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# Highly selective synthesis of glyceryl monostearate via lipase catalyzed esterification of triple pressed stearic acid and glycerin

Ahmad Mustafa [a,b,*], Rehab Ramadan [c], Fumiya Niikura [d], Abrar Inayat [e], H. Hafez [f]

[a] Faculty of Engineering, October University for Modern Sciences and Arts (MSA) 12566, Egypt
[b] Center of Excellence, October University for Modern Sciences and Arts (MSA) 12566, Egypt
[c] Survey of Natural Resources Department, Environmental Studies and Research Institute, University of Sadat City, P.O. 32987, Egypt
[d] Process Engineering Research Laboratories, Lion Corporation, 7-2-1 Hirai, Edogawa-ku, Tokyo 132-0035, Japan
[e] Department of Sustainable and Renewable Energy Engineering, University of Sharjah, 27272 Sharjah, United Arab Emirates
[f] Nanotech. Dept., Environmental Studies and Research Institute, University of Sadat City, P.O. 32987, Egypt

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ABSTRACT

The synthesis of value-added products from glycerin is an attractive research area that aims to valorize this abundant by-product of the biodiesel industry. Thus, raising the economic feasibility and mitigating the environmental consequences. In this work, an alternative green, energy-efficient, and selective enzymatic (ENZ) esterification of triple-pressed stearic acid (TPSA) and glycerin was carried out to produce glyceryl monostearate (GMS). Response surface methodology (RSM) was used to optimize the reaction conditions; the optimum conditions were a 6:1 glycerin to TPSA molar ratio, 8% w/w Lipozyme 435 amount, and 350% w/w solvent amount. It is worth mentioning that the solvent addition greatly enhanced the yield of GMS compared to the conventional autocatalytic esterification (AUT) process. The proposed ENZ approach was also economically assessed, and the findings were compared to those of the AUT method. Considering a plant capacity of 4,950 t year$^{-1}$ and an interest of 11%, the total capital investment of the ENZ GMS production was 1.8 times cheaper than the AUT process, suggesting a favorable investment opportunity. In addition, the positively obtained net present value (NPV) and return on investment (ROI) for the ENZ process's total production costs reveal the proposed method's economic feasibility. The suggested approach for synthesizing GMS can be seen as a baseline for a cleaner large-scale monoglycerides synthesis.

## Introduction

The biodiesel industry generates considerable quantities of glycerin as a byproduct. With the increasing production of biodiesel, the market has become saturated with such polyalcohol [1,2]. The annually produced glycerin is already higher than the market need by six times [3]. Therefore, converting glycerin to other value-added products is of great importance. Glyceryl monostearate (GMS) is one of the most commonly used monoglycerides globally that finds many applications in cosmetics, personal care, and food sectors [4]. GMS is commercially produced by esterification or transesterification processes. In the transesterification process, hydrogenated palm stearin is reacted with glycerin, while in the esterification process, triple pressed stearic acid (TPSA) is esterified with glycerin [5]. The two approaches can be performed using enzymatic process (ENZ), chemical catalyzed process (CHEM) or autocatalytic process (AUT) that is performed without any catalyst.

The commercially available GMS is now being produced using CHEM or AUT technologies. Both processes use elevated reaction temperatures, resulting in random reactions with many byproducts [6,7]. Among the byproducts, di and triglycerides are generated instead of monoglycerides [8]. However, pure monoglycerides are often required to satisfy practical demands. Therefore, a distillation step is necessary to obtain pure monoglycerides [9]. However, conventional distillation can't be applied because of the low vapor pressure of glycerides [10,11]. Presently, to produce pure monoglycerides, the expensive short-path distillation unit is required [12]. Besides the considerable high investment of such a step, it requires high energy requirements resulting in significant environmental implications. Nevertheless, both CHEM and AUT processes are currently used for GMS production due to the accepted manufacturing cost owing to the cheap cost of catalyst/energy

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[*] Corresponding author at: Faculty of Engineering, October University for Modern Sciences and Arts (MSA) 12566, Egypt.
*E-mail address:* ammhamed@msa.edu.eg (A. Mustafa).

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[13].

Concerning the AUT process, the esterification reaction is catalyzed autocatalytically without using any catalysts. Instead, the effect of high temperature shifts the reaction toward completion. The CHEM esterification process, on the other hand, uses an acid-catalyzed process. Strong mineral acids, such as sulfuric acid and hydrochloric acid, are mainly used. In addition, heterogeneous catalysts such as cation-exchange resins [14] and zeolites are also employed [15].

On the other hand, lipases display cleaner esterification/transesterification reactions since the employed reaction temperature is much lower than the AUT process [16]. In addition, the selective mechanism offered by the enzymatic reaction yields pure monoglycerides instead of diglycerides and triglycerides, as in the AUT process. Thus avoiding the need to apply the subsequent energy-intensive short-path distillation process [17]. The lower energy consumption, equipment pieces, and land required for the plant suggest a simple and competitive enzymatic process that can compete with CHEM and AUT processes [18].

The solvation strategy is an interesting approach to the selective production of monoglycerides. Introducing a solvent into the reaction system enhances the miscibility and interaction of the reactants, namely, hydrophobic fatty derivatives and hydrophilic glycerin, thus promoting the reaction selectivity [19]. Many organic solvents, such as *tert*-pentanol, *tert*-butanol, acetone, acetonitrile, dioxane, n-hexane, and *iso*-octane, were used in enzymatic monoglycerides production [20]. The highest MAG yield was obtained with tertiary alcohols such as tert butanol [21]. Previous studies have suggested monoglycerides percentage improvement when solvents are introduced to the reaction mixture [22]. For instance, Monoolein was enzymatically synthesized in a high yield of 93.3% using a combination of a binary solvent of tertiary-pentanol and tertiary-butanol at a ratio of 20:80 v/v in a two-step process [5]. Glyceryl monostearate was also produced with a yield of 82.5% using a binary solvent solution (isopropanol/*tert*-butanol, 20:80, wt./wt.) in the presence of Lipozyme 435 [23].

To further prove the applicability of a proposed method, a study should evaluate if the proposed investment is economically feasible [24]. This article analyses the economic analysis in conjunction with relevant indicators, namely return on investment (ROI) and net present value (NPV). The values of these two economic analysis measures are highly suggestive of a system's profitability and viability [25]. This investigation considers a shelf life of 15 years, encompassing the years leading up to and beyond 2030, a turning point for sustainable development. The SDGs aim to provide universal access to modern, reliable, affordable energy services by 2030 [26,27]. This paper provides an integrated techno-economic evaluation for synthesizing glyceryl monostearate using ENZ and AUT methods. The capital and production expenses, net present value, and return on investment were calculated for both methodologies. As far as we are aware, no published study has examined the technical and economic feasibilities of producing glyceryl monostearate from triple-pressed stearic acid utilizing the ENZ process.

## Materials and methods

### *Materials*

Triple pressed stearic acid (60% palmitic acid and 40% stearic acid) was purchased from IOI Oleochemical Company (Pulau Pinang, Malaysia). Glycerin with a purity of 99.7% was donated by Oleo Misr for Oleochemicals Company (Sadat City, Egypt). Tertiary butanol, molecular sieves, 8–12 mesh beads, 4 Å, and GC standards (1 stearoyl-*rac*-Glycerin, Stearic acid, palmitic acid, 2-Monostearoyl, Glycerin, 1,3 glyceryl distearate, *rac*-1-Palmitoylglycero, and, 2-Monopalmitoyl Glycerin) were purchased from Sigma (St. Louis, MO, USA). Immobilized lipase, Lipozyme 435, *Candida antarctica* lipase, supported on a macroporous acrylic resin, 10,000 PLU/g donated by Novozymes A/S (Copenhagen-Denmark). All additional chemicals and solvents were of analytical grade.

### *Synthesis of glyceryl monostearate*

In the ENZ technique, the biocatalyst was Lipozyme 435; however, no catalyst was utilized in the AUT. The reaction time for the two processes (ENZ and AUT) was selected to be 3 h as per our previous study (18). In the esterification process, the alcohol molecule reacts with the carboxylic functional group to create a blend of mono, di, and triglycerides of both stearic and palmitic acids [28].

### *Enzymatic formation of glyceryl monostearate*

The esterification reaction between glycerin and TPSA was carried out in a 100 mL batch reactor for 3 h and 150 rpm. Three reaction conditions were optimized using RSM based on a central composite design. TPSA conversion to glycerides has been monitored through titration and confirmed by GC analysis. The titration method used 0.1 N of NaOH as titrant against the phenolphthalein endpoint. Aliquots of 200 μL were periodically withdrawn and immediately dissolved in a mixture of ethanol acetone (50%:50% v/v). The percentage conversion was calculated based on equation (1).

$$Conversion\ to\ ester(\%) = \frac{N - N^o}{N} \times 100 \tag{1}$$

Where N represents the volume of NaOH consumed without adding lipase, while $N^o$ is the volume of NaOH consumed at the end of the esterification reaction.

The selectivity of the enzymatic reaction toward monoglycerides formation was also calculated using GC analysis as per equation (2)

$$Selectivity(\%) = \frac{\%MAG}{\%MAG + \%DAG + \%TAG} \times 100 \tag{2}$$

Where MAG is monoglycerides (Alpha monostearin and alpha monopalmitin), DAG is diglycerides, and TAG is triglycerides.

All experiments in this work were analyzed in triplicate and the average conversion represents the mean measured readings. In addition, the conversion values obtained by GC were in good agreement with those obtained by titration, with a maximum error of 5%.

After the esterification time was elapsed, a simple purification step was conducted as per Mustafa et al. [17]. First, molecular sieves and immobilized enzymes were separated by filtration. Vacuum distillation was then used to separate tert butanol at 60 °C. The remaining mixture of glycerin and glycerides were separated by gravity due to the difference in densities. The heavy glycerin phase was separated from the bottom of the separating funnel and was used for further esterification processes. In contrast, the upper glycerides phase was sent for analysis.

### *Glyceryl monostearate synthesis by autocatalytic route*

TPSA and glycerin were esterified at molar ratios ranging from 1:1 to 4:1 at a reaction time of 3 h and a temperature of 190 °C. The lower molar ratio of 1:1 was selected as this is the exact reaction stoichiometry to form monoglycerides. While the higher molar ratio of 4:1 was considered as it was reported that higher glycerin to fatty acid molar ratios could drive the reaction toward completion [4]. The temperature of 190 °C was selected after preliminary runs. It was found that at less than 190 °C, the reaction proceeded very slowly, while higher temperatures are not generally recommended to maintain the yield of monoglycerides.

One liter four-necked round flask was used as a batch reactor, and it was heated using a heating mantle and agitated at 250 rpm using mechanical agitator. During the reaction course, purging a nitrogen blanket was applied to continuously separate the formed water vapor (by-product) along the reaction course. When the water vapor left the reactor, it was condensed by employing a condenser attached to the esterification reactor. After passing the esterification time, the reactor's content underwent a settling process in a separating funnel to separate glycerin (heavy layer) and glycerides (light layer). The glycerides layer was then sent for analysis to determine the conversion and monoglycerides content via titration and GC, respectively.

### *Examining the impact of solvent addition on the fatty acid conversion and monoglycerides yield (Enzymatic Route)*

First, a blank run without adding solvent was performed utilizing the following conditions: enzyme amount of 8% w/w, the molar ratio of 6:1 glycerin to TPSA, temperature of 70 °C, molecular sieves of 10% w/w, and reaction time of 3 h. Then, the impact of adding tert butanol as an organic solvent to the reaction mixture was examined against the percentage of fatty acids conversion and the yield of monoglycerides. The preliminary investigation was conducted using tert butanol amounts of 50, 100, 200, 400, and 600% w/w. The same reaction conditions in the case of solvent-free reactions were used with the solvated experiments. After the reaction time has been elapsed, the purification was conducted using the methodology described in Section 2.2.1. After purification, the yield of monoglycerides was measured by GC.

### *Product specification*

#### *Analyses via gas chromatography*

The SHIMADZU GC-2025 gas chromatograph was used to analyze the reaction blend. The GC has an automatic injector (AOC-20i). For the purpose of chromatographic separation, Agilent Inc.'s DB-1HT (non-polar, ID 0.25 mm, 30 m, and film thickness of 0.1 m) column was used. At a flow rate of 7.9 mL/min, helium was employed as carrier gas. The oven was set to 50 °C, held for 15 min, followed by a temperature ramp of 10 °C each minute to 395 °C, and then kept for 15 min. The temperatures of the detector and injector were kept at 400 °C and 380 °C, respectively. The silylation procedure was employed to derivtize materials. Monoglycerides, diglycerides, and triglycerides samples were dissolved in 20 mg/mL pyridine and portions of 1000 μL were allowed to react for 30 min at 80 °C with 500 μL N,O-bis(trimethylsilyl)trifluoroacetamide. 1 μL of the resulting solution was then injected with a split ratio of 1:10 into the GC column.

### *Experimental design*

The interactive impact of solvent quantity, enzyme amount, and molar ratio on the conversion of triple-pressed stearic acid to GMS was explored using RSM. The experimental design generated a total of twenty runs. First, the glycerin to TPSA molar ratio of 6:1 to 1:1 was considered. Enzyme amount was also optimized in the range from 2% to 8% w/w. The minimum limit of 2% w/w was selected because most literature experiences low conversion at lower than 2% w/w enzyme amount. While to maintain the economic feasibility of the proposed method, a maximum enzyme amount limit of 8% was considered. The solvent (tertiary butanol) amount was the third studied parameter. It was noticed that no previously published works have considered the solvation of the esterification reaction between TPSA and glycerin. Therefore, to identify the solvent investigation limit, preliminary experiments were performed. After conducting the solvent study, it was possible to identify the solvent range of 50%-350% w/w. The enzymatic esterification reaction temperature and the amount of molecular sieves are well discussed in the literature. Their effect on the enzymatic esterification and transesterification reactions has been well established. Thus, a molecular sieved percentage of 10% w/w and a temperature of 70 °C have been directly selected for all experiments in this paper. A polynomial equation of the second degree (equation (3) was constructed to fit the obtained data:

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} \sum_{i\leqslant j\leqslant 3} b_i b_j x_i x_j \tag{3}$$

Where Y is the fraction of glyceryl stearate produced, where bi, $b_{ii}$, $b_{ij}$, and $b_o$ are constant coefficients, and $x_{i,j}$ are uncode independent factories. Design Expert (version 11) was used for the response surfaces, the ANOVA, and the subsequent regression analysis.

### *Economic evaluation and model validation*

The economic assessment's fundamental goal is determining the project implementation and operation cost. In addition, this study can predict the project's feasibility by analyzing the cash flows. Therefore, the proposed techno-economic assessment can be considered a baseline to help the stockholders interested in investment in the oleochemicals business.

### *Summary of total capital expenditure*

The total capital investment can be the sum of the fixed capital investment, the working capital required and the starting expenses. For example, the cost of building, developing, installing, and modifying a factory is a fixed capital investment, an essential consideration when launching a new plant [29]. To prevent budgetary and operational failures, reliable estimates of these costs are necessary. This budget may be estimated by combining the costs associated with inside battery limitations (ISBLs), outside battery restrictions (OSBLs), engineering, and contingencies.

Consequently, the estimated ISBL might affect the total design cost of a process. Therefore, care must be taken to prevent miscalculations and accurately identify the ISBL's target. ISBL comprises essential manufacturing equipment, pipelines, instruments, pipes, and valves [30]. Numerous methodologies are employed to calculate the ISBL cost depending on the production capacity and industry. Taylor, Gore, Stallworthy, Bridgewater, Klumpar, Fromme, and Brown have commonly used estimation approaches [31]. The Bridgewater method was utilized in this work to determine the ISBL cost. To generate an accurate result, three variables must be calculated carefully: the plant capacity, the reactor conversion, and the number of main units. The plant's annual production capacity was set at 4,950 tonnes. Through laboratory studies, the conversion was observed. Using Bridgewater's method, the cost of ISBL was estimated as per equation [4].

$$C = 280,000\,N\left(\frac{Q}{s}\right)^{0.3} \tag{4}$$

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

The cost of off-site improvements and advancements required for the functioning of the facility must be considered. The aforementioned costs can be consolidated into the OSBL cost. These costs include infrastructure activities like water and gas connections with the plant. A percentage that ranges from 10% to 100% of the ISBL cost is granted for this cost [32]. In the proposed study, a percentage of 30% was selected.

The contingency budget is the amount to cover unanticipated project expenses. The average contingency proportion is 10% of the total cost of capital (ISBL + OSBL). A percentage of 10% was thus evaluated for AUT technology, whereas 15% was assigned for ENZ technology. This is because working with enzymes to produce oleochemicals in industrial scale is a new technique. The working capital cost includes the cost of buildings, commissioning, pre-commissioning, and plant operation. The working capital of the proposed study was considered 15% of the direct capital costs [29]. Lastly, the percentage of the expected startup expenses were considered 10% of ISBL plus OSBL.

### *Operating expenditures*

Among the operational expenditures, both variable and fixed

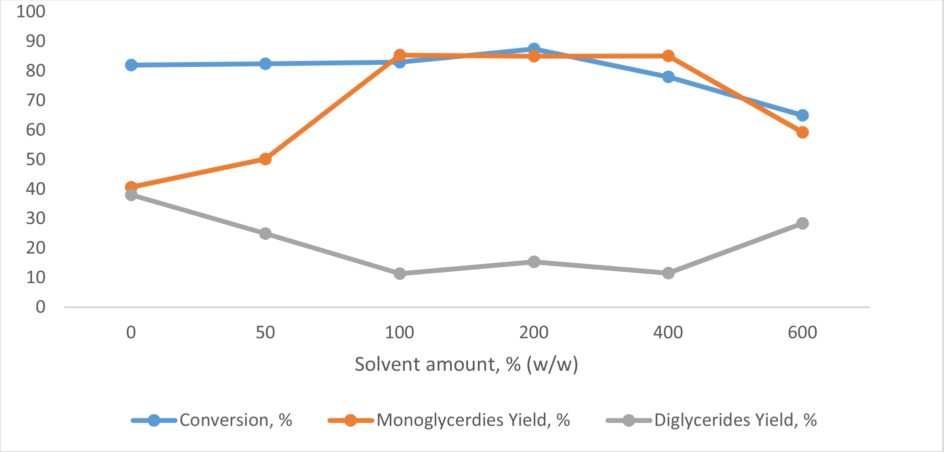

**Fig. 1.** Effect of solvent addition on conversion, monoglycerides yield, and diglycerdies yield (reaction time of 3 h, and molar ratio of 6:1 alcohol to fatty acid).

production costs are recognized. The fixed production costs include maintenance, labor, administrative overhead, property insurance and taxes, environmental fees, and land rent. These expenses are present regardless of a project's level of performance. In comparison, the variable product costs involve physical raw materials/products (e.g., glycerin, triple-pressed stearic acid, T-butanol, and lipase). It also includes the cost of utilities and services required for production (e.g., cooling water, electricity, and steam for heating). Other variable production costs include packaging, product shipping, and waste stream disposals. Optimal resource use, like decreased raw material losses, energy saving, and more prolonged enzyme usage, may thus assure an overall reduction in variable costs.

The calculations of the mass and energy balance were carried out considering the following:

- The AUT method consists of three operating units: TPSA esterification, deglycerination, and short-path distillation. After deglycerination, the recovered glycerin is returned and mixed with the fresh glycerin.
- After short-path distillation, the separated DAG and TAG are blended with the TPSA and glycerin to start a new reaction.
- The AUT selectivity toward monoglycerides formation is 42% (obtained experimentally). This means that the remaining fractions are DAG (dipalmitin and distearin) and TAG (tripalmitin and tristearin).
- For the AUT process, the power and energy consumptions of 22677 MJ/d, and 1650 kWh were directly provided by Oleo Misr for Oleochemicals Company, Egypt.
- The ENZ consists of two units, namely esterification and purification. In addition, the ENZ selectivity towards MAG formation is 88.3% (obtained experimentally)
- The AUT process's losses are 2.12 times higher than the ENZ process. Such a factor was considered based on dividing the MAG yield of the ENZ process over the AUT process.
- The use of lipase was considered to be 1 kg for every three tones of glyceryl monostearate produced. This consumption was approximated as per the reported operational stabilities of Lipozyme 435 [8,33].

### *Economic viability indicators*

First, investment revenues are the total income from selling the main and untargeted products [34]. In our study, no significant byproduct losses were considered due to considering di and triglycerides as part of the final product. Gross margin is an additional indicator of economic viability. This may be calculated by deducting the revenues of the products' sales from the consumed raw materials. Therefore, the gross margin figure gives more idea into the retained sales income unrelated to manufacturing expenses. In the proposed research, the cost of raw materials accounts for around 80% of the production cash cost, which has a substantial impact on the gross margin (CCOP). Gross margin is an additional indicator of economic viability. This may be calculated by deducting the revenues of the products' sales from the consumed raw materials.

The declining balance depreciation approach is useful for calculating depreciation costs when cash flow dominates. This research assumed a 10% depreciation rate for a 5-year project recovery period. This study also considers that the ENZ and AUT recovery times for the proposed project are 15 years. This recovery time is crucial because it demonstrates investors' thorough understanding of the operation's economic sustainability [27]. Therefore, the recommended economic study accounted for an 11% discount rate.

The net present value (NPV) represents the difference between the current values of cash inflows and cash outflows [32]. This value considers the time value of money by annualizing the present value using the interest rate. NPV was determined using equation S1 in supplementary:

Return on investment, or ROI, is the net income ratio to the amount invested. A high ROI percentage indicates that an investment's earnings exceed its costs. ROI is used to assess the effectiveness of different investments as a measure of investment success. ROI was computed using the equation S2 in supplementary.

## Results and discussion

### *Effect of solvation on conversion and monoglycerides yield*

The effect Solvation on conversion and monoglycerides yield is one of the main objectives of this work. When the enzymatic esterification was performed without medium solvation, the percentages of conversion and monoglycerides (Monostearin + Monopalmitin) were 82% and 40.65 %, respectively. It can be observed that the monoglycerides percentage was as same as that obtained using the AUT method. This suggests that Lipozyme 435 exhibited no selectivity towards MAG formation without medium solvation. Figs. S1 and S2 (supplementary) show the GC chromatograms of AUT and ENZ approaches, respectively.

The solvent addition to the reaction mixture was then examined, starting from 50% w/w and ending at 600% w/w (concerning the total weight of the medium) following the optimum conditions. Fig. 1 shows that the conversion reached its maximum value of 87.5% at a solvent amount of 200% w/w. This is mainly due to the reduced medium viscosity and, therefore, the better enzyme access to substrates offered by solvation [35]. However, the conversion decreased slowly with further

**Table 1**

Experimental and predicted values of conversion based on the experimentally designed 20.

| Run | Glycerin/stearic acid Molar ratio | Enzyme Load, % (w/w) | Solvent amount, % (w/w) | Experimental values of conversion, % | Predicted values of conversion, % | Residuals, % |
|---|---|---|---|---|---|---|
| 1 | 3.5 | 2 | 350 | 72.5 | 72.7 | −0.0002 |
| 2 | 6 | 2 | 200 | 75 | 77.9 | −2.90 |
| 3 | 1 | 2 | 200 | 72.7 | 72.7 | 0.0000 |
| 4 | 3.5 | 2 | 50 | 69 | 72.7 | −3.70 |
| 5 | 3.5 | 5 | 200 | 70.2 | 69.67 | 0.5250 |
| 6 | 3.5 | 5 | 200 | 61.5 | 59.61 | 1.89 |
| 7 | 3.5 | 5 | 200 | 84.5 | 83.79 | 0.7125 |
| 8 | 6 | 5 | 50 | 82.5 | 82.99 | −0.4875 |
| 9 | 3.5 | 5 | 200 | 70 | 72.7 | −2.70 |
| 10 | 6 | 5 | 350 | 87 | 86.66 | 0.8375 |
| 11 | 1 | 5 | 350 | 90 | 85.86 | 4.14 |
| 12 | 1 | 5 | 50 | 64 | 60.91 | 3.09 |
| 13 | 3.5 | 5 | 200 | 60 | 61.54 | −1.54 |
| 14 | 3.5 | 8 | 350 | 77 | 75.65 | 1.85 |
| 15 | 1 | 8 | 200 | 62 | 60.24 | 1.76 |
| 16 | 6 | 8 | 200 | 66 | 67.57 | −1.57 |
| 17 | 3.5 | 8 | 50 | 71 | 72.7 | −1.70 |

increasing the solvent amount until it reached its lowest value of 65% at a solvent amount of 600% w/w. Such a decrease in conversion comes in agreement with many previously published literature that reported enzyme deactivation at high solvent percentages [36].

The selectivity of the enzymatic reaction was also studied in the presence of tert butanol. Fig. 1 shows that the MAG yield increased from 40.65% to 50.19%, then dramatically reached more than 85% at solvent amounts 0%, 50%, and 100% w/w, respectively. This indicates that the high viscosities haven't favored the formation of MAG, evidenced by the result obtained at the solvent percentage of 0% and 50% w/w. The highly reduced viscosity at the solvent percentage of 100, 200, and 400% w/w facilitated the selective reaction between fatty acid and the *sn*-1 position of glycerin [37]. Unlike the conversion, the MAG percentage maintained its value at high solvent percentages till 400% w/w and finally dropped to less than 60% at 600% w/w of solvent amount. The sum of diacylglycerides (DAG) (dipalmitin, distearin, and 1,3 dipalmitin stearin) resulting from the enzymatic esterification was also evaluated compared to the conventional method. The DAG percentage was found to decrease with increasing solvent percentage. Fig. 1 shows that the DAG percentage was only 11.4% at a solvent amount of 100% w/w, whereas the MAG was more than 85% with no presence of triacylglycerides (TAG). On the other hand, a DAG percentage of 36.7% resulted in the AUT approach. Such a result further confirms the specificity of Lipozyme 435 toward MAG formation in the presence of tert butanol. These findings indicate that adding solvent has favored MAG formation more than two times compared to solvent-free reactions. In addition, neither conversion nor selectivity was favored at a very high solvent amount. Many previous works come into agreement with our study but with other esters. Bellot et al. [38] obtained a monoglycerides percentage of 94% when glycerin monooleate was produced using a mixture of n-2-methyl-2-butanol and hexane in a *Rhyzomucor miehei* lipase-catalyzed reaction. In addition, Chen et al. [23] reported a Lipozyme 435 catalyzed esterification of methyl laurate and glycerin. The authors obtained a mass fraction of glycerin monolaurate of 80.8% when the enzymatic reaction was solvated by a mixture of isopropanol:tert-butanol, 80:20, wt./wt. The variations in MAG with the aforementioned studies might be due to the different types of substrates, enzymes, and solvents [39].

### Analysis of variation (ANOVA)

Design expert software was used to obtain the ANOVA. Table 1 displays the predicted and experimental results of Lipozyme 435 catalyzed production of glyceryl monostearate. The comparatively low residual values show that the error is well distributed. This indicates that the expected and actual outputs are correlated. As indicated in Table 2, the model's F-value is 29.93, suggesting its significance, with only a 0.01% probability that an F-value is attributable to noise.

Moreover, the comparatively high value of the determination coefficient $R^2 = 0.9698$ with 97% confidence suggests that there is a statistical link between the given parameters and the response. This indicates that the current model cannot account for only a total variance of 3%. Thus, minor extra variables affecting the TPSA and glycerin reaction might have influenced the reaction conversion.

**Table 2**

Analysis of variance of the ENZ production of *Glyceryl monostearate.*

| Source | Sum of Squares | Degree of freedom | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Model | 1258.27 | 6 | 209.71 | 27.73 | <0.0001 | Significant |
| A-Molar ratio | 1176.13 | 1 | 1176.13 | 155.51 | <0.0001 | |
| B-Enzyme load | 0.0113 | 1 | 0.0113 | 0.0015 | 0.9700 | |
| C- Solvent amount | 9.46 | 1 | 9.46 | 1.25 | 0.2895 | |
| AB | 4.00 | 1 | 4.00 | 0.5289 | 0.4838 | |
| AC | 2.25 | 1 | 2.25 | 0.2975 | 0.5974 | |
| BC | 66.42 | 1 | 66.42 | 8.78 | 0.0142 | |
| $A^2$ | | | | | | |
| $B^2$ | | | | | | |
| $C^2$ | | | | | | |
| **Residual** | 75.63 | 10 | 7.56 | | | |
| Lack of fit | 65.50 | 6 | 10.92 | 4.31 | 0.0894 | Significant |
| Pure error | 10.13 | 4 | 2.53 | | | |
| Corrected Total | 1333.90 | 16 | | | | |

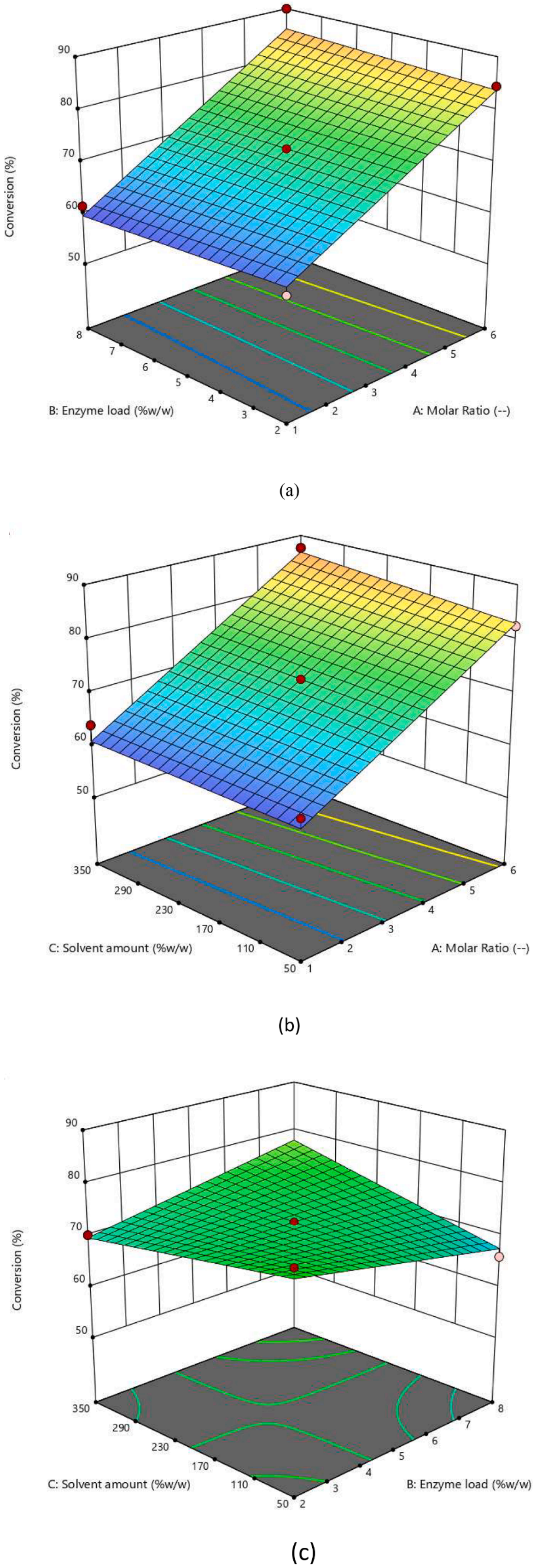

**Fig. 2.** Three-dimensional graphs of the interactive effect of (a) Molar ratio and enzyme load, (b) Molar ratio and solvent amount, and (c) Enzyme load and solvent amount versus conversion. Fixed reaction conditions: agitation of 150 rpm, reaction time of 3 h, and molecular sieves amount of 10% w/w.

### *Regression analysis*

Each dataset was submitted to regression analysis to generate coefficient sets according to the model's equations. The adequate precision value of >4 is favorable for acceptable models; the adequate precision value of 18.271 (which measures the signal-to-noise ratio) in the suggested model shows that it may be utilized to explore the design space. In addition, the comparatively low coefficient of variation value of 5.52 % implies that the performed runs are dependable and accurate.

The "Prob > P" values of less than 0.0500 indicate that this study's model term is significant. In this study, the molar ratio's parameter Prob > P value is <0.0001, indicating that the model term is significant. While the Prob > P value of enzyme load and solvent amount were 0.9875 and 0.2710, respectively, indicating that both terms are insignificant. Based on the aforementioned Prob > P values, the contribution of parameters on response can be ordered as follow: molar ratio, solvent amount, and enzyme amount. Equation (5) was generated to estimate response values when changing the uncoded screened parameters at any point.

$$Y = +72.64 + 12.13A + 0.0375B + 1.09C + 1.0000AB + 0.7500AC + 4.07BC \tag{5}$$

Where Y is the conversion and A, B, and C are the molar ratio, enzyme load, and solvent amount, respectively.

### *Response surface plots in three dimensions*

The plotting of response surfaces was performed using equation [3] and regression analysis.

#### *Effect of varying the enzyme amount with molar ratio on conversion*

It is obvious from Fig. 2a that a high conversion is obtained at a high Lipozyme 435 amount and glycerin to TPSA molar ratio. In contrast, a reduced conversion of 60% resulted from a low enzyme load and molar ratio. Additionally, the graph demonstrates that conversion decreases dramatically with increasing molar ratios and decreasing enzyme loads, and vice versa. The ANOVA found that the amount of enzymes was not a significant variable. Table 2 displays the significance of enzyme load and molar ratio when the P-value is 0.9875 and <0.0001, respectively. Unlike the traditionally catalyzed reactions, lipases cannot catalyze esterification or transesterification with a precise reaction stoichiometry molar ratio. Typically, a greater molar ratio of alcohol to fatty acids is required for the conversion process. This may be considered a disadvantage of the ENZ processes. This is simply because the esterification reaction of TPSA with glycerin requires only one mole to complete the reaction. The added excess alcohol serves only for the reaction to proceed and occupies a useless volume in the esterification reactor. This certainly reduces the practical reactor volume and productivity or increases the plant's fixed cost of the required productivity.

On the other hand, conversion was not favored at low enzyme loading (i.e., 1% w/w). Many authors have suggested that 4% w/w or above of Lipozyme 435 can favorably proceed the reaction toward equilibrium [40]. Putting into account that enzymes should be utilized several times to maintain the process's feasibility [41].

#### *Effect of changing molar ratio and solvent amount on conversion*

Fig. 2b illustrates that molar ratio change has a greater effect on conversion values than solvent variation. As per equation [5], the conversion decreased dramatically from 87.5% (at 6:1 M ratio) to 64% (at 1:1 M ratio). However, when the solvent content decreased from 350% w/w to 50% w/w at constant molar ratio of 6:1, the conversion values decreased slightly from 87.5% to 82.5%. According to Table 2, the P-value for the molar ratio is 0.0001, whereas the P-value for the solvent amount is 0.27. The solvent concentration of 350% w/w yielded a slightly greater conversion because at high solvent amounts, the

**Table 3**
Summary of total capital investment.

| Cost parameter | Cost of AUT plant (USD) | Cost of ENZ plant (USD) |
|---|---|---|
| ISBL | 3,290,165 | 1,751,374 |
| OSBL | 987,050 | 525,412 |
| **Direct capital investment cost (ISBL + OSBL)** | 4,277,215 | 2,276,786 |
| Cost of engineering | – | – |
| Cost of contingency | 427,721 | 341,518 |
| **Fixed capital cost** | 4,704,936 | 2,618,304 |
| Startup expenses | 427,721 | 227,679 |
| Working capital | 641,582 | 341,518 |
| **Total capital investment** | 7,852,966 | 4,335,001 |

**Table 4**
Summary of total cost of production.

| Reactants | Cost ($) | Enzymatic route | | Autocatalytic route | |
|---|---|---|---|---|---|
| | | Amount (t) | Price ($) | Amount (t) | Price ($) |
| Raw materials | | | | | |
| Glycerin[a] (25 of plant capacity) | 1.1/kg | 4.016 | 4417.21 | 4.313 | 4743.75 |
| Stearic acid (75% of plant capacity) | 1.3/kg | 12.047 | 15,661 | 12.937 | 16,818.8 |
| T-Butanol21 ton/y (Closed circuit) 0.5% loses/day | 1.36/kg | 0.105 | 142.8 | – | – |
| Novozym 435 (1 kg/3tons of Monostearin) | 700/kg | 5 | 3500 | – | – |
| Sodium hydroxide | 3/kg | – | 6 | – | – |
| Washing water | 0.00227/kg | – | 15 | – | 15 |
| Utilities | | | | | |
| Steam | 0.0227/MJ* | 1713 MJ | 38 | 22677 MJ$^{**}$ | 515 |
| Electric power | 0.136/kWh* | 125 kWh | 17 | 1650 kWh$^{**}$ | 225 |
| Variable production cost/day | | | **23,797** | | **22,317** |
| Packing | 1% | | 238.000 | | 223.175 |
| Repair and maintenance | 1% | | 238.000 | | 223.175 |
| Waste stream disposal | 1% | | 238.000 | | 223.175 |
| Total production cost/day | | | **24,511 $/day** | | **22,987 $/day** |
| Total production cost/ton | | | **1,634 $/t** | | **1,532 $/t** |
| Total production cost/year | | | **8,088,604 $/y** | | **7,585,718 $/y** |
| Gross Profit/d | | | **989.076 $/d** | | **2513.00 $/d** |
| Gross Profit/y | | | **326,395 $/y** | | **829,282 $/y** |

Market price of Glyceryl Monostearate is 1700 $/t.
*source: (Mustafa et al., 2016).
**Source: (Oleo Misr for Oleochemicals Company).

medium's viscosity decreases, mass transfer is boosted, and substrates have better access to the enzyme's active sites [42].

*Influence of solvent amount and enzyme load on conversion*

Fig. 2c indicates that increasing the quantity of solvent at low enzyme concentrations reduces the conversion. At a 2% w/w enzyme load, the conversion decreased from 77.5 to 70.2% as the solvent concentration changed from 50% to 350%, respectively. The decline in conversion may be due to the denatured enzymes and lower activity brought on by the increasing solvent additions. However, with a high enzyme load of 8% w/w, increasing the quantity of solvents resulted in a favorable reaction. 75% conversion was accomplished at a solvent concentration of 350% w/w as contrasted to 66% conversion at a solvent concentration of 50% w/w.

In conclusion, increasing the quantity of solvent had a negative impact on conversion if the amount of enzyme was low owing to enzyme denaturation. However, when the enzyme load is high, increasing the quantity of solvent had a favorable impact. As indicated in Equation (5), a maximum conversion of 90% is possible with a moderate solvent amount of 200% w/w, an enzyme load of 8%, and a molar ratio of 6:1 glycerin to fatty acids.

*Economic assessment*

Manufacturers must select their investment direction based on the total cost of capital investment, with the ISBL plant cost being the first cost to consider. As stated in Table 3, the ISBL cost for the AUT and ENZ was calculated to be $3,290,165, and $1,751,374 using Bridgewater's technique, based on a production capacity of 4,950 t/year of glyceryl monostearate. The cost of the AUT facility exceeds 1.80 times that of the ENZ facility. This is mainly because the AUT plant includes a costly short-path distillation unit for extracting glyceryl monostearate from the reaction mixture. This disparity in investment costs predicts a prosperous future for industrial ENZ units in oleochemical synthesis. Similar results were reported by Mustafa, Karmali [18] who indicated that a traditional monolaurin manufacturing facility incurs 20% higher ISBL costs than an enzymatic unit. In this research, however, the proportion of monoglycerides in the AUT process is 2.1 times lower than in the ENZ process, requiring an increase in unit volume compared to ENZ equipment to maintain 15 tpd of glyceryl monostearate output.

The ENZ approach is notably favorable in mitigating greenhouse gases due to its lower energy consumption. Only 1713 MJ/d has been consumed in the ENZ method as opposed to 22677 MJ/d for the AUT method, as shown in Table 4. The energy-intensive short-path distillation step and high reaction temperature of 190 °C affected the AUT process's excessive energy consumption. This significant difference in energy use makes the ENZ process more sustainable and ecologically benign than the AUT approach. This result is consistent with sustainable development goal No. 12 about responsible consumption and production (Sachs, 2012). This SDG emphasizes resource and energy efficiency by "doing more with less and doing it better." In this context, the ENZ approach has lower energy consumption and lower losses.

In addition to the ISBL cost, the total production cost is vital for determining a method's feasibility. The total production cost of GMS is $1,634/t for the ENZ process and $1,532/t for the AUT method, as shown in Table 4. This result reveals that the ENZ method is around 6.6% more costly than the AUT method. Jagannathan et al. (2011) found that the cost of manufacturing biodiesel using an ENZ process was 2.1 times that of an alkali-catalyzed approach. The immobilized enzyme accounted for approximately 14.7% of the variable production costs. On the other hand, the energy consumption in the AUT process contributed to 3.3% of the variable production cost.

Nevertheless, the authors believe the ENZ and AUT technologies should refrain from competing in production costs. Since the ENZ approach often results in higher manufacturing costs than traditional procedures, one should not expect the contrary to be true. This is mainly

**Table 5**
Summary of NPV for Glyceryl Monostearate production using the autocatalytic technology.

| Project Year | Investment | Capacity | Charge of Depreciation | investment | Expenses of Operation | Gross Profit | Expenses of Depreciation | Tax income | Tax paid | Cash flow | Discounted Cash flow | pretax Cash Flow |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 |
| 2 | 100 | 0 | 0 | -$7,852,965.82 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 | -$7,852,965.82 | -$7,074,743.98 | -$7,852,965.82 |
| 3 | 0 | 100 | 0.2 | | -$7,585,718.25 | $829,281.75 | $165,856.35 | $663,425.40 | $149,270.72 | $8,265,729.29 | $6,708,651.31 | $8,116,458.57 |
| 4 | 0 | 100 | 0.32 | | -$7,585,718.25 | $829,281.75 | $265,370.16 | $563,911.59 | $126,880.11 | $8,288,119.89 | $6,043,830.01 | $8,161,239.78 |
| 5 | 0 | 100 | 0.192 | | -$7,585,718.25 | $829,281.75 | $159,222.10 | $670,059.65 | $150,763.42 | $8,264,236.58 | $5,444,891.90 | $8,113,473.16 |
| 6 | 0 | 100 | 0.1152 | | -$7,585,718.25 | $829,281.75 | $95,533.26 | $733,748.49 | $165,093.41 | $8,249,906.59 | $4,905,308.02 | $8,084,813.18 |
| 7 | 0 | 100 | 0.1152 | | -$7,585,718.25 | $829,281.75 | $95,533.26 | $733,748.49 | $165,093.41 | $8,249,906.59 | $4,419,196.42 | $8,084,813.18 |
| 8 | 0 | 100 | 0.0576 | | -$7,585,718.25 | $829,281.75 | $47,766.63 | $781,515.12 | $175,840.90 | $8,239,159.10 | $3,981,258.03 | $8,063,318.20 |
| 9 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $3,586,718.95 | $8,041,823.21 |
| 10 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $3,231,278.33 | $8,041,823.21 |
| 11 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $2,911,061.56 | $8,041,823.21 |
| 12 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $2,622,577.98 | $8,041,823.21 |
| 13 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $2,362,682.87 | $8,041,823.21 |
| 14 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $2,128,543.12 | $8,041,823.21 |
| 15 | 0 | 100 | 0 | | -$7,585,718.25 | $829,281.75 | $0.00 | $829,281.75 | $186,588.39 | $8,228,411.61 | $1,917,606.42 | $8,041,823.21 |
| | | | | | **Cumulative profit** | **$10,780,662.75** | **ROI = 9.15%** | | | | **NPV** | **$43,188,860.95** |

**Table 6**
Summary of NPV for α Glyceryl Monostearate 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 | -$4,335,001.39 | $0.00 | $0.00 | $0.00 | $0.00 | $0.00 | -$4,335,001.39 | -$3,905,406.66 | -$4,335,001.39 |
| 3 | 100 | 0 | 0.2 | | -$8,088,604.88 | $326,395.12 | $65,279.02 | $261,116.10 | $58,751.12 | $8,356,248.88 | $6,782,119.05 | $8,297,497.76 |
| 4 | 100 | 0 | 0.32 | | -$8,088,604.88 | $326,395.12 | $104,446.44 | $221,948.68 | $49,938.45 | $8,365,061.55 | $6,110,017.16 | $8,315,123.09 |
| 5 | 100 | 0 | 0.192 | | -$8,088,604.88 | $326,395.12 | $62,667.86 | $263,727.26 | $59,338.63 | $8,355,661.37 | $5,504,519.96 | $8,296,322.73 |
| 6 | 100 | 0 | 0.1152 | | -$8,088,604.88 | $326,395.12 | $37,600.72 | $288,794.40 | $64,978.74 | $8,350,021.26 | $4,959,026.99 | $8,285,042.52 |
| 7 | 100 | 0 | 0.1152 | | -$8,088,604.88 | $326,395.12 | $37,600.72 | $288,794.40 | $64,978.74 | $8,350,021.26 | $4,467,591.89 | $8,285,042.52 |
| 8 | 100 | 0 | 0.0576 | | -$8,088,604.88 | $326,395.12 | $18,800.36 | $307,594.76 | $69,208.82 | $8,345,791.18 | $4,024,857.56 | $8,276,582.36 |
| 9 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $3,625,997.80 | $8,268,122.20 |
| 10 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $3,266,664.68 | $8,268,122.20 |
| 11 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $2,942,941.16 | $8,268,122.20 |
| 12 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $2,651,298.34 | $8,268,122.20 |
| 13 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $2,388,557.06 | $8,268,122.20 |
| 14 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $2,151,853.21 | $8,268,122.20 |
| 15 | 100 | 0 | 0 | | -$8,088,604.88 | $326,395.12 | $0.00 | $326,395.12 | $73,438.90 | $8,341,561.10 | $1,938,606.49 | $8,268,122.20 |
| | | | | | **Cumulative profit** | **4,243,136.55** | **ROI = 6.53%** | | | | **NPV** | **$46,908,644.69** |

because the less energy consumed in the ENZ method was inadequate to outweigh the cost of the commercial enzyme. However, contemporary customers are more aware of green products and ready to buy them. To entice customers, several manufacturers started labeling their goods as "green." As a result, consumers may pay a little extra for eco-friendly items [28].

The current emphasis is on Net Present Value (NPV), which is the difference between the present values of cash inflows and cash outflows over time (in this study, 15 years). A technique must have a positive NPV to be economically viable. The NPV summary for AUT and ENZ are shown in Tables 5 and 6, respectively. Year 1 of the project represents the commencement of cash flow and the design phase. Year 2 includes the building and installation phases, for which the total fixed capital expenditures for the project are provided. Finally, in Year 3, the plant operates at full depreciation expenditures and capacity is subtracted from the gross profit.

Tables 5 and 6 show that the AUT method's NPV and ROI are $43 million and 9.15%, respectively, whereas the ENZ method's NPV and ROI are $47 million and 6.53%, respectively. This indicates the profitability and appropriateness of investments in both technologies.

## Conclusion

This work reports glyceryl monostearate synthesis through the ENZ and AUT procedures. The technical, as well as economic considerations have been discussed. ENZ esterification of glycerin and triple-pressed stearic acid in the presence of *tert*-butanol was optimized using RSM. The optimum conditions were a 6:1 glycerin to TPSA molar ratio, 8% w/w Lipozyme 435 amount, and 350% w/w solvent amount. The ENZ process yielded 88.3% MAG at optimum conditions, while the AUT technique yielded only 41.6%. Total capital expenditures for the AUT and ENZ processes were $7,852,966 and $4,350,000, respectively, demonstrating that the ENZ approach provides substantial investment potential. However, the projected ROI and NPV for the ENZ procedure were around 6.53 % and $47 million, as opposed to 9.15% and $43 million for the AUT method. This indicates that the suggested ENZ method is less profitable than the AUT method. This demonstrates that more research is necessary to enhance commercial enzymes' reusability and operational stability. Nevertheless, the cleaner nature of the ENZ process makes it an appealing option for manufacturers.

## CRediT authorship contribution statement

**Ahmad Mustafa:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Rehab Ramadan:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Fumiya Niikura:** Formal analysis, Investigation, Methodology. **Abrar Inayat:** Investigation, Methodology, Writing – review & editing. **H. Hafez:** Conceptualization, Data curation, Methodology, Visualization, Writing – review & editing.

## 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.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seta.2023.103200.

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