



Techno-economic assessment of benzyl benzoate clean production using conventional heating or microwaves

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ABSTRACT

Benzyl benzoate is an important anti-scabies agent, so finding sustainable production processes is essential. This work involved the techno-economic assessment of benzyl benzoate production in a solventless system with conventional heating or microwave-assisted. The proposed processes' conditions were optimized by transesterifying methyl benzoate and benzyl alcohol in a solventless system using the Lipozyme 435 lipase as the catalyst. The optimized conditions were an ester/alcohol molar ratio of 1:6, a temperature of 73 °C, and enzyme loading of 10% and 16% (w/w), for conventional heating and microwave-assisted, respectively. Under these conditions, the two reactions reached conversions greater than 90% in 24 h and 82% in 7 h. The tests on lipase reusability showed that the ester production remains stable for up to 4 use cycles. Gas chromatography and proton NMR confirmed that benzyl benzoate could be produced biocatalytically, and a high purity can be obtained by simple distillation. The economic analysis of the process showed that the total capital investment was favorable, suggesting a promising investment opportunity. Furthermore, a production total cost showed a favorable positive net present value and returned on investment for benzyl benzoate production. Hence, the proposed clean production of benzyl benzoate can be considered for industrial scale-up.

1. Introduction

Recent data from the literature report an increasing incidence of scabies worldwide and in Europe, especially in the last 10–20 years (Aždajić et al., 2022). Scabies, a superficial skin condition caused by the mite *Sarcoptes scabiei* var *hominis*, is a significant public health problem worldwide. It is estimated that it affects 130 to 300 million people annually, and the World Health Organization (WHO) recognized it as a neglected tropical disease (Lajarin-Reinares et al., 2022; Meyersburg et al., 2022). However, a prevalence of 0.2–71% is still estimated depending on the geographic location (Rosumeck et al., 2018). European Academy of Dermatology and

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Venerology recommends its treatment with 5% permethrin cream or 10–25% benzyl benzoate (BB) lotion or oral ivermectin 200 µg/kg (Salavastru et al., 2017).

Since the 1930s, when it was first used to treat scabies, BB has been used, mainly in emerging countries, due to its high efficiency and lower cost than other scabies treatment methods (Heukelbach and Feldmeier, 2006; Ly et al., 2009). In addition, this benzyl ester is widely used as a fragrance (Api et al., 2020), spasmolytic agent (Rivero-Cruz et al., 2007), larvicidal (Seo et al., 2012), cleaning tissues (Molbay et al., 2021; Tian et al., 2021) and reducing vegetable oil viscosity (Moosasaït et al., 2021). Recently, Aydın et al. (2021) suggested that benzyl esters, including BB, have the potential as a targeted drug against the Covid-19 virus due to the possible host bromodomain protein inhibition, which may inhibit its binding to the protein E of the virus SARS-CoV-2.

BB is an aromatic ester compound found in essential oils of *Myroxylon balsamum* (L.) Harms var. *pereirae* (66%) (Seo et al., 2012), *Populus tremula* L. (45%) (Okinczyk et al., 2018), *Acca sellowiana* (O. Berg) Burret (Peng et al., 2019), *Cynomorium songaricum* Rupr. (Zhou et al., 2009), *Cinnamomum zeylanicum* Blume (Senanayake et al., 1978). However, in nature, its availability is scarce (de Meneses et al., 2020), and traditional synthetic methods are employed to carry out its production on an industrial scale (Saber and Hashemi, 2018; Tharp et al., 2002). Therefore, it is essential to develop economic, efficient, and sustainable processes based on the exploitation of green catalysts, reducing waste that is harmful to human health and the environment (Remonatto and Lerin, 2023).

Biocatalysis using enzymes such as lipases in a solventless system has become an excellent alternative for esters synthesis as it follows several principles of green chemistry (Anastas and Eghbali, 2009; Mustafa et al., 2023; Mustafa and Niikura, 2022) and can be described as a chemical click reaction (Devaraj and Finn, 2021). Lipases work well under mild temperature and pressure conditions and can be used in a broad palette of reactions (Hosney et al., 2020b; Hosney and Mustafa, 2020). In addition, they are non-toxic, biocompatible, biodegradable, and produced from inexpensive and renewable resources (Alcántara et al., 2022; de Meneses et al., 2020; Sheldon et al., 2021). These biocatalysts are usually relatively stable and used in diverse media, like aqueous, organic solvents, solventless, ionic liquids, deep eutectic solvents, and supercritical fluids, which may be associated with technologies to intensify these actions, such as ultrasound and microwaves. This way, lipases offer many possibilities in enzymatic synthesis (Khan and Rathod, 2018; Lerin et al., 2014; Loss et al., 2015; Ortiz et al., 2019).

The non-use of solvents in enzymatic reactions catalyzed by lipases has several advantages, such as reduced reaction volume, high concentration of reagents, high reaction rate, reduction of product separation steps, drastic reduction of environmental contamination and production costs. However, the mass transfer of reactants can be impaired in a solventless system. Consequently, using an excess reagent, in general, alcohol, acts as a solvent shifting the equilibrium of the reaction towards the synthesis for thermodynamic reasons, but may affect its behavior and yield due to these same reasons. Therefore, the effects on the reaction will depend on the amount and nature of the excess reagent (Sousa et al., 2021; Wang et al., 2022).

Reactions catalyzed by lipases for synthesizing BB are a bit explored, although the literature presents some works related to this topic. The main works report the enzymatic acylation using an activated acyl donor, such as benzoic anhydride, with and without solvent, reaching conversions ranging from 32 to 92% (de Meneses et al., 2020; Shiki et al., 2022; Song and Chang, 2022). On the other hand, the transesterification approach, using methyl benzoate (MB) as an acylating agent, was carried out with and without solvent, under conventional heating and/or intensified by microwaves irradiation, with conversions between 79 and 87% (Gryglewicz et al., 2000; Shinde and Yadav, 2014). However, evaluation studies of process variables for optimizing reaction conditions using transesterification still need to be performed.

These studies on BB production only focused on technical the feasibility. However, other important points to be evaluated are the environmental and economic aspects of the process. Ecological elements, especially potential environmental impacts, are increasingly crucial for developing and implementing new or improved processes. In this sense, the specific aspects of the process or system from an environmental and economic perspective must be appropriately evaluated (Mata et al., 2018; Mustafa et al., 2022). Therefore, an integrated techno-economic assessment of bioprocesses makes it possible to determine investment areas, opportunities, and challenges for both industry and researchers (Mustafa et al., 2022).

Based on the above reasons, this study aimed at the techno-economic evaluation of the enzymatic BB production through the enzymatic transesterification of MB and benzyl alcohol (BA). The production was optimized in batch mode, in a solventless system, using conventional heating (water bath) or microwave-assisted in order to maximize the conversion facilitating downstream processes and reducing production costs. Afterwards, an economic evaluation was carried out to investigate the dependence of critical profitability indicators on production capacity. A sensitivity analysis was also carried out to determine the effect of raw materials and product prices on the return on investment (ROI) of the process.

2. Materials and methods

2.1. Materials

For this study, the commercial lipase Lipozyme® 435, lipase B from *Candida antarctica* immobilized on acrylic resin (declared activity 9000 PLU g⁻¹) was supplied from Novozymes SA. The reagents used as substrates were methyl benzoate (99%, Sigma-Aldrich) and benzyl alcohol (99.8%, Riedel-de Haën). Molecular sieves bead 5 Å (8–12 mesh) was supplied from Sigma-Aldrich. Cyclohexane (Carlo Erba), ethyl acetate (Carlo Erba), acetone (Carlo Erba), acetic acid (Sigma-Aldrich), and deuterated chloroform (Sigma-Aldrich) were of analytical grade and used without further purification.

2.2. BB quantification

With some modifications, the BB quantification was carried out as described previously by Shiki et al. (2022). First, all samples were filtered with filter paper and centrifuged (14,000 rpm for 2 min), and then the supernatant was diluted in ethyl acetate (1:100

v/v) for gas chromatography analysis. A Thermo Focus GC with a flame ionization detector (FID) and a MEGA-SE-52 column (30 m length \times 0.32 mm internal diameter \times 0.1–0.15 μ m film thickness) were used. The oven temperature ramp was programmed to 100 °C for 2 min, and then to 300 °C with a rate of 10 °C min⁻¹. Injector and detector temperatures were maintained at 175 °C and 250 °C, respectively. The injection mode selected was the split ratio (1:50), and a volume of 1 μ L was injected, using He as the carrier gas. The ester production was calculated as the ratio of the peak area of BB and the sum of the areas of the BB and the limiting reagent (BA) (Giovannini et al., 2019; Remonato et al., 2022).

2.3. Conventional heating reactor (CHR)

The conventional heating reactions were carried out in a concave bottom glass reactor with a total volume of 10 mL hermetically closed by a screw lid with a Teflon seal. The reactor was placed in a thermostatic bath with automatic temperature control and equipped with a magnetic stirring system.

2.4. Microwave reactor (MR)

Microwave-assisted enzymatic reactions were performed using a Biotage Initiator™, as described by Venturi et al. (2023).

2.5. Optimization of BB production in CHR and MR

The optimization of the reaction conditions to produce BB for the CHR and MR was performed by the experimental designs (DOE) methodology (Rodrigues and Iemma, 2014). A 2³ central composite rotatable design (CCRD), at four levels of combinations (+ α , +1, 0, -1, - α) and 3 replicates on the central point, totalizing 17 independent experiments (Table 1), was applied to optimize the independent variables of the process. The CCRD investigated the influence of the BA to MB molar ratio (mmol/mmol), temperature (°C) and enzyme concentration (% concerning the mass of BA). All assays were conducted in batch mode using Lipozyme 435 lipase as a biocatalyst with 700 rpm magnetic stirring for the CHR. For the MR, a 30-s magnetic pre-agitation and, during the reaction, stirring and high absorption level were kept constant.

The reaction time (4 h) was determined by evaluating the reaction kinetics using the experimental conditions of the central point (Table 1, trials 15–17). No BB formation was observed without adding the enzyme in 72 h (data not shown). At predetermined reaction times, aliquots of the reaction medium were treated and analyzed by GC, as described in section 2.2.

Molecular sieve (5 Å) was used to remove the co-produced methanol. The molecular sieve mass required was calculated by the maximum absorption capacity of methanol (200 mg g⁻¹) based on the maximum theoretical mass of methanol produced during each reaction.

The significance of the coefficients and the fit of the model were evaluated by analysis of variance (ANOVA) using the online software Protimiza Experimental Design (<http://experimentaldesign.protimiza.com.br/>), considering a confidence level of 10% ($p \leq 0.1$) in both reaction systems.

Table 1

2³ CCRD matrix showing the coded and real values (in parentheses) of the independent variables and experimental and model-predicted responses obtained regarding BB production after 4 h in CHR and MR using Lipozyme 435 lipase.

Trial	RM ^a	T (°C)	E (wt%) ^b	CHR			MR		
				Experimental (%)	Predicted ^c (%)	RE ^d (%)	Experimental (%)	Predicted ^c (%)	RE ^d (%)
1	-1 (1:2)	-1 (65)	-1 (5)	11	11.7	6.6	17	16.2	2.3
2	1 (1:10)	-1 (65)	-1 (5)	29	31.0	6.7	30	33.6	12.6
3	-1 (1:2)	1 (80)	-1 (5)	17	11.7	31.0	24	16.2	31.5
4	1 (1:10)	1 (80)	-1 (5)	28	31.0	10.5	42	33.6	20.3
5	-1 (1:2)	-1 (65)	1 (15)	61	54.1	11.3	47	43.3	7.1
6	1 (1:10)	-1 (65)	1 (15)	47	48.8	3.9	48	60.7	26.5
7	-1 (1:2)	1 (80)	1 (15)	54	54.1	0.2	46	43.3	5.8
8	1 (1:10)	1 (80)	1 (15)	48	48.8	1.7	70	60.7	12.9
9	-1.68 (1:0.7)	0 (73)	0 (10)	14	20.2	44.3	8	15.3	91.7
10	1.68 (1:12.7)	0 (73)	0 (10)	37	31.9	13.7	46	44.6	3.0
11	0 (1:6)	-1.68 (60)	0 (10)	38	42.2	11.2	48	48.2	0.4
12	0 (1:6)	1.68 (85)	0 (10)	46	42.2	8.2	35	48.2	37.6
13	0 (1:6)	0 (73)	-1.68 (1.6)	19	18.2	4.0	10	16.1	61.3
14	0 (1:6)	0 (73)	1.68 (18.4)	67	68.8	2.7	62	61.8	0.4
15	0 (1:6)	0 (73)	0 (10)	53	51.3	3.3	50	48.2	3.7
16	0 (1:6)	0 (73)	0 (10)	50	51.3	2.5	50	48.2	3.7
17	0 (1:6)	0 (73)	0 (10)	51	51.3	0.5	53	48.2	9.1

^a Molar ratio benzyl alcohol to methyl benzoate.

^b Enzymes loading as % weight concerning the mass of BA.

^c Calculated according to Equations (6) and (7).

^d Relative Error (RE, %) = $\left(\frac{\text{Experimental conversion} - \text{Predicted conversion}}{\text{Experimental conversion}} \right) * 100$.

2.6. Effect of enzyme concentration in CHR and MR

A kinetic study of the enzyme concentration effect on BB production in CHR and MR was carried out using the optimized conditions for each reactor. For both reactors, the optimized condition presented a molar ratio of 1:6 (BA to MB) and a temperature of 73 °C. Enzyme concentrations were 2.5, 5 and 10%, and 10, 16 and 20% for CHR and MR, respectively. Aliquots of the reaction medium (20 µL) were taken at predetermined times up to 70 and 8 h for the CHR and MR, respectively, and then treated and analyzed by GC as described in section 2.2.

2.7. Lipase reusability in CHR and MR

Lipozyme 435 lipase reusability was performed in the maximized condition for each system of reaction heating. For the reactions carried out in CHR and MR, the conditions were BA to MB molar ratio of 1:6, temperature of 73 °C, 10 and 16% lipase, 700 rpm agitation and 24 and 8 h, respectively. At the end of each cycle of lipase use, the reaction medium was filtered twice, the first using a 7-mesh filter to remove the molecular sieve (5 Å) and the second using filter paper to recover the enzyme. The lipase was then washed with acetone to remove substrates from the enzyme support (de Meneses et al., 2020) and dried under vacuum (30 mmHg) at 40 °C for 30 min. After drying, the lipase was used in a new reaction cycle. The BB production in each cycle was used to evaluate the lipase performance. All assays were performed in triplicate, and significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$).

2.8. Purification and spectroscopic characterization of BB

The reaction medium was evaporated under reduced pressure (10 mmHg at 50 °C) until the substrates had evaporated entirely to obtain pure BB (99.9% purity). Next, an aliquot of BB was diluted in chloroform deuterate (CDCl₃) to acquire ¹H and ¹³C NMR spectra, operating at 400 MHz and room temperature. ¹H NMR δ (ppm): 8.09 (d, $J = 7.5$ Hz, 2H, Ar), 7.56 (t, $J = 7.5$ Hz, 1H, Ar), 7.48–7.32 (m, 7H, Ar), 5.38 (s, 2H, –OCH₂); ¹³C NMR, δ (ppm): 166.4, 136.1, 133.0, 129.7, 128.6, 128.4, 128.2, 128.1, 66.7.

2.9. BB production simulation

ASPEN PLUS version 10 software from Aspen Tech Inc was used to simulate the BB production. The methods used for modelling and simulation are described in this section.

2.9.1. Process modelling

The two reactants used for BB production are MB and BA. The stoichiometric reaction is shown in Equation (1):



The physical properties of all reactants and products are shown in Table S1. Thermodynamic packages like UNIQUAC, NRTL, and UNIFAC were extensively used in the literature for esters production (Mustafa, 2021) and were found to fit the proposed model. Therefore, the UNIQUAC property method was used in this study.

Properties of the streams, including mass and energy balances can be viewed in Table S2. The conversion and reaction conditions obtained from the conventional heating study (namely: conversion of 91%, reaction temperature of 73 °C, and BA to MB molar ratio of 1:6) were fed to ASPEN software. RStoic reactor was chosen as an appropriate type to perform the reaction in the simulation since the conversion per cent is known. The simulation results were generated based on an annual plant capacity and productivity of 94 t and 19.7 t, respectively.

2.10. Model analysis and economic evaluation

Economic studies' primary goals are to determine potential revenue streams and project management and implementation costs. The feasibility of a project is also predicted by these studies, which also analyze financial flows. In order to help the community of esters producers make decisions, the current techno-economic research provides a thorough overview that may be used as a tool.

2.10.1. Total investment summarized

The sum of the working capital need, the fixed capital investment, and the initial expense is the project's total capital investment (Mustafa et al., 2022). An important aspect to take into account when starting a plant is the cost of the fixed capital investment, which comprises the full cost of building, planning, installing, and altering a factory (Sinnott and Towler, 2019). Therefore, accurate cost estimations are essential for planning and avoiding operational failure. By adding the expenses of contingencies, engineering, inside battery limitations (ISBLs), and outside battery limits (OSBLs), this cost may be estimated.

Therefore, the predicted ISBL may have an impact on the financing of a process design. Miscalculations have to be avoided, and the ISBL's target should be appropriately identified with great care. ISBL contains the machinery, pipelines, tools, pipes, valves, and other auxiliary components required to begin production. Depending on the industry and production capacity, a variety of approaches are employed to determine the ISBL cost. The methods of Bridgewater, Taylor, Gore, Stallworthy, Klumpar, Brown, and Fromme are a few examples of common estimating techniques (Tsagkari et al., 2016). These estimating techniques provide a solid understanding of the ISBL cost. Therefore, Bridgewater's method is used to evaluate the ISBL cost of the BB manufacturing facility. To get an accurate answer using this approach, three terms - the number of main units, plant capacity, and reactor conversion - must be properly specified. As illustrated in Fig. 1, an ASPEN PLUS PFD had been created to effectively determine the quantity of units and provide a more comprehensive understanding of the suggested response. Based on the generated ASPEN calculations, the plant's capacity and productivity were 94 and 19.7 t/d, respectively. The experimental findings showed that 91% of the conversion occurred.

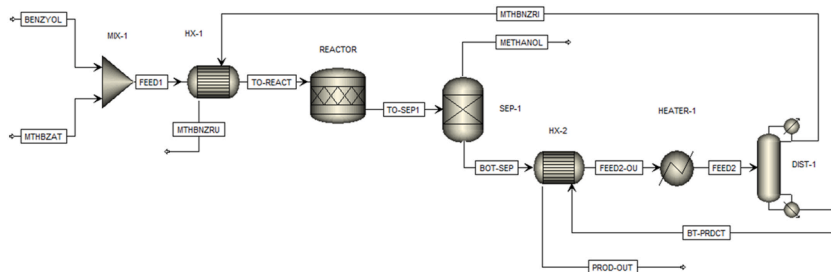


Fig. 1. Process flow diagram of BB production using ASPEN PLUS.

The reaction section and the distillation section had been assumed to be the two units in the proposed BB plant, and the ISBL cost had been calculated using Bridgewater's approach (Equation (2)).

$$C = 280,000N \left(\frac{Q}{s} \right)^{0.3} \quad (2)$$

where N is the number of main units, S is the reactor's conversion, and Q is the plant's capacity (t/year), where C is the ISBL's capital cost (in £).

The expense of off-site upgrades and changes needed for the plant to function is covered by the OSBL. Infrastructure improvements related to connecting gas and water, among other things, to the primary plant are part of these advancements. The proportion of the ISBL used to determine the OSBL value, which can be anywhere between 10 and 100% (Mata et al., 2018). Using 30% as the OSBL proportion for this investigation.

The fee for the engineering package, which includes the operating handbook, pipelines and instrumentation drawings, equipment lists, and constructive drawings, is covered by the engineering cost. In this context, 10% of the entire capital cost is considered. The expenditure to cover any unforeseen project charges is referred to as the contingency amount. The normal contingency proportion (ISBL + OSBL) is 10% of the capital cost. This proportion is often considered using proven technology. As a result, a value of 10% is considered for the suggested technology.

The cost of operating, constructing, and commissioning a plant in accordance with its design is included in the working capital. In the most recent work, working capital was defined as 15% of the direct capital cost (OSBL + ISBL) (Hosney et al., 2020a). The start-up cost has been finally determined as 10% of ISBL + OSBL.

2.10.2. Operational costs

Variable and fixed production costs are two categories under which the operational expenditures may be divided. As a whole, the following expenses are included in the fixed production costs: labour, administrative expenditures, maintenance, property taxes and insurance, land rent, and environmental fees. Whether a project operates at its peak efficiency or not, these expenses still persist (Thoppil and Zein, 2021). Contrarily, there is a clear link between the output and production rate and the variable production costs. This cost includes expenses for consumables, product shipping costs, packing, and waste stream disposals such as used lipases. It also includes charges for raw materials (such as MB, BA, and biocatalyst), utilities (such as electricity, steam for heating, and cooling water), and any other applicable costs. Therefore, efficient resource usage, such as reducing energy use and raw material losses, can ensure a decrease in variable costs overall.

2.10.3. Indicators of economic feasibility

The money from product sales for the desired primary output and any additional co-products acquired make up the revenues connected with an investment. In our instance, credits for the methanol produced by the transesterification of BA and MB were calculated.

Gross margin is an additional indicator that influences economic viability. To determine this, subtract the cost of the raw ingredients from the sales proceeds from the product. As a result, the gross margin number sheds lighter on the profits kept from sales that are unrelated to the cost of production. In the suggested research, the cost of the raw materials (MB and BA) has a major impact on the gross margin since they account for around 86% of the cash cost of production (CCOP).

To get more in-depth information about a project's economic viability, profit must be determined. Here, the CCOP becomes crucial in determining the profit value. The total fixed and variable production costs are also included in the CCOP. After then, the profit is determined by deducting the CCOP from the biodiesel sales. The revised profit estimate still represents a gross profit. To get the net profit, the corporation tax is subtracted from the gross profit. Depending on the year and the nation where the project is carried out, the corporation tax rate varies. A corporation tax rate of 22.5% was taken into account in this analysis. Equation (3) can be used to compute the amount of tax paid.

$$\text{Amount of tax paid} = \text{tax rate} \times \text{taxable income} \quad (3)$$

Anyone can determine their taxable income by subtracting their tax allowance from their whole earnings. The cost of depreciation is a well-known example of a tax allowance.

Where cash flow will predominate, the depreciation charges can be calculated using the falling balance depreciation technique. For the purposes of this analysis, a depreciation rate of 10% and a 5-year project recovery period were considered. The 15-year recovery period for the suggested project was also considered in this assessment. Such a recovery period has always been necessary and has shown that the investors fully comprehend the process's economic viability (Apostolakou et al., 2009). Therefore, a discount rate of 11% was considered in the suggested economic analysis.

An economic indication known as the net present value (NPV) calculates the difference between the current values of cash inflows and outflows (Mata et al., 2018). In order to account for the time worth of money, this value annualizes the present value using the interest rate. Equation (4) is used to estimate the NPV.

$$NPV = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i)^n} \quad (4)$$

where CF_n is the cash flow in years, i is the discount rate, and t is the project life in years.

Net income to investment is expressed as a ratio, or ROI. An investment's returns on investment (ROI) are likely to outperform its expenses if the ROI % is high. ROI is a ratio used to assess the effectiveness of various investments as an indicator of investment profitability. ROI is estimated using the following Equation (5):

$$ROI = \frac{\text{Cumulative net profit}}{\text{Plant life} \times \text{Initial investment}} \times 100 \quad (5)$$

3. Results and discussion

3.1. Optimization of BB production in CHR and MR

Knowing the kinetics of the reactions is essential to evaluate the behavior of the biocatalyst and the response over time. In addition, it is a fundamental parameter for enzymatic catalysis once the formation of the product increases with time. Therefore, time as an independent variable can mask the effects of other variables. Thus, the CHR and MR reaction kinetics were evaluated using the central point conditions (Table 1, tests 15–17) of 2^3 CCRD to determine the reaction time suited to perform the optimization study for BB production.

Fig. 2 shows the kinetics of enzymatic transesterification of MB and BA in CHR and MR, wherein it is possible to note that, unless the two ways of heating, the reaction kinetics was the same, presenting after 7 h a BB production of 65%. Similar results were found in the study of acylation catalyzed by Novozym 435 of BA with benzoic anhydride under conventional heating and an ultrasound bath where a production of approximately 60% of BB was achieved in 8 h (de Menezes et al., 2020). It is worth of note that, Shinde and Yadav (2014) performing the transesterification in a microwave system with organic solvent, reached a maximum conversion of 79% in 6 h; that is only 14% higher than that obtained in the systems without solvent under study. In virtue of this, for the other CCRD assays, 4 h was chosen since it presented production above 50%.

Once defined the reaction time, the effects of other process variables such as molar ratio, temperature, and enzyme concentration, on the BB production, were evaluated by a CCRD for both heating systems. The results are shown in Table 1 and highlight BB's excellent production, with conversion above 61% in 4 h for both systems. However, the MR showed a slightly higher production (70%) compared to the CHR (67%). The excellent reproducibility of the tests performed is demonstrated by the repetition of the central point (Table 1, runs 15–17), which showed very low pure errors (CHR $(4.67/4467.01) \times 100 = 0,10\%$; MR $(6/4127.73) \times 100 = 0,14\%$) and tends towards zero for the CHR and MR.

The CCRD results were treated statistically by analysis of variance (ANOVA) to evaluate the effect of independent variables and verify whether the empirical model can reproduce the experimental data of the BB production in CHR and MR. From the ANOVA, an F test was performed, indicating significant differences between the means ($p \leq 0.1$) since the results were F_{calc} equal to 37.7 and 17.4 for the CHR and MR, respectively. The F_{tab} was 2.4 for both systems, presenting F_{calc} 15 and 7 times greater than F_{tab} . The percentage of

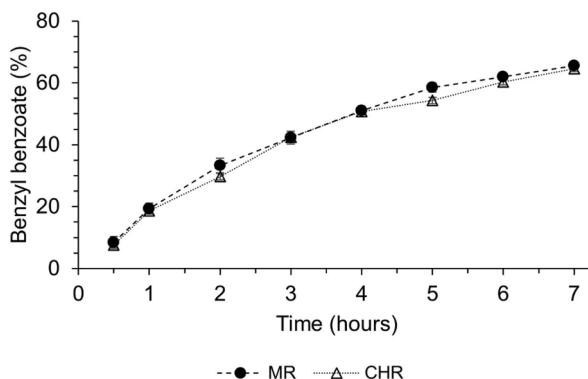


Fig. 2. Kinetics of BB enzymatic production in CHR and MR performed under CCRD central point conditions (Table 1, trials 15–17).

model variation was evaluated through R^2 , showing 96 and 85% values for CHR and MR, respectively (Table 2). From the results obtained, it can be concluded that there is a relationship between the variables, whose ANOVA showed an excellent fit of the model with the experimental data. Furthermore, the value of R^2 shows that Equations (6) and (7) were able to explain the variability of the experimental data for the BB production:

$$Y_1 = 51.27 + 3.49RM - 8.93RM^2 - 3.17T^2 + 15.06E - 2.74E^2 - 6.12RME \quad (6)$$

$$Y_2 = 48.17 + 8.78RM - 6.45RM^2 + 13.58E - 3.27E^2 \quad (7)$$

where Y_1 and Y_2 are BB production (%) in CHR and MR, respectively, RM is BA to MB molar ratio, T is temperature, and E is enzyme concentration.

The good fit of the models to the experimental data is observed by the BB production predicted by Equations (6) and (7) and by the low relative errors found for the CHR and MR (Table 1).

Evaluating the effect of the independent variables on the BB production in Equations (6) and (7), it is observed that the molar ratio and enzyme concentration in the linear function presented a significant positive effect for both heating systems, where the increase in these led to more excellent production. However, for the quadratic function of these variables, the effect was significantly negative; that is, after a certain point, the increase of these variables did not occur an increase in the BB production. Furthermore, the quadratic function of temperature and the interaction between molar ratio and enzyme concentration significantly negatively affected the reactions in CHR.

With Equations (6) and (7), was possible to generate the response surfaces to produce BB in CHR and MR. Figs 3a, b and c show the response surfaces for the CHR and Fig. 3d for the MR. In Fig. 3a, it is observed that the region of maximum BB production (67%) was obtained around the conditions of the central point with molar ratio and temperature close to 1:6 and 73 °C, respectively. Figs 3b and c show the region of maximum production at the highest enzyme concentrations. However, when using an excess of the enzyme (trial 14, Table 1), no significant increase in the BB production was observed.

A similar behavior was observed for the reactions conducted in microwaves and shown in Fig. 3d, where the region of maximum BB production (70 and 62%) was close to the central point for the molar ratio and at the highest enzyme concentrations (trials 8 and 14, Table 1). According to Gryglewicz et al. (2000) and Shinde and Yadav (2014), the use of excess of MB to alcohol in transesterification reactions resulted in a higher benzoate esters conversion. Likewise, for Shiki et al. (2022), reactions conducted with solvent and an excess of benzoic anhydride led to higher conversions. On the other hand, de Meneses et al. (2020) and Song and Chang (2022) concluded that an excess of alcohol is essential for the solubilization of the benzoic anhydride, thus facilitating its diffusion through the pores of the enzyme support.

Temperature is an essential parameter in enzymatic reactions since each enzyme has an optimal temperature range; however, temperatures above the optimum can result in the loss of tertiary structure, causing enzyme inactivation. Furthermore, temperature aids in the solubilization of reactants improving the molecular-collision interface (Badgujar and Bhanage, 2014; Sá et al., 2017). In this work, it was observed that the optimal temperature for the BB production was in the central point (73 °C) for the CHR (Fig. 3a,c) while, it was of 80 °C for the MR (Table 1). For both heating systems at extreme temperatures, higher or lower than the optimum, a decrease in BB production was observed.

Based on the results presented by the CCRD, the optimized conditions for the enzymatic transesterification of MB and BA in a solventless system in 4 h of reaction time were a molar ratio of 1:6 and 1:10, the temperature of 73 and 80 °C and enzyme concentration of 18.4 and 15% for CHR and MR, respectively (trials 8 and 14, Table 1).

3.2. Effect of enzyme concentration in CHR and MR

The CCRD results showed that increasing enzyme concentration increases BB production for both heating systems. In solventless systems, lipase activity may be directly or indirectly affected by the molar ratio of substrates, and the magnitude of these effects on li-

Table 2
Analysis of variance (ANOVA) of the estimated model for optimizing BB's CHR and MR enzymatic synthesis.

Variation source	Sum of square	Degrees of freedom	Mean square	F _{calc}	P-value
CHR					
Regression	4467.01	6	744.50	37.77	2.63E-06
Residuals	197.10	10	19.71		
Lack of Fit	192.44	8	24.05	10.31	0.09139
Pure Error	4.67	2	2.33		
Total	4664.12	16			
Regression coefficient: $R^2 = 96\%$; $F_{0.90; 6; 10} = 2.46$					
MR					
Regression	4127.73	4	1031.93	17.44	6.12E-05
Residuals	710.16	12	59.18		
Lack of Fit	704.16	10	70.42	23.47	0.041536
Pure Error	6.00	2	3		
Total	4837.88	16			
Regression coefficient: $R^2 = 85\%$; $F_{0.90; 4; 12} = 2.48$					

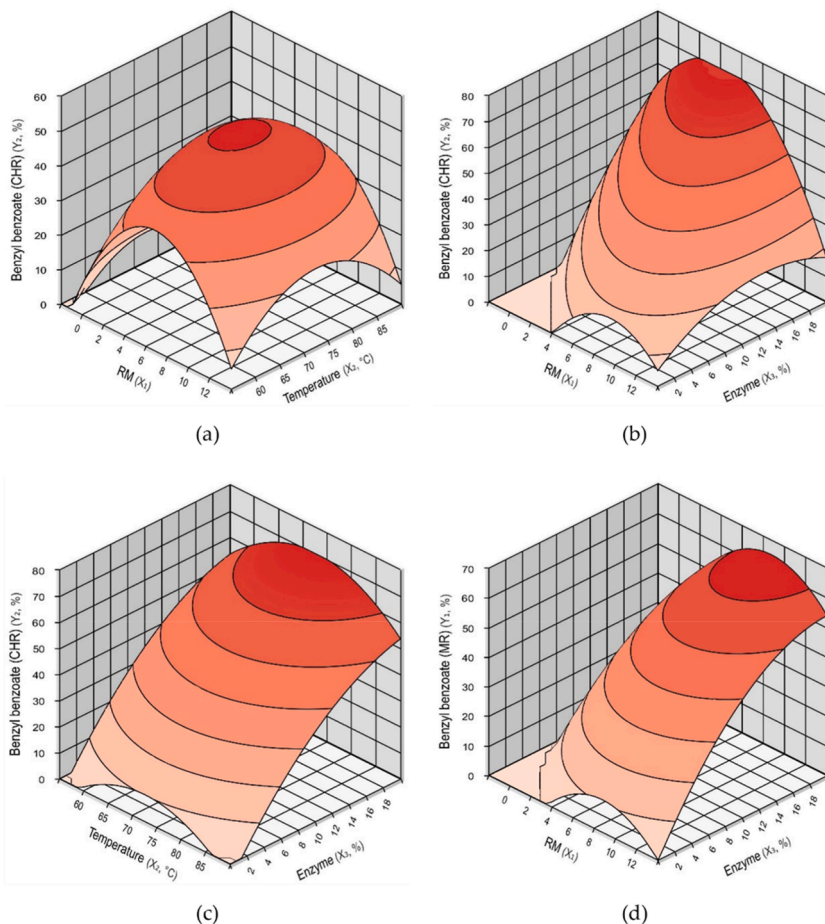


Fig. 3. Response surface for the BB enzymatic production in CHR (a, b, c) and MR (d) in solventless system.

pase activity may be modulated by the enzyme concentration present in the reaction medium. In this style of reaction system, the concentration of substrates is very high and, consequently, the influence on the enzyme of these properties of the substrates (Sousa et al., 2021). Therefore, the effect of enzyme concentration was evaluated by a kinetic study performed under experimental conditions taken from points on the response surfaces, in order to validate the model and understand the kinetic behavior of the reaction in CHR and MR (Figs. 4 and 5). The results for the CHR (Fig. 4) show that with increasing enzyme concentration, the reaction rate is higher. However, after 48 h the reactions at enzyme concentrations of 5 and 10% presented a difference in the BB production lower than 3%,

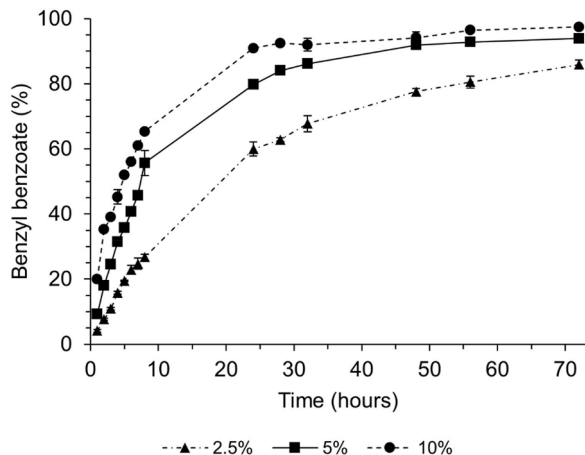


Fig. 4. Effect of enzyme concentration on reaction kinetics for BB production in CHR.

which reached 94 and 97% after 72 h, respectively. Even with a 50% reduction in enzyme concentration (5%), a high production of BB was obtained after 72 h (86%), which may represent a significant reduction in the production cost. In addition, in CHR, the production in 4 h (16, 31, and 45% for 2.5, 5, and 10% of enzyme, respectively) was very close to those calculated by Equation (6) (22, 33, and 51% for the tested enzyme concentrations), proving that the equation provided by the CCRD is predictive for the range of conditions studied.

Fig. 5 shows the kinetic study performed in MR, and how the production of BB increased with the enzyme concentration. However, the production obtained using 16 and 20% enzyme concentrations had very similar kinetic behavior with an average difference in the BB production of less than 5%. After 8 h, the production achieved with the two higher enzyme concentrations was around 82%, with a difference of less than 2%. The BB production in 4 h of reaction (51, 63 and 68% for 20, 16 and 10% of enzyme, respectively), was very similar to that predicted by Equation (7) (48, 60, and 63% for the same concentration of enzyme), confirming the excellent fit of the experimental data with the model. This same behavior of the enzymatic kinetics obtained for the two reaction systems, where from a certain amount of biocatalyst, its increase does not lead to a significant increase in production, was observed by Venturi et al. (2023).

The results for maximising BB production by enzymatic transesterification in a solventless system showed excellent (97%) and good (82%) productivity for CHR and MR, respectively, compared to data already available in the literature, as seen in Table 3.

3.3. Lipase reusability in CHR and MR

The reuse of enzymes is an important parameter to be evaluated in bioprocesses, as it must keep process efficiency and will directly influence the process cost. With this, the Lipozyme 435 reuse was evaluated in the maximized conditions in CHR and MR to investigate the enzyme stability under the two heating systems. The results show that in both heating systems, the enzyme presented excellent operational stability up to the fourth cycle of use, with no significant decrease in the BB production (Fig. 6). However, from the fourth cycle to the seventh, a production loss of approximately 32% is observed for both heating systems. The decrease in ester pro-

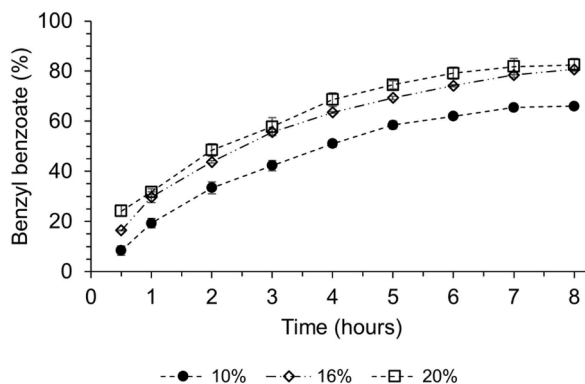


Fig. 5. Effect of enzyme concentration on reaction kinetics for BB production in MR.

Table 3

A comparative profile of the enzymatic production conditions of BB.

Substrate ^a	Molar ratio	Lipase	Enzyme amount	T (°C)	Agitation (rpm)	Solvent	Heating mode ^b	Time (h)	Production (%)	Reference
MB + BA	1:2	Novozym 435	0.3 g	55	–	Solventless	C	100	87	Gryglewicz et al. (2000)
MB + BA	1:2	Novozym 435	0.02 g cm ⁻³	60	300	n-heptane	M	6	79	Shinde and Yadav (2014)
BAn + BA	1:6	Lipozyme TL IM	6%	50	150	Solventless	C	6	92	de Meneses et al. (2020)
	1:9	Lipozyme RM IM	10%	50	150	Solventless	C	24	73	
	1:9	Novozym 435	10%	50	150	Solventless	C	24	60	
	1:9	Lipozyme TL IM	10%	50	–	Solventless	U	8	76	
	1:9	Lipozyme RM IM	10%	50	–	Solventless	U	8	88	
BAn + BA	1:5	Novozym 435	10%	60	150	<i>t</i> -butanol	C	24	32	Shiki et al. (2022)
	1:5	Lipozyme RM IM	10%	40	150	<i>t</i> -butanol	C	24	51	
BAn + BA	1:9	Silica cross-linked CalB particles	0.5 g	50	n.d.	Solventless	C	24	67	Song and Chang (2022)
	1:9	Native CalB	0.185 g	50	n.d.	Solventless	C	24	75	
MB + BA	1:6	Lipozyme 435	10%	73	700	Solventless	C	72	97	This study
	1:6	Lipozyme 435	16%	73	–	Solventless	M	8	82	

^a MB = methyl benzoate, BAn = Benzoic anhydride, BA = benzyl alcohol.

^b C = Conventional, M = Microwave, U = Ultrasound. n.d. = not described.

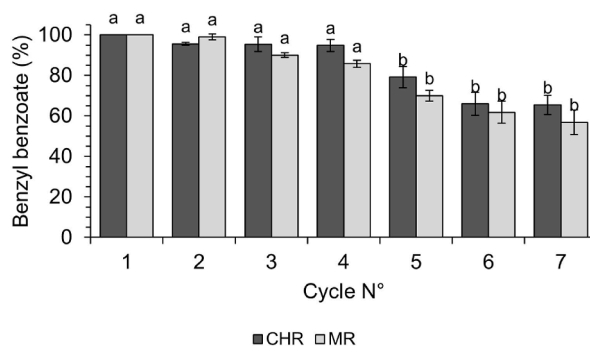


Fig. 6. Reusability of Lipozyme 435 lipase in the BB production in CHR and MR in solventless systems.

duction is correlated with lipase deactivation/denaturation due to thermal effects, to the contact with the substrates and the product, allied with the long reaction time and the decrease in the integrity of the solid support that can affect lipase binding (de Sousa et al., 2022; Lerin et al., 2011).

3.4. Economic considerations for BB production in CHR

3.4.1. Process description

The enzymatic production of BB from the transesterification reaction of MB and BA was simulated in ASPEN PLUS with the flow sheet proposed in Fig. 1. The two reactants (BA and MB) are mixed in a molar ratio of 1:6 in Mix-1. The resultant mixture is then heated to a temperature of 73 °C in heat exchange HX-1. The preheated blend is then sent to the main enzymatic transesterification reactor at a flow rate of 94 t/d. After the enzymatic reaction time is elapsed, the main product BB undergoes two main downstream processes to be purified to a concentration of 99.9%. The first purification step is carried out through separator SEP-1; the main function of this separator is removing the formed methanol during the reaction course. The separated methanol co-product of 3 t/d can be considered a credit contributing to the process's profitability. After methanol removal, the mixture is sent to two-step heating in HX-2 and HEATER for further heating the product-containing mixture from 125 °C to 300 °C to remove MB in Dist-1. Such temperature is typically determined by maximising the separation efficiency, in other words, by achieving the desired product purity, which is > 99% in our case. In this study, ASPEN PLUS showed that the feed stage should be at 6.7 (approximately 7). Fig. S4 shows a feed temperature of 300 °C matches the feed stage 6.7. The previously calculated data ensure two functions: (1) an optimum separation efficiency by obtaining BB at >99% and (2) the lowest reboiler duty. To obtain pure BB of >99%, one distillation column (Dist-1) is used to separate BA efficiently. The column (Dist-1) has a reflux ratio of 0.723 and consists of 18 stages, and it produces BB with a concentration of 99.9%. It should also be mentioned that the MB excess exits the column (Dist-1) from the top and returns to the reactor upstream.

It is worth mentioning that the proposed design involves an interesting heat recovery system, where the hot condensed MB (stream MTHBNZRI) is used to heat the fresh raw materials HX-1. This results in heating the raw materials from 25 °C to 73 °C without additional energy requirements. The same concept is applied to heat the mixture entering HX-2 by the hot BB (BT-PRDCT) being cooled simultaneously. The final product is cooled from 322 °C to 120 °C without an additional cooling requirement.

3.4.2. Economic evaluation

For manufacturers, determining the direction of their investment choice is greatly influenced by the entire cost of the capital expenditure. The initial primary cost to consider is hence the cost of the inside battery limits (ISBL) plant. Thus, the suggested enzymatic process resulted in an ISBL cost of \$3,928,655 as determined by Bridgewater's technique, as shown in Table 4.

The overall cost of production, in addition to the ISBL cost, is a key factor in establishing a process's viability. As an illustration, Table 5 reveals that the entire cost of manufacturing for BB is 2847 \$/t.

The difference between the present values of the cash inflows and outflows over a period (in this study, 15 years) is known as the net present value (NPV). To be economically feasible, a procedure needs a positive NPV. An overview of the NPV for BB production is

Table 4
Summary of total capital investment.

Cost Parameter	Cost (\$)
ISBL	3,928,655.50
OSBL	1,178,596.65
Total Capital Cost	5,107,252.16
Contingency Cost	510,725.22
Engineering Cost	510,725.22
Fixed Capital Cost	6,128,702.59
Working Capital	766,087.82
Start-up Expenses	510,725.22
Total Capital investment	9,034,729.06

Table 5
Summary of total cost of BB production.

Reactants	Cost (\$)	Enzymatic Process	
		Amount (t)	Price (\$)
Raw materials			
Benzyl Alcohol (55% of plant capacity)	2.15/kg	10.056	21,620.62
Methyl Benzoate (45% of plant capacity)	2.03/kg	12.644	25,667.12
Lipozyme 435 (1kg/3 tons of BB)	1082/kg	6.567	7105.13
Utilities			
Steam	0.037/KWh	16,232.88	600.6
Variable production cost/day			54,993.5
Packing	1.0%		550
Repair and maintenance	0.5%		275
Waste stream disposal	0.5%		275
Total production cost/day			56,093.35 \$/d
Total production cost/ton			2847.38 \$/t
Total production cost/year			18,510,806.00 \$/y
Gross Profit/d ^a			986.65 \$/d
Gross Profit/y ^{**}			325,594.00 \$/y

^a BB price is 2800 \$/t, methanol price is 640 \$/t. ^{**} One year is assumed to contain 330 days.

shown in Table 6. The designing stage of the project begins in year 1 and cash flow begins. The building and installation stages of the project, which take place in year 2, are presumptively included. As of year 3, the facility is operating at full capacity, and the gross profit has started to be reduced by depreciation costs.

According to Table 6, the proposed approach has a positive NPV and ROI of \$118 million and 46.85%, respectively. This indicates a successful and valuable investment.

Table 6
Summary of NPV for BB production using the enzymatic technology.

Project year	Capacity	Investment	Charge of depreciation	Investment (\$)	Expenses of operation (\$)	Gross profit (\$)	Expenses of depreciation (\$)	Tax income (\$)	Tax paid (\$)	Cash flow (\$)	Discounted cash flow (\$)	Pre-tax (\$)	
1	0	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
2	0	100	0	-9,034,729.06	0.00	0.00	0.00	0.00	0.00	-9,034,729.06	-8,139,395.55	-9,034,729.06	
3	100	0	0.2	0	-18,510,806.01	325,593.99	65,118.80	260,475.20	58,606.92	18,777,793.08	15,240,478.11	18,719,186.16	
4	100	0	0.32	0	-18,510,806.01	325,593.99	104,190.08	221,403.92	49,815.88	18,786,584.12	13,730,160.46	18,736,768.24	
5	100	0	0.192	0	-18,510,806.01	325,593.99	62,514.05	263,079.95	59,192.99	18,777,207.01	12,369,513.93	18,718,014.02	
6	100	0	0.1152	0	-18,510,806.01	325,593.99	37,508.43	288,085.57	64,819.25	18,771,580.75	11,143,706.24	18,706,761.50	
7	100	0	0.1152	0	-18,510,806.01	325,593.99	37,508.43	288,085.57	64,819.25	18,771,580.75	10,039,374.99	18,706,761.50	
8	100	0	0.0576	0	-18,510,806.01	325,593.99	18,754.21	306,839.78	69,038.95	18,767,361.05	9,044,481.98	18,698,322.10	
9	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	8,148,181.96	18,689,882.70	
10	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	7,340,704.47	18,689,882.70	
11	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	6,613,247.27	18,689,882.70	
12	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	5,957,880.42	18,689,882.70	
13	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	5,367,459.84	18,689,882.70	
14	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	4,835,549.41	18,689,882.70	
15	100	0	0	0	-18,510,806.01	325,593.99	0.00	325,593.99	73,258.65	18,763,141.35	4,356,350.82	18,689,882.70	
					Cumulative profit	4,232,721.93	ROI=	46.85%			NPV	117,669,378.25	

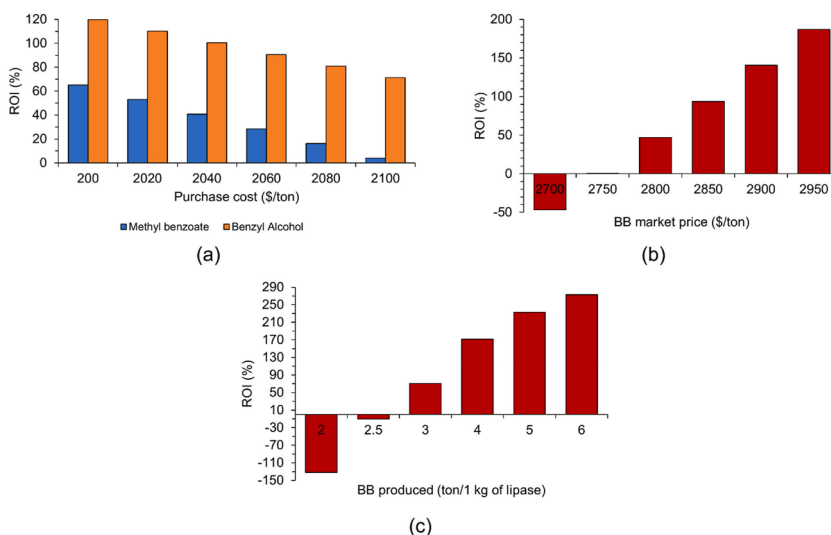


Fig. 7. Sensitivity of methyl benzoate and benzyl alcohol purchasing price on ROI (a); ROI dependency on BB market price (b), and Sensitivity of BB quantity produced per 1 kg of Lipozyme 435 on ROI (c).

3.4.3. Sensitivity analysis

The sensitivity analysis offers a comprehensive economic understanding of cost variation in the BB production process. The economic factor, namely ROI, was the focus of this investigation. The feedstock price played a significant role in the cost of producing BB, where MB contributed 47%, BA 40% and lipase 13% of the total costs. Also, the market price of BB potentially affected the process profitability. The positive ROI indicates a profitable production process. The sensitivity analysis of MB and BA purchase costs on ROI is shown in Fig. 7a, respectively. When studying the effect of MB price variation on ROI, the BA cost was fixed at 2150 \$/t, while when studying the impact of BA price variation, the MB price was set at 2030 \$/t. The price of Lipozyme 435 was fixed in all cases at 1082 \$/kg.

Fig. 7a shows that the ROI per cent decreased dramatically from 65.22% to 3.98% by varying the price of MB from 2000 \$/t to 2100 \$/t, respectively. Also, the ROI decreased from 119.9% to 71.2% when ranging the BA price from 2000 \$/t to 2100 \$/t, respectively. It can be noted that the ROI per cent value is more affected by varying the price of MB than the price of BA, mainly because of the higher mass stoichiometric portion of MB (about 55%) compared to BA (45%) while feeding the esterification plant. Likewise, the sensitivity analysis of BB price on the ROI was investigated. As shown in Fig. 7b, not surprisingly, the ROI increased as the market price of the final product increased at fixed MB and BA prices.

Nevertheless, it is a common practice that the BB price is directly affected by the price of MB and BA. Therefore, the ROI may not be significantly affected by the BB price variation, as the relation between BB, MB, and BA prices is directly proportional.

Finally, regarding Lipozyme 435, it was crucial to study the sensitivity of enzyme consumption on ROI. In this sense, the quantity of BB produced was varied against consuming 1 kg of Lipozyme 435. Fig. 7c shows the sensitivity of the amount of product per one unit mass of Lipozyme 435. The tested range of BB production quantity was from 2 t to 6 t. The study considered the following market prices of 2150 \$/t, 2030 \$/t, 2800 \$/t, and 1082 \$/kg for BA, MB, BB, and Lipozyme 435, respectively. Fig. 7c shows a negative ROI value at 2 and 2.5 t of product per 1 kg of lipase; the negative ROI illustrates that the project is not profitable. Therefore, the production quantity of 2.65 t of product per 1 kg of lipase was seen as the minimum quantity that gave a positive ROI.

4. Conclusions

The results of this study show high BB production with more significant conversion values with respect to those reported in the literature (Table 3). The biocatalytic production of benzyl benzoate is technically feasible using commercial lipases in a solventless system, achieving conversions greater than 90% and 80% with CHR and MR, respectively, with proper adjustment of the enzyme load. The commercial enzyme showed good reusability, remaining stable for up to 4 reaction cycles with both reactor types. The economic assessment of the enzymatic transesterification process of MB with BA in CHR suggested a promising investment opportunity, with an investment cost of \$9,034,729.06. This fact is confirmed by the positive NPV and ROI calculations of \$117,669,378.25 and 46.85%, respectively, indicating that the assumptions and conditions here investigated and presented are a profitable process. Overall, the suggested enzymatic process proved to be an environmentally friendly and economically profitable method for cleanly synthesizing an essential agent against scabies.

CRedit authorship contribution statement

Simona Aprile: methodology, formal analysis, validation, data curation; Valentina Venturi: methodology, validation; Francesco Presini: formal analysis, validation; Ahmad Mustafa: methodology, software, data curation, writing – original draft preparation, writ-

ing – review & editing; M. Shaaban Sadek: formal analysis, software; Abrar Inayat: validation, software; Daniela Remonato: data curation, writing – original draft preparation; Pier Paolo Giovannini: conceptualization, writing – original draft preparation, writing – review & editing; Lindomar Alberto Lerin: conceptualization, methodology, formal analysis, validation, software, data curation, writing – original draft preparation, writing – review & editing, project administration, funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scp.2023.101257>.

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