Journal of Bioprocessing & Biotechniques

Research Article Open Access

Alternative Technology of Enzymatic Production of Eugenyl and Linalyl Acetate Esters

Adriana BV[1], Bruna A[1], Glaucia F[1], Bruna MS Puton[2], Rogerio LC[2], Natalia P[2] and Debora O[3] Vanin

[1]*University of the West of Santa Catarina, Exact and Technological Sciences Area, Getúlio Vargas Street, Joaçaba, Brasil*
[2]*Post-Graduate Program in Food Engineering, Universal Regional University of Upper Uruguay and Missions, Brazil*
[3]*Department of Chemistry and Food Engineering, Federal University of Santa Catarina, Florianópolis, Brazil*

Abstract

Flavors production by enzymatic esterification is an important scientific and technological issue due to the numerous inconveniences related to the acid catalysis, mainly due to not be a clean technology. In this sense, this work used an alternative technology that describes the maximization of the enzymatic production of eugenyl acetate and linalyl acetate esters via enzymatic esterification of eugenol, a major component of clove oil (*Caryophyllus aromaticus*), and tertiary alcohol linalool, by using acetic anhydride and immobilized enzyme Novozym 435 as the catalyst in an organic solvent-free system. The best reaction condition with 100% of eugenyl acetate conversion was obtained at 60°C with an excess of acetic anhydride (1:5 molar ratio) and 10% of enzyme concentration (w/w in relation to the substrates). Higher yields of linalyl acetate (5.6%) were achieved with the enzyme concentration of 5%, at 70°C and molar ratio acid to alcohol of 9:1, with 6 hours of reaction time for the both esters production. The data analysis showed a positive effect of molar ratio associated with the temperature to the eugenyl acetate conversion, and the effect of the temperature to the linalyl acetate conversion. The tertiary alcohol characteristic in esterification it was also discussed.

Keywords: Eugenol; Linalool; Eugenyl acetate; Linalyl acetate; Esterification

Introduction

Esters are organic compounds often found in nature, obtained from the reaction between carboxylic acids and alcohols, with usage in the synthesis of drugs, fine chemicals, pharmaceuticals, solvents and plasticizers, cosmetic and food industries [1]. The concern about food production is increasingly focused on the final product quality that has been demanding a better quality of the final product, attending the concept of sustainability, health, and natural resources [2,3]. Aromas from natural sources obtained via enzymatic catalysis or via biotransformation are considered natural, which provides a greater acceptance. In this context, biotechnology has contributed to the development of research on flavor production, encouraging industries to use enzymes and/or microorganisms in the flavor production. The biotechnological processes can produce complex systems with many of the necessary compounds for the flavor characterization, which commonly are not obtained in the synthetic route of production, making it an economically unfeasible route. In general, esters of essential oils containing terpene alcohols are most commonly used in the perfume and food industry due they are more aromatic [4]. Esterification process of alcohols is used particularly to obtain molecules with lower molecular weight from fatty acid esters. The acetylation of alcohols can be accomplished easily under heat and by using acetic acid, or acetic anhydride or via enzymatic catalysis in a solvent-free system [2,5-9]. However, the acylation process of tertiary alcohols sterically hindered is yet difficult [10]. Since linalool is a tertiary alcohol with an ethylenic double bond on the carbons α and β to the tertiary carbon atom, its esterification shows some difficulties characteristic of these alcohols. The free acid (used as the esterifying agent or formed as a by-product when the acid anhydride is used) avoid that the temperature of esterification reaction increase, and more important induces side reactions, as for instance, dehydration and isomerization [11,12]. In this sense, the objective of this work was to evaluate the enzymatic synthesis of eugenyl and linalyl acetate using a commercial Novozym 435 lipase as a biocatalyst and to compare the effect of the eugenol structure (phenylpropanoid) and linalool (tertiary monoterpene alcohol) on the esters production.

Materials and Methods

Enzymatic production of esters

The reaction medium consisted of clove (*Caryophyllus aromaticus*) (Viafarma, 85% of eugenol) or linalool (Sigma-Aldrich, 97%), acetic anhydride (Vetec, 97%), immobilized Novozym 435 lipase from Candida antarctica (Novozymes Brazil), with a total reaction volume of 5 mL. All experiments were performed in a shaker with constant agitation of 150 rpm and reaction time fixed in 6 hours. After the reaction time, the biocatalyst was separated from the substrates by simple filtration and the reaction quantified by gas chromatography (GC).

Optimization of enzymatic production of esters

For the determination of experimental conditions that maximized the esters synthesis, a central composite design (2^3) with triplicate in the central point was carried out. The temperature (T), the molar ratio (RM) and enzyme concentration ([E]) (w/w in relation to the substrates) ranges are shown in Table 1. The results of the enzymatic esterification are shown in terms of conversion percentage and were treated statistically with multivariate analysis of principal component

***Corresponding author:** Adriana B. Vanin, University of the West of Santa Catarina, Exact and Technological Sciences Area, Getúlio Vargas Street, Joaçaba, Brasil, Tel: 35512000; E-mail: adriana.vanin@unoesc.edu.br

Received September 05, 2019; **Accepted** October 25, 2019; **Published** October 31, 2019

Citation: Adriana BV, Bruna A, Glaucia F, Puton BMS, Rogerio LC, et al. (2019) Alternative Technology of Enzymatic Production of Eugenyl and Linalyl Acetate Esters. J Bioprocess Biotech 9: 345.

analysis (PCA) and cluster analysis using correlation as similarity measure and paired group algorithm from Past software version 1.81.

Quantification of reaction conversion

Ester quantification was performed by gas chromatography on Shimadzu GC-2010 equipment. The analyses were carried out using a WAX fused silica capillary column (30 m × 250 μm), 0.25 μm film thickness, FID detector, with the following temperature programming: 40-180°C (3°C/min), 180-230°C (20°C/min), 230°C (20 min), injector temperature 250°C, detector at 275°C, split injection mode, split ratio 1:100, H_2 entrainment gas (56 KPa). The samples were diluted in n-hexane (1:10) and a volume of 0.4 μL was injected. Eugenyl acetate or linalyl acetate conversion was performed following the reduction of the limiting agent peak (eugenol or linalool).

Level	Temperature (°C)		Molar ratio (mol/mol) (anydride/alcohol)		Enzyme Concentration (wt%)	
	Eugenol	Linalool	Eugenol	Linalool	Eugenol	Linalool
-1	40	50	1:1	3:1	1	5
0	50	60	3:1	6:1	5.5	10
1	60	70	5:1	9:1	10	15

Table 1: Variables and levels studied in the central composite design 2^3 for enzymatic esters production.

Results and Discussion

Table 2 shows the matrix of the central composite design 2^3 with the real and coded values of the independent variables and the conversions in terms of eugenyl and linalyl acetate. In relation to the eugenyl acetate, it can be observed from the results that high conversions were obtained at all levels studied at different enzyme concentrations, molar ratio, and temperature range. Higher conversion (~100%) was obtained at 60°C, excess of acetic anhydride and 10 wt% of enzyme concentration (assay 8). The results of eugenyl acetate conversion were statistically treated, but it was not possible to validate an empirical

Run	Temperature (ºC)		Molar ratio Anhydride/alcohol		Enzyme concentration (wt%)		Conversion to acetate (%)	
	Eugenol	Linalool	Eugenol	Linalool	Eugenol	Linalool	Eugenyl	Linalyl
1	-1 (40)	-1 (50)	-1 (1:1)	-1 (3:1)	-1 (1%)	-1 (5%)	60.12	0.65
2	1 (60)	1 (70)	-1 (1:1)	-1 (3:1)	-1 (1%)	-1 (5%)	87.85	3.48
3	-1 (40)	-1 (50)	1 (5:1)	1 (9:1)	-1 (1%)	-1 (5%)	89.78	0.55
4	1 (60)	1 (70)	1 (5:1)	1 (9:1)	-1 (1%)	-1 (5%)	93.77	5.64
5	-1 (40)	-1 (50)	-1 (1:1)	-1 (3:1)	1 (10%)	1 (15%)	46.53	0.73
6	1 (60)	1 (70)	-1 (1:1)	-1 (3:1)	1 (10%)	1 (15%)	83.38	3.95
7	-1 (40)	-1 (50)	1 (5:1)	1 (9:1)	1 (10%)	1 (15%)	92.55	0.92
8	1 (60)	1 (70)	1 (5:1)	1 (9:1)	1 (10%)	1 (15%)	99.97	0.75
9	0 (50)	0 (60)	0 (3:1)	0 (6:1)	0 (5.5%)	0 (10%)	96.78	1.13
10	0 (50)	0 (60)	0 (3:1)	0 (6:1)	0 (5.5%)	0 (10%)	96.86	0.98
11	0 (50)	0 (60)	0 (3:1)	0 (6:1)	0 (5.5%)	0 (10%)	97.01	0.99
At 150 rpm and 6 h of reaction time.								

Table 2: Matrix of experimental design 2^3 (coded and real values) with responses in terms of conversion in eugenyl and linalyl acetate

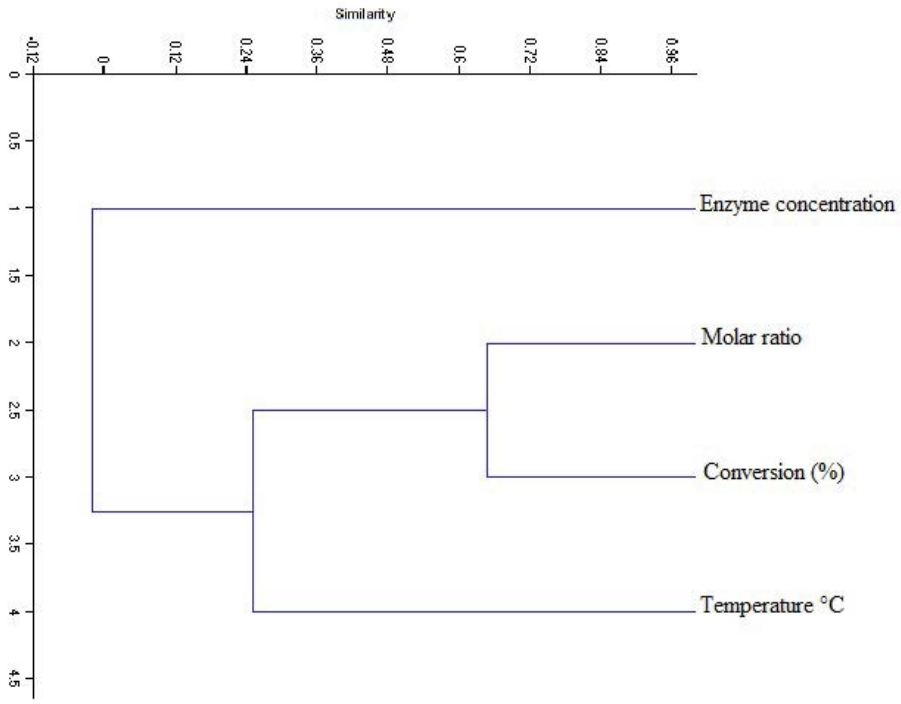

Figure 1: Cluster analysis between analyzed variables and eugenyl acetate conversion.

model describing the conversion of eugenyl acetate for the studied variables range. Thus, the results were studied in a multivariate cluster analysis to measure the degree of similarity between the groups (Figure 1). It was observed that the enzymatic conversion of eugenol to eugenyl acetate had a high correlation with substrates molar ratio and temperature, indicating that higher conversions are obtained at higher temperatures and in an excess of acetic anhydride, whereas the enzyme concentration showed lower correlation with the conversion. Thus, high conversion rates can be obtained with lower enzyme concentrations, indicating cost reduction during the process. The acid/alcohol molar ratio is one of the most important parameters in esterification reactions since the reaction is reversible. In esterification reactions, alcohol excess (nucleophile/acyl receptor) leads to higher conversion levels due to the availability of nucleophile for substrate transfer [13]. However, in some reactions, there is an inhibitory effect of alcohol excess. Hoffmann et al. [14] also observed lower esterification yields of (R,S)-1-phenylethanol with alcohol excess, showing the effect of substrate inhibition in the catalyst decreasing conversion degree. Temperature also has high importance in the reaction systems. Firstly, due to increased collisions between substrates and biocatalyst [15], and secondly, the enzymes have an optimum temperature, in this case, for Novozym 435 a temperature range from 40°C to 70°C [6]. In relation to linalyl acetate, it can be observed that low conversions were related at all studied levels at different enzyme concentrations, substrates molar ratio, and temperature. After conversion results analysis (Table 2), it was observed that only the temperature showed a correlation with the conversion (Figure 2) indicating that at higher temperature ranges (70°C) would lead to higher esters conversions. However, temperatures above 70°C could reduce enzyme activity. The endothermic nature of the esterification reactions allows concluding that the positive effect of temperature on both reactions is consistent with those reported in the literature. Positive enthalpies were observed by different authors in the ester syntheses, such as isoamyl oleate [16] methyl palmitate, methyl stearate, methyl oleate, and hexyl levulinate [17]. In this context, the increase in temperature provides a displacement in the reaction system equilibrium a favoring products formation and increasing reaction yield. The principal component analysis confirmed the effect of molar ratio and temperature on the enzymatic esterification of eugenol and linalool (Figure 3). Lakoud and Djuerourou [18] reported the esterification and etherification of steroid and terpene by using different alcohol type. The reactivity of primary alcohols was compared to secondary, and with tertiary alcohol, and no reaction was observed. Furthermore, since linalool undergoes dehydration, cyclization, and isomerization with great ease, ordinary methods of esterification such as heating with acetic acid in the presence of inorganic acids cannot be used in this case [19]. In the linalool molecule, the OH group is attached to a carbon atom to which three other carbon atoms are attached. These tertiary terpene alcohols are particularly difficult to esterify and obtain high yields without decomposition. Once linalool is very degradable to free acid when higher temperatures are used to obtain higher reactions yields, the linalool changes to geraniol or terpineol which is esterified. And in this case, the esters formed are undesirable in the linalool ester final product and are not easily to be separated by fractionation [20]. Since linalool has an asymmetric carbon atom, it can exist in optically active forms, and it is a good natural source of a tertiary alcohol for the study of problems related to optical isomerism. Some of the reactions, such as cyclization to an optically active terpineol, are not well understood and offer interesting fields of research to the organic chemistry. In view of this large natural occurrence and peculiar structure, it is possible that there is a fundamental relationship between

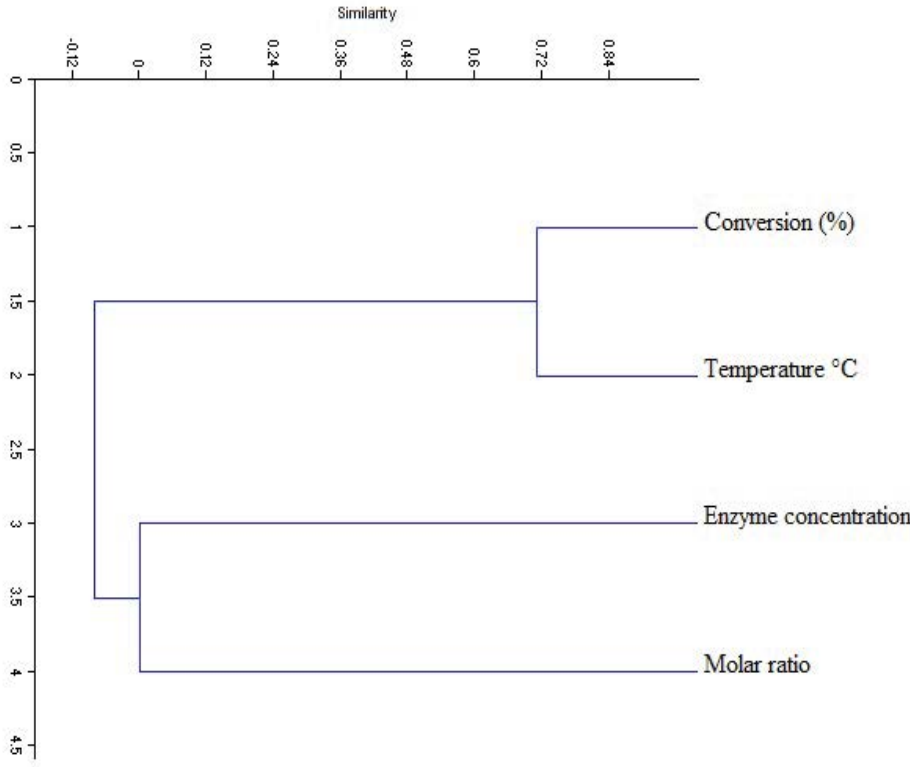

Figure 2: Cluster analysis between analyzed variables and the linalyl acetate conversion.

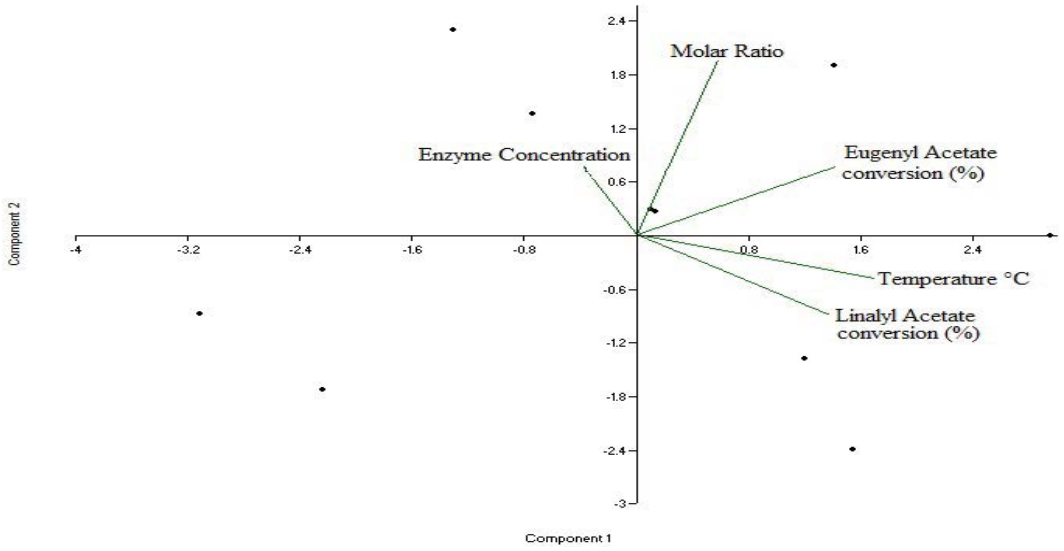

Figure 3: Principal component analysis (PCA) of molar ratio, temperature and enzyme concentration on eugenyl and linalyl acetate conversion.

linalool and other terpenes, and that linalool plays an important role in the biogenesis of essential oils [21]. The isomerization of linalool to geraniol and the opposite is of great academic interest and consist in a classic example of an ionotropic or allylic rearrangement. This type of rearrangement is common to the allyl type of alcohols. In the case of linalool, the rearrangement to geraniol may be conducted by prolonged boiling with acetic anhydride [21].

Conclusion

Eugenol showed high esterification capacity (~100%) due to the chemical structure and the flat aromatic ring does not prevent an approximation between substrates and biocatalyst, and due to the higher acidity of the phenolic compounds in relation to the alcohols allowing a release of the hydroxyl group from the ring with lower energy loss. Alcohol excess affected negatively the enzymatic reaction due to the inhibition effect. The low conversion of tertiary linalool alcohol (~5%) was related to the steric hindrance, and the only positive effect observed was in relation to the temperature that in some enzymatic reactions can result in the reduction of enzyme activity and undesirable products.

Acknowledgements

The authors thank the CNPq, CAPES, FAPERGS, URI and UNOESC by financial support.

Declaration of Interest

The authors report no declarations of interest.

References

1. Rajabi F, Abdollahi M, Luque R (2016) Solvent-free esterification of carboxylic acids using supported iron oxide nanoparticles as an efficient and recoverable catalyst. Materials 9: 557-566.
2. Laroque DA, Loss RA, Silva MJA (2015) Synthesis of eugenyl acetate in solvent-free acetylation: Process optimization and kinetic evaluation. J Chem Eng Process Technol 6: 1-8.
3. Bndes (2011) National economic and social development bank.
4. Sá AGA, Meneses AC, Araújo PHH, Oliveira D (2017) A review on enzymatic synthesis of aromatic esters used as flavor ingredients for food, cosmetics and pharmaceuticals industries. Trends Food Sci Technol 69: 95-105.
5. Pätzold M, Weimer A, Liese A, Holtmanna D (2019) Optimization of solvent-free enzymatic esterification in eutectic substrate reaction mixture. Biotechnol Rep (Amst) 22: e00333.
6. Sbardelotto CR, Piazza SP, Nyari NLD (2018) Optimization of solvent-free geranyl butanoate production using novozyme 435 and homemade polyurethane immobilized novozyme nzl-102-lyo-hq as catalysts. Quím Nova 41: 905-911.
7. Vanin AB, Orlando T, Piazza SP (2014) Antimicrobial and antioxidant activities of clove essential oil and eugenyl acetate produced by enzymatic esterification. Appl Biochem Biotechnol 174:1286-1298.
8. Chiaradia V, Paroul N, Cansian RL (2012) Synthesis of eugenol esters by lipase-catalyzed reaction in solvent-free system. Appl Biochem Biotechnol 168: 742-751.
9. Paroul N, Grzegozeski LP,Cansian RL (2011) Erratum to solvent-free geranyl oleate production by enzymatic esterification. Bioprocess Biosyst Eng 34: 331-337.
10. Oromí-Farrús M, Torres M, Canela R (2012) Acylation of chiral alcohols: A simple procedure for chiral GC analysis. J Anal Methods Chem 2012: 1-11.
11. Paroul N, Biasi A, Rovani AC (2010) Enzymatic production of linalool esters in organic and solvent-free System. Bioprocess Biosyst Eng 33: 583-589.
12. Gradefl PS, Finet B (1969) Process for esterification of tertiary terpenic alcohols. United States Patent.
13. Azudin NY, Shukor SRA (2018) Solvent-Free Isoamyl Acetate Production via Enzymatic Esterification. Intech Open London, UK.
14. Hoffmann I, Silva VD, Nascimento MG (2011) Enantioselective resolution of (R,S)-1-phenylethanol catalyzed by lipases immobilized in starch films. J Braz Chem Soc 22: 1559-1567.
15. Shinde SD, Ganapati D, Yadav GD (2015) Insight into microwave-assisted lipase catalyzed synthesis of geranyl cinnamate: optimization and kinetic modeling. Appl Biochem Biotechnol 175: 2035-2049.
16. Lage FA, Bassi JJ, Corradin MC (2016) Preparation of a biocatalyst via physical adsorption of lipase from Thermomyces lanuginosus on hydrophobic support to catalyze biolubricant synthesis by esterification reaction in a sovent-free system. Enzyme Microb Technol 84: 56-67.
17. Badgujar KC, Bhanage BM (2015) Imobilization of lipase on biocompatible co-polymer of polyvinyl alcohol and chitosan for synthesis of laurate compounds in supercritical carbon dioxideusing response surface methodology. Process Biochem 50: 1224-1236.
18. Lakoud SG, Djerourou A (2016) Esterification and etherification of steroid and terpene under Mitsunobu conditions. Arab J Chem 9: 889-892.

19. Tsitsishvili V, Ivanova I, Ramishvili P (2017) Catalytic conversion of linalool on micro-mesoporous BEA-type zeolite. Bulletin of the Georgian National Academy Of Sciences 11: 79-87.

20. Aeschbach ME (1947) Esterification of terpene alcohols. United States Patent 2423545.

21. Fajdek-Bieda A, WróblewskaA, Miądlicki P (2019) Influence of technological parameters on the isomerization of geraniol using sepiolite. Catal Lett pp: 1-11.