatized fatty esters in industry.

In summary, the use of the Pseudomonas lipase enzyme as a catalyst proved highly efficient in the synthesis of phenylethyl esters, promoting the esterification of all the fatty acids present in licuri fixed oil. Furthermore, this study represents the first report of the production of phenylethyl esters from the fixed oil of *Syagrus coronata*, expanding its application potential and reinforcing its socioeconomic and cultural importance for the region of Pernambuco, Brazil.

5. Conclusions

Based on the results obtained, it is concluded that the methodology employed for the synthesis of phenylethyl fatty esters by enzymatic esterification was effective, allowing not only the characterization of the chemical composition of licuri oil but also the successful production of esters with a floral aroma characteristic of 2-phenylethanol. The process, conducted with Syagrus coronata fixed oil, 2-phenylethanol, and Pseudomonas lipase under the established conditions, proved effective.

Further studies are needed to optimize the esterification reaction parameters, including optimal temperature, reaction time, and reagent concentrations, to identify the ideal conditions for maximizing yield in Pseudomonas lipase-catalyzed reactions. In parallel, it is recommended to investigate the biological activities of these esters, considering the bioactive potential of licuri oil and 2-phenylethanol, with a view to expanding their industrial and pharmacological applications.

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Institutional Review Board Statement:

Informed Consent Statement:

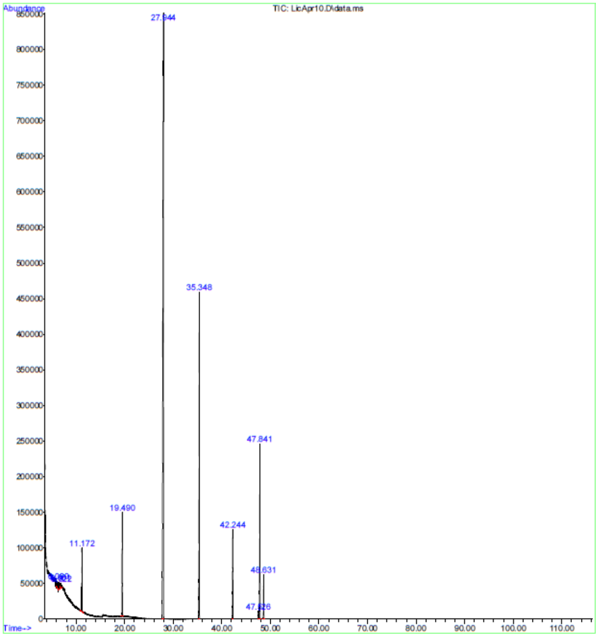
Data Availability Statement: The data presented in this study are available upon request to the corresponding author due to the development of other studies related to this one.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Appendix A.1. Original Chromatogram Obtained for Characterization of the Fixed Oil of Syagrus coronata

Attached is the original chromatogram obtained with GC-MS, without any treatment, for the characterization of licuri fixed oil. It should be noted that the choice to present a chromatogram treated in the ORIGIN program was intended to facilitate viewing, reading, and interpretation by readers, since the original chromatogram, due to the proportions of the components, is difficult to read.



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