I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil

OPTIMIZATION OF THE ENZYMATIC PRODUCTION OF SOLKETAL ESTERS FROM A WASTE OIL BY HYDROESTERIFICATION

José Miguel Jr.[1], João V. B. Dimas[1], Milson S. Barbosa[2], Cleide M. F. Soares[3], Adriano A. Mendes[1]*

[1] *Institute of Chemistry, Federal University of Alfenas, Alfenas – MG, Brazil.*
[2] *Federal Institute of Education, Science and Technology of Paraíba, Cajazeiras – PB, Brazil.*
[3] *Tiradentes University, Aracaju – SE, Brazil.*
** Corresponding author's email address: adriano.mendes@unifal-mg.edu.br*

ABSTRACT

The aim of this new study consists in the optimization of the enzymatic production of solketal esters, a valuable class of oleochemicals, from used soybean cooking oil (USCO) via a two-step process (hydroesterification). This process comprises a complete hydrolysis of USCO into free fatty acids (FFAs) catalyzed by a non-specific lipase extract from *Candida rugosa* (CRL). The resulting FFAs were used as raw material in the esterification step with solketal in a solvent-free system. In this step, a low-cost lipase (Eversa® Transform 2.0 – ET2.0) immobilized by physical adsorption on pre-treated epicarp particles from *Acrocomia aculeata* (macauba) was used as heterogeneous biocatalyst. The esterification reaction was optimized by a central composite rotatable design (CCRD) achieving a FFAs conversion of 71% after 150 min of reaction at 46 °C using a biocatalyst concentration of 17% m m^{-1}, and FFAs:solketal molar ratio of 1:1.6. This study shows the promising application of two agro-industrial wastes for production of valuable materials (immobilization support and solketal esters).

Keywords: Solketal esters. Agro-industrial wastes. Enzymatic hydroesterification. Optimization.

1 INTRODUCTION

Solketal esters are a valuable class of compounds used as oxygenated fuel additives and monomers for polymers production.[1,2] They have been preferentially produced by esterification or transesterification reactions catalyzed by acid or basic homogeneous catalysts. In general, these reaction systems require high temperatures and complex reaction schemes using high amounts of organic solvents to improve the miscibility of the raw materials.[1–4] On the other hand, the production of these compounds using lipases is a highly interesting and sustainable option, since it requires milder experimental conditions, which reduce energy demand, and their high specificity and selectivity reduces the formation of undesirable byproducts that, thus, minimizes the generation of wastes in the process.[5]

In this context, the main objective of this study was to develop a sustainable process for the production of solketal esters from waste oils, e.g. used soybean cooking oil (USCO), by a two-step enzymatic process known as hydroesterification. The first step consisted in the complete hydrolysis of USCO performed in an emulsifier-free and buffer-free system catalyzed by a non-specific lipase from *Candida rugosa* (CRL). Subsequently, the resulting FFAs were separated from the reaction mixture and esterified with solketal in a solvent-free system catalyzed by lipase Eversa® Transform 2.0 (ET2.0) immobilized via physical adsorption on a renewable and low-cost support – treated *Acrocomia aculeata* (macauba) epicarp particles. The effect of three relevant parameters (FFAs:solketal molar ratio, reaction temperature and biocatalyst concentration) on the esters production via esterification of FFAs was evaluated using a full central composite rotatable design (CCRD) approach.

2 MATERIAL & METHODS

2.1. Materials – Macauba fruits were harvested from the Cerrado biome at the Federal University of São João del-Rei, *Campus* Sete Lagoas (Sete Lagoas, MG, Brazil). Lipases Eversa® Transform 2.0 (ET2.0) and *Candida rugosa* (CRL) and solketal were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). USCO was collected after being used once for french fries preparation in a restaurant at the Federal University of Alfenas (Alfenas, MG, Brazil). All other chemicals and solvents were of analytical grade acquired from Synth® (São Paulo, SP, Brazil).

2.2. General immobilization procedure of ET2.0 on pre-treated macauba epicarp particles – The heterogeneous biocatalyst used in this study (immobilized ET2.0 via adsorption on treated macauba epicarp particles) was prepared at pH 5.0 (5 mmol L^{-1} sodium acetate solution) using an initial protein loading of 40 mg g^{-1} of support.[6] Under such conditions, a protein loading of 25.2 ± 1.3 mg g^{-1} of support was achieved after 15 h of contact at 25 °C.[7]

2.3. General procedure of enzymatic hydrolysis of USCO – The enzymatic hydrolysis step occurred in batch mode using a closed 350 mL-plastic flask immersed in thermostatic water bath at 40 °C under continuous mechanical stirring (1500 rpm) using a mixture of USCO:water at 40% m m^{-1} of oil. Experimental conditions for a full hydrolysis were set as follows: 3.2 g L^{-1} of CRL and 3 h of contact time.[6] The resulting mixture of FFAs were recovered in a separation funnel, followed by washing with distilled water and drying with anhydrous sodium sulfate.

2.4. General procedure of enzymatic solketal esters production – The second step involved the esterification of the resulting FFAs from USCO and solketal in a solvent-free system using 100 mL open glass bottles containing 6 g of reaction mixture. The desired

amount of biocatalyst was added and the reaction mixture was then immersed in a thermostatic water bath under fixed mechanical stirring (240 rpm). Samples (0.1 mL) were periodically collected, diluted in 10 mL of ethanol solution at 70% (m m^{-1}), and titrated with a 40 mmol L^{-1} NaOH solution using phenolphthalein as indicator to determine FFAs conversion percentage.[6,8]

2.5. CCRD optimization – A full CCRD with three independent variables such as FFAs:solketal molar ratio (1:1 – 1:4), reaction temperature (40 – 70 °C) and biocatalyst concentration (5 – 20% m m^{-1}) was proposed to optimize the enzymatic production of solketal esters. The dependent variable (response) was FFAs conversion percentage determined at 40 min of reaction under experimental conditions above described. The data were analyzed at 95% confidence level using software Protimiza Experimental Design (https://experimental-design.protimiza.com.br) to obtain 2D contour plots.

3 RESULTS & DISCUSSION

According to results summarized in Table 1, experimental FFAs conversion percentage values varied from 6.2 ± 3.5% (Run #1) to 55.7 ± 2.4% (Run #14) at 40 min of reaction. Moreover, experimental and predicted (theoretical values determined according to Eq. (1)) response values were very similar (Table 1), which shows the high adequacy of the model.

Table 1 CCRD matrix for the analysis of the effect of independent variables on the enzymatic production of solketal esters.

Runs	Independent variables Coded (actual)			FFAs conversion (%)	
	FFAs:solketal molar ratio	Temperature (°C)	Biocatalyst (% m m^{-1})	Experimental	Predicted[a]
1	–1 (1:1.6)	–1 (46)	–1 (8)	6.4	6.7
2	+1 (1:3.4)	–1 (46)	–1 (8)	30.7	26.4
3	–1 (1:1.6)	+1 (64)	–1 (8)	23.4	23.0
4	+1 (1:3.4)	+1 (64)	–1 (8)	25.7	27.0
5	–1 (1:1.6)	–1 (46)	+1 (17)	43.2	42.8
6	+1 (1:3.4)	–1 (46)	+1 (17)	40.5	41.5
7	–1 (1:1.6)	+1 (64)	+1 (17)	43.3	47.5
8	+1 (1:3.4)	+1 (64)	+1 (17)	30.3	30.4
9	–1.68 (1:1)	0 (55)	0 (12.5)	21.4	20.3
10	+1.68 (1:4)	0 (55)	0 (12.5)	22.6	22.6
11	0 (1:2.5)	–1.68 (40)	0 (12.5)	29.2	31.7
12	0 (1:2.5)	+1.68 (70)	0 (12.5)	37.2	36.1
13	0 (1:2.5)	0 (55)	–1.68 (5)	19.7	20.4
14	0 (1:2.5)	0 (55)	+1.68 (20)	53.2	53.6
15	0 (1:2.5)	0 (55)	0 (12.5)	33.6	32.6

[a] Values calculated according to Eq. (1).

The analysis of variance (ANOVA) shows that the mean, quadratic term of FFAs:solketal molar ratio (x_1^2), linear term of biocatalyst concentration (x_3), and all interactions ($x_1.x_2$, $x_1.x_3$, and $x_2.x_3$) were statistically significant at 95% confidence level (*p*-values < 0.05). The non-significant parameters (*p*-values > 0.05) were excluded from the model. The significant terms were then used to obtain the quadratic polynomial equation to explain how these independent variables influence the response in terms of coded values, as shown in Eq. (1).

$$Y(\%) = 32.64 - 4.30x_1^2 + 9.88x_3 - 3.96x_1.x_2 - 5.27x_1.x_3 - 2.92x_2.x_3 \quad (1)$$

where x_1, x_2, and x_3 represent the FFAs:solketal molar ratio, reaction temperature and biocatalyst concentration, respectively.

The coefficient of determination (R^2) was used to demonstrate the adequacy of the model. In this study, a R^2 value of 0.9577 was obtained. This adequacy of the model was also confirmed by Fisher's *F*-test – the calculated *F*-value (20.11) was greater than the tabulated *F*-value at 5% significance level (3.39), thus showing its significance. Therefore, these results show that the proposed model can be used to predict and explore the contour plots to determine the optimal reaction conditions (Figure 1(A)–(B)).

Figure 1(A) shows the influence of FFAs:solketal molar ratio and biocatalyst concentration and their interaction on the reaction. An increase in FFAs conversion percentage by increasing biocatalyst concentration from 5% m m^{-1} (coded value = –1.68) to 20 m m^{-1} (coded value = +1.68) was observed. In fact, the highest coefficient value reported in Eq. (1) was obtained for the linear term of biocatalyst concentration (+9.88x_3), thus showing its positive and significant effect on the ester production. These results shows that maximum FFAs conversion can be obtained using an excess of solketal in the reaction medium – FFAs:solketal molar ratio between 1:1.6 (coded value = –1) and 1:2.5 (coded value = 0) Their interaction was also significant at 95% confidence level with a negative effect on the reaction (–5.27$x_1.x_3$ – Eq. (1)). Therefore, maximum ester production can be achieved using a biocatalyst concentration above 17% m m^{-1} of reaction mixture (coded values of ≥ +1) and FFAs:solketal molar ratio between 1:1 (coded value of –1.68) and 1:1.6 (coded value of –1).

An increase in reaction temperature positively improves the miscibility of raw materials and reduces the reaction mixture viscosity that, thus, improves mass transfer effects.[5] Although linear and quadratic terms of temperature were not statistically significant at 95% confidence level (*p*-values > 0.05), a gradual increase of FFAs conversion by increasing temperature from 40 °C (coded value = –1.68) to 46 °C (coded value = –1) can be detected in Figure 1(B). It is also possible to note that maximum FFAs conversion percentage can be achieved above 17% m m^{-1} of biocatalyst (coded value ≥ +1), as illustrated in Figure 1(A). The interaction between these two individual parameters was also significant at 95% confidence level with a negative coefficient value ($–2.92x_2.x_3$) – Eq. (1)). This shows a positive influence on the reaction for biocatalyst concentration values above 17% m m^{-1} (coded value ≥ +1) and reaction temperature up to 46 °C (coded value ≤ –1).

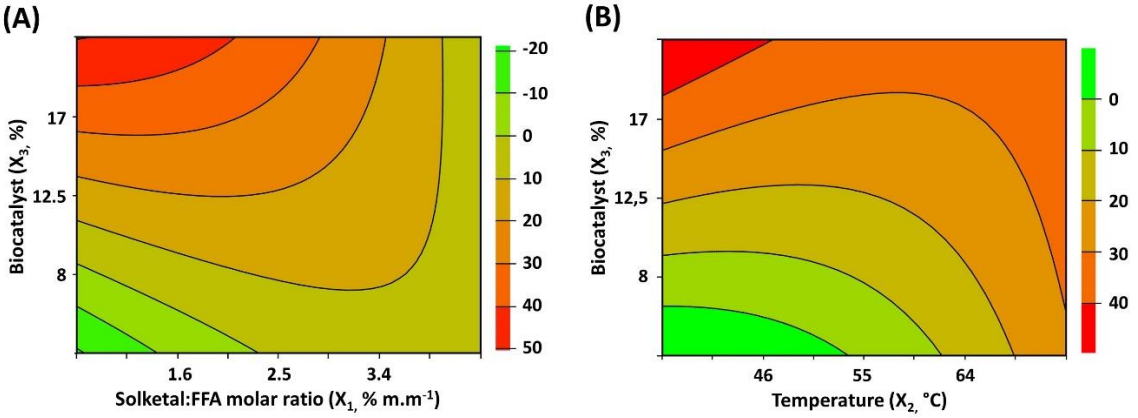

Figure 1 Contour plots for the enzymatic production of solketal esters. Effect of biocatalyst concentration and FFAs:solketal molar ratio (A), and biocatalyst concentration and reaction temperature (B) on the FFAs conversion percentage.

Therefore, the second-order polynomial model was validated by conducting runs under optimal conditions obtained from the analyses of 2D contour plots: FFAs:solketal molar ratio of 1:1.6 (coded value = –1), reaction temperature of 46 °C (coded value = –1) and 17% m m^{-1} of biocatalyst (coded values = +1). A maximum experimental FFAs conversion percentage of 44.5 ± 0.5% at 40 min of reaction was observed, which was consistent with predicted value (42.8%). This correspondence between experimental and predicted values confirms the satisfactory adequacy of the developed model to the experimental data.

The influence of reaction time on the FFAs conversion percentage catalyzed by immobilized ET2.0 was evaluated under optimal experimental conditions established by CCRD. According to results, the highest FFAs conversion percentage (71%) at 150 min of reaction was achieved, thus confirming the satisfactory catalytic performance of the biocatalyst prepared in this study.

4 CONCLUSION

This study clearly showed that the resulting heterogeneous biocatalyst was highly active in esterification reactions conducted under moderate experimental conditions. This is a relevant aspect of the proposed process, since it has a low energy demand for the production of an important class of oleochemicals. The development of an efficient and sustainable process using a waste oil as raw material and a lignocellulosic biomass waste as lipase support material can be considered a promising field for environmental researchers. Further studies will be conducted in our research group aiming at the application of solketal esters as plasticizers for the production of flexible poly(vinyl chloride) (PVC) films.

REFERENCES

1 MARIAM, N.M.D.N.S., HOONG, S.S., ARNIZA, M.Z., ISMAIL, T.N.M.T., YEONG, S.K., YUSOP, M.R. 2022. J. Oil Palm Res. 34 (1) 524-534.
2 YANG, J., MA, W.J., LI, N., ZHOU, J.H., SUN, H.Z. 2014. Adv. Mater. Res. 941-944 (8) 1021-1025.
3 KENAR, J.A., KNOTHE G. 2008. J. Am. Oil Chem. Soc. 85 (2) 365-372.
4 SULTANOVA, R.M., KHUSNUTDINOVA, N.S., BORISOVA, Y.G., RASKILDINA, G.Z., MESHCHERYAKOVA, S.A., SAMORODOV, A.V., ZLOTSKY, S.S. 2023. Russ. J. Gen. Chem. 93 (1) 1–7.
5 SOUSA, R.R., SILVA, A.S., FERNANDEZ-LAFUENTE, R., FERREIRA-LEITÃO, V.S. 2021. Catal. Sci. Technol. 11 (5) 5696-5711.
6 SABI, G.J., GAMA, R.S., FERNANDEZ-LAFUENTE, R., MENDES, A.A. 2022. Enzyme Microb. Technol. 157 (1) 110019.
7 BRADFORD, M.M. 1976. Anal. Biochem. 72 (1) 248-254.
8 ALVES, M.D., ARACRI, F.M., CREN, É.C., MENDES, A.A. 2017. Chem. Eng. J. 311 (1) 1-12.

ACKNOWLEDGEMENTS

The present study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Brazil – Finance Code 001. The authors are also thankful for the financial support of Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) – Brazil (Process APQ-01691-21). Adriano A. Mendes thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research fellowship (PQ 2 – CA BI – Grant 306253/2023-2). José Miguel Jr. thanks the FAPEMIG for the student fellowship.