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Production of Steryl Esters Using Alternative Sources of Sterols and Free Fatty Acids: Modeling and Guidelines

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Supporting Information

ABSTRACT: Enzymatic esterification reactions for the production of steryl esters were conducted using different sources of sterols and free fatty acids from mixtures of vegetable oil deodorizer distillates and refined oil. This work discusses the possibility of using free fatty acids from alternative and less expensive sources, determining their influence on the yield of steryl ester production as well as on the validity of a kinetic model previously developed for defined reaction medium composition. The extrapolation capacity of such a model was investigated, identifying the cases where a lack of model fitting was observed and establishing the range of conditions for model applicability. Based on a sensitivity analysis, guidelines to obtain acceptable yields (>80%) were established. This paper shows that the use of alternative sources of sterols and free fatty acids for the production of steryl esters is possible; however, there are constraints and guidelines that have to be taken into account.

INTRODUCTION

Deodorizer distillates are a residual stream produced by the industry of vegetable oil refining. Their high content in bioactive compounds such as sterols, tocopherols, and squalene may vary between 2 and 20%;[1] however, regardless the interesting composition of deodorizer distillates, they have a very limited commercial value due to their high content in pesticides.[2,3]

The attempts for valuing deodorizer distillates have been mainly focused on the recovery of sterols and tocopherols. Classical methods described in the literature include solvent extraction, chemical treatment, crystallization, complexation, molecular distillation,[4] and hydrogenation.[5] Recently, greener technologies have been proposed such as lipase-catalyzed methyl or ethyl esterification of soybean oil deodorizer distillate (SODD) to transform free fatty acids into their corresponding fatty acid methyl or ethyl esters coupled to molecular distillation and/or supercritical fluid extraction. These strategies improve the separation between tocopherols, sterols, and free fatty acids.[4,6] Alternatively, enzymatic esterification of the sterols with the fatty acids already present in the deodorizer distillates makes the separation of tocopherols and sterols easier using short-path distillation or supercritical fluid extraction.[7] The authors applied and optimized the latter strategy in order to make easier their recovery by membrane technology (work under development).

Steryl esters are relevant ingredients in the preparation of functional foods, being recognized by the European Food Safety Authority (EFSA) as cholesterol-lowering agents[8] with higher bioactivity than free sterols.[9] Moreover, the higher molecular weights of steryl esters (650 < MW < 800 g/mol) in comparison to those of their free sterols (400−415 g/mol) and contaminant pesticides (150 < MW < 400 g/mol) opens a potential use of separation processes based on size exclusion such as pressure-driven membrane processes.

In a previous work, the authors showed that the lipase from *Candida rugosa* is able to esterify sterols and free fatty acids (FFA) from deodorizer distillates, producing steryl esters under optimized conditions.[10] According to this study, in order to obtain a satisfactory esterification degree of phytosterols (above 80%) in 24 h at T = 40 °C, the concentration of enzyme and the water activity of the reaction medium (a_W) must be within the ranges 0.25 < [Enz] < 0.50% (w/w) and 0.45 < a_W < 0.85, respectively. Moreover, in the same study it was shown that there is no significant advantage in performing control of water activity during the enzymatic reaction, as long as the water activity is within the same range (0.45 < a_W < 0.85). On the other hand, it is important to ensure that the molar ratio of the reactants (free fatty acids:sterols, FFA:S) is higher than 6.0, in order to drive the reaction toward esterification. The excess of free fatty acids may be adjusted by the addition of oleic acid, as is also proposed by other authors.[11−13]

The use of alternative sources of sterols and free fatty acids may be very interesting from an economic and environmental point of view, but it increases the complexity of the reaction system. The mathematical model established in a previous work[10] provided the kinetic constants of the relevant involved reactions, enabling, consequently, the prediction of the composition profile during reaction (once characterized the initial composition of the reaction mixture). This model was obtained using different deodorizer distillates as a source of sterols and oleic acid as additional sources of free fatty acids. Even though the origin of the deodorizer distillates was different, the initial composition of the reaction mixtures was rather similar. It is expected that this situation may be different if alternative sources of free fatty acids with completely different

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compositions are considered to adjust the FFA:S molar ratio (in place of oleic acid). This paper discusses the possibility of using free fatty acids from alternative and less expensive sources, determining their influence on the yield of steryl ester production as well as on the validity of the previous model developed. Additionally, it is aimed at the setting of guidelines for the preparation of the reaction mixture, in order to obtain yields above 80%.

Depending on the operating conditions of the deodorization step, the composition of the respective distillates vary in terms of sterols, tocopherols, steryl esters, free fatty acids, and acylglycerols.[14] Since the enzymatic hydrolysis of acylglycerols is a potential source of free fatty acids, a deodorizer distillate, even poor in sterols, may be used to ensure the required excess of fatty acids. Deodorizer distillates from olive oil are typically poor in sterols and rich in free fatty acids, making this byproduct an alternative source with an additional high oxidation stability due to their high content in squalene and tocopherol.[6,15,16] Refined oils are products mainly constituted by triglycerides, easily available to the producers of edible oil and less expensive than the oleic acid itself. Therefore, three alternative sources of free fatty acids were considered in our study: a deodorizer distillate and a refined oil from sunflower, rich in triglycerides, and a deodorizer distillate from olive oil.

The applicability of the mathematical model developed in a previous work[10] using sunflower deodorizer distillates with different initial compositions was verified by evaluating the quality of the fitting of the model. The present work aims to determine the validity of this model when using alternatives and cheaper sources of free fatty acids and sterols. To accomplish this objective, enzymatic reactions using distinct sources were carried out under optimal conditions (defined in a previous work[10]). Deviations of the predicted yield of production of steryl esters from the data acquired experimentally were analyzed and discussed, and the limitations to the model extrapolation were assessed. Finally, guidelines for the preparation of the initial reaction mixture were established in order to obtain yields above 80%.

MODELING OF THE ENZYMATIC REACTION

The extent of the esterification of sterols (S) can be measured by the value of the kinetic constants, since the equilibrium between species will be subsequently affected by the capacity of the enzyme to transfer the acyl group from free fatty acids (FFA) to sterols (S), releasing steryl esters (SE) and water (W). The reaction can be described as

$$\mathrm{S} + \mathrm{FFA} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SE} + \mathrm{W} + \mathrm{E} \quad \text{(I)}$$

where k_1 and k_{-1} are the kinetic constants that describe the forward reaction and the reverse reaction, respectively.

Simultaneously, the hydrolysis of acylglycerides (glycerol (G), monoglycerides (MG), diglycerides (DG), and triglycerides (TG)) occurs, since water (W) is available to react, as described by the following equations:

$$\mathrm{MG} + \mathrm{W} + \mathrm{E} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{G} + \mathrm{FFA} + \mathrm{E} \quad \text{(II)}$$

$$\mathrm{DG} + \mathrm{W} + \mathrm{E} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{MG} + \mathrm{FFA} + \mathrm{E} \quad \text{(III)}$$

$$\mathrm{TG} + \mathrm{W} + \mathrm{E} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \mathrm{DG} + \mathrm{FFA} + \mathrm{E} \quad \text{(IV)}$$

The rate equations for all reactant compounds can be established easily. For instance, the kinetics of sterols (S) and of steryl esters (SE) can be expressed as

$$\frac{1}{[\mathrm{E}]}\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = k_{-1}[\mathrm{SE}][\mathrm{W}] - k_1[\mathrm{S}][\mathrm{FFA}] \quad (1)$$

$$\frac{1}{[\mathrm{E}]}\frac{\mathrm{d}[\mathrm{SE}]}{\mathrm{d}t} = k_1[\mathrm{S}][\mathrm{FFA}] - k_{-1}[\mathrm{SE}][\mathrm{W}] \quad (2)$$

The kinetic constants determined in a previous work[10] are listed in Table S1 (Supporting Information).

The rate equations described by a system of differential equations can be solved numerically, since the kinetic constants and the initial concentration of each compound are known. Therefore, the composition of the reaction mixture in each instant can be estimated.

MATERIALS AND METHODS

Materials. *Sources of Sterols and Free Fatty Acids.* Sunflower deodorizer distilates were obtained from Lesieur (France) and Sovena (Portugal). The refined oil with high content in triolein (>90%) and olive oil deodorizer distillate were also a gift from Sovena (Portugal). Food-grade oleic acid with an acid value of 196.0−204 mg of KOH/g was purchased from Sigma-Aldrich (Belgium). The characterization of deodorizer distillates was made according to the methods described under Analytical Methods, being summarized in Table S2 (Supporting Information). Other compounds that were not quantified may comprise hydrocarbons, aldehydes, ketones, pesticides, herbicides, and breakdown products of tocopherols and free phytosterols.[17]

Enzyme. *Candida rugosa* lipase, Type VII, was obtained from Sigma (Saint Quentin, France). The activity of this lipase as indicated by the supplier is 1535 U/mg of solid. One unit (U) of lipase activity is defined as the amount of enzyme that hydrolyzes 1.0 μequiv of fatty acid from a triglyceride in 1 h at pH 7.2 and at 37 °C using olive oil (30 min of incubation).

Chemicals. Analytical-grade chloroform, food-grade oleic acid, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) solution (from Fluka), and pyridine were obtained from Sigma-Aldrich (Belgium).

All analytical-grade standard substances, squalene (99.3% purity), stigmasterol (97% purity), β-sitosterol (99% purity), campesterol (99% purity), cholesteryl stearate (96% purity), monoglyceride olein (>99% purity), diglyceride olein (99.7% purity) and triglyceride olein (99.6% purity) were purchased from Sigma (France). A tocopherol kit consisting of α-, β-, γ-, and δ-tocopherols was obtained from Merck (>95% purity).

The internal standard heptadecanyl stearate (HDS) was prepared by condensation of heptadecanol and stearoyl chloride, both obtained from Aldrich (Belgium), as described by Verleyen et al.[18]

Analytical Methods. *Analysis of Acylglycerides, Tocopherols, Sterols, and Steryl Esters.* The procedure to analyze acylglycerides, tocopherols, sterols, and steryl esters was described elsewhere.[10] Briefly, the sample treatment involved its derivatization, using *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) solution and pyridine as derivatizating and

silylation agent. The analysis was performed in a Chrompack CP9001 series gas chromatograph with an on-column injection at a set temperature of 60 °C and with a flame ionization detector at 360 °C. A capillary column Zebron-5MS 15 m × 0.25 mm, 0.25 μm (Phenomenex, USA), was used. The temperature program of the oven was the following: increasing at 30 °C/min from 60 to 140 °C and continuing the oven heating at a rate speed of 5 °C/min to 235 °C with a 7 min hold and further heating at 15 °C/min to 340 °C with a 30 min hold. Helium was used as a carrier gas at a pressure of 100 kPa. The peaks were integrated using Borwin software (JMBS Developments, France).

Analysis of Free Fatty Acids. The free fatty acids (FFA) were determined by titration according to the NF.EN.ISO 660-1999 standard. The method was developed and implemented in a titration workstation TitraLab 856 from Radiometer (Denmark). The result was expressed as grams as oleic acid per 100 g.

Analysis of Water Content. A Karl Fischer titration system from Metrohm AG, Herisau, Switzerland (Model 756 KF coulometer), was used to analyze the water content. The determinations of the water content were conducted with Hydranal-Coulomat CG as catholyte reagent, free of halogenated hydrocarbons, and with Hydranal-Coulomat Oil as anolyte reagent, both specific for coulometric Karl Fischer titration. Samples were previously weighed (≃1 g) and injected into the KF titrator. The result was expressed as parts per million.

Experimental Procedures. Enzymatic reactions were carried out in a 100 mL hermetically sealed and jacketed vessel in order to maintain the temperature constant (40 °C). The vessel was initially filled with 50 g of the standard mixture, the water activity was adjusted to $a_W = 0.8$, and 0.5% w/w lipase from *C. rugosa* was added. The procedure to adjust the water activity is described in detail elsewhere.[10] Briefly, the water activity was adjusted adding the corresponding water amount given by the water adsorption isotherm. This curve for deodorizer distillates was determined by the pre-equilibration of the mixture with a saturated salt solution at a fixed temperature and in a closed vessel. The isotherm enables the determination of the water amount needed to obtain a specific water activity. All the reaction mixtures were maintained in equilibrium overnight with the respective salt to ensure that same water activity. Using this procedure, eight different mixtures consisting of sunflower deodorizer distillate and an additional source of fatty acids were prepared accordingly to obtain a molar ratio of free fatty acids (FFA) to sterols (S) of 7.0, considering the deodorizer distillate as the single source of sterols. It should be noted that no exogenous solvent was added in the preparation of these mixtures. The proportion of sunflower deodorizer distillate and source of free fatty acids used for the preparation of the reaction mixtures are summarized in Table S3 (Supporting Information).

Over the time course of the reaction, samples of ≃2 g were periodically removed from the reaction vessel for FFA, Karl Fischer, and gas chromatographic analysis.

Numerical Methods. *Modeling prediction and Sensitivity Analysis.* The dynamic system under study was simulated and analyzed using the SimBiology toolbox of MatLab (2013a version).

The software calculated local sensitivities by combining the original ordinary differential equation (ODE) system for a model (defined by the rate equations (eqs 1 and 2) with the auxiliary differential equations for the sensitivities. This method is sometimes called "forward sensitivity analysis" or "direct sensitivity analysis".[19] The sensitivity of the species x with respect to species k was calculated with a full normalization, defined as

$$\frac{k}{x(t)}\frac{\mathrm{d}x(t)}{\mathrm{d}k} \tag{3}$$

This normalization allowed data to be dimensionless and a fair comparison to be obtained between them.

SimBiology sensitivity analysis uses "complex-step approximation" to calculate derivatives of reaction rates. This technique yields accurate results for the vast majority of typical reaction kinetics.

The sensitivity analysis was conducted to determine the influence of the initial concentration of each compound in the production of steryl esters (after 24 h). The initial composition of the reaction mixture was assumed to be the average of the reaction mixtures used to develop the model (established in a previous work[10]).

Quality of Fit of the Model. The least-squares objective function, S_y, was used to measure the quality of fit of the model. For n compounds and j data points, the function S_y compares the values predicted by the model ($\hat{\theta}$) and the values actually observed (θ), being expressed as

$$S_y = \sqrt{\frac{\sum_1^n \sum_1^j (\theta - \hat{\theta})^2}{jn - 1}} \tag{4}$$

Lower values of S_y indicate a good agreement between experimental data and model predictions.

RESULTS AND DISCUSSION

The experimental kinetic constants (Table S1, Supporting Information) enable the description of the transient evolvement of the enzymatic reactions given that the initial composition of the reaction medium is known beforehand, independently of the source of each compound.

Table S4 (Supporting Information) shows the initial composition of the reaction mixtures, as well as the range of concentrations for each compound, where the kinetic constants were validated. In all reactions, there is at least one compound out of the validation range. Even so, the kinetic constants were used in a mass-balance model to describe the profile of concentration of the various compounds during reaction and, ultimately, to determine the yield of production of steryl esters.

The predicted yields of steryl ester production for reactions 3, 6, and 8 show a high deviation from the corresponding experimental values (Table 1). Figure 1 compares the profile of the concentration of sterols and steryl esters during the kinetics of reactions 2 and 6, which are examples of a good fitting and a bad fitting of the model, respectively. Interestingly, in both cases olive oil (C; Table S3 in the Supporting Information) was used as a source of free fatty acids, which suggests that the lack of model fitting is not due to the nature of the source. Instead, the reason for the lack of model fitting may be related to the initial concentration of compounds out of the validated range of the model. In fact, reaction 3 showed concentrations of triglycerides, sterols, and free fatty acids significantly out of the validated range. In reaction 6, concentrations of diglycerides, triglycerides, and free fatty acids were outside the validated range, while in reaction 8, diglycerides, triglycerides, steryl

Table 1. Experimental and Predicted Yield of Steryl Ester Production and Corresponding Quality of Model Fitting Measured as S_y[a]

	yield after 24 h (%)		
reaction	exptl	pred	S_y[b]
1	83.2	76.2	7.8
2	85.9	84.8	3.2
3	83.8	74.2	23.4
4	87.2	86.0	2.7
5	73.3	73.9	6.1
6	51.6	64.9	29.8
7	76.2	79.2	8.8
8	51.6	64.9	19.9

[a]See eq 4. [b]Calculated taking into account the amount of steryl esters produced during 24 h of reaction.

esters, and free fatty acids were outside the validated range. The complexity of the system does not allow to conclude directly which compounds may be responsible for the deviation of the model in predicting the yield of production of steryl esters, since the hydrolysis of acylglycerols releases free fatty acids but is also a consumer of water from the system, affecting consequently the equilibrium state of the esterification of sterols.

A sensitivity analysis of the model is therefore considered to be critical for identification of the relevant compounds for the production of steryl esters. The ordinary differential equations (ODEs) defining the sensitivity of steryl ester production (x) with respect to the initial concentration of each compound present in the reaction mixture (k) were obtained by using eq 3 (the detailed procedure and assumptions to solve these ODE system are described under Numerical Methods).

Figure 2 shows that the production of steryl esters after 24 h of reaction is affected mainly by the initial concentration of sterols, followed by the free fatty acids, steryl esters, and, finally, triglycerides. The influence of the remaining compounds was found to be not significant. As can be observed, the increase of sterols, free fatty acids, and triglycerides has a positive impact on the production of steryl esters, while for an increased initial concentration of steryl esters it is negative. These results suggest that the production of steryl esters is favored in a reaction mixture rich in sterols, free fatty acids, and triglycerides, and with a low initial concentration of steryl esters.

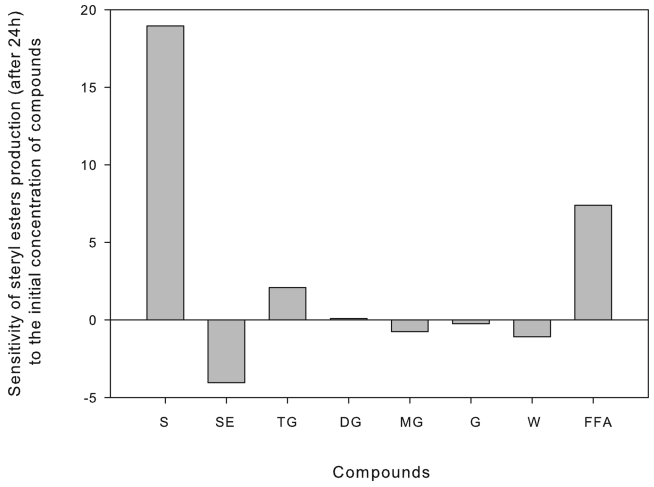

Figure 2. Sensitivity of steryl ester production (after 24 h) to variation of the initial concentration of each reactant compound.

Figure 3 shows the normalized response of steryl ester production to a spike in the initial concentrations of other individual compounds. A spike in the initial concentrations of sterols and triglycerides has a positive effect in the production of steryl esters (upward trend), while a spike of steryl esters is

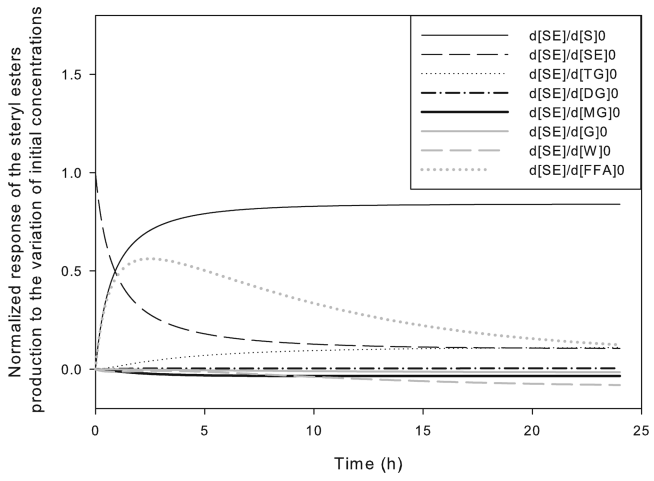

Figure 3. Normalized response of steryl ester production, during the reaction, to variation of the initial concentration of each reactant compound.

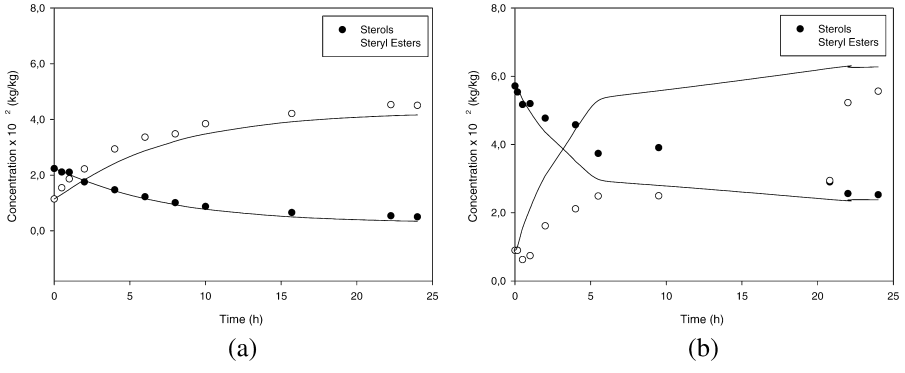

Figure 1. Examples of (a) good and (b) bad model fitting to the profile of concentrations of the sterols and steryl esters during reaction: reactions 2 and 6, respectively.

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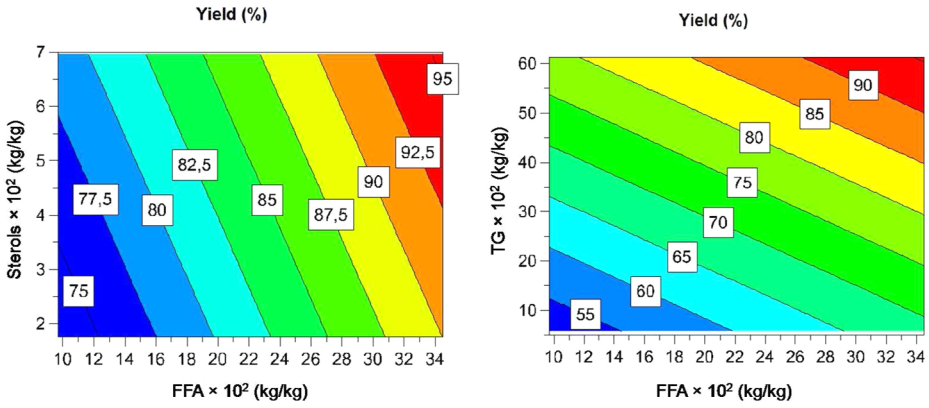

Figure 4. Yield of steryl ester production as a function of the initial concentration of free fatty acids (FFA) and (a) sterols (fixing the concentration of TG at 0.61 kg/kg) and (b) triglycerides (TG) (fixing the concentration of S at 6.96×10^{-2} kg/kg).

negative (downward trend). The trend of the curve for a spike in free fatty acids shows an initial increase in production of steryl esters until ≈2 h of reaction time, but then the effect decreases with time (although remaining positive for a 24 h time frame). The influence of the remaining compounds was observed to be not as significant.

The nature of the impact of the relevant compounds could be anticipated. A high initial concentration of steryl esters drives the equilibrium toward the hydrolysis of steryl esters, having a negative impact on the overall production of steryl esters. Additionally, a high initial concentration of sterols favors the equilibrium toward esterification. On the other hand, the lipase from *C. rugosa* is reported to be specific for the hydrolysis of triglycerides, which are their natural substrates. Therefore, once the reaction starts, the enzyme is expected to hydrolyze the triglycerides, releasing molecules of free fatty acids which may be used in the production of steryl esters.

The sensitivity analysis performed in this work suggests that the model is sensible to the variation of the initial concentrations of the compounds identified in reactions 3, 6, and 8 as being out of the validated range. Aligned with this observation, the model presented a lack of fit for those reactions, predicting a lower yield for reaction 3 and a higher yield for reactions 6 and 8. An inhibition effect due to a high initial concentration of free fatty acids (>0.34 kg/kg), not captured in the developed model, may be the reason behind the lack of fit. This inhibition could explain a higher prediction of yield in reactions with a low content of free fatty acids (reaction 3) and a lower prediction of yield in reactions with a high content of free fatty acids (reactions 6 and 8).

Based on simulations and experimental observations, it is possible to define guidelines to obtain yields of steryl ester production above 80% in 24 h. These guidelines are hereby presented on the basis of the parameters for which the yield is more sensitive (e.g., steryl esters, sterols, free fatty acids, and triglycerides). Therefore, the initial concentration of steryl esters should be as low as possible, to avoid their hydrolysis. This reaction can be mitigated by using an excess of free fatty acids (FFA) and, consequently, driving the equilibrium toward the esterification of sterols. However, the concentration of FFA should not exceed 0.34 kg/kg; otherwise, an inhibition by excess of substrate may occur. In order to avoid this, it is possible to use a reaction medium rich in triglycerides, since their hydrolysis produces free fatty acids needed for the target esterification. Figure 4 shows contour plots of the yield of steryl ester production for a range of concentrations of the three most important components, i.e., triglycerides (TG), sterols (S), and free fatty acids (FFA). Figure 4a indicates the relationship between S and FFA that should be taken into account to obtain a desirable yield, while Figure 4b indicates the relationship between TG and FFA (both sources of free fatty acids). For the ranges studied, yield shows a monotonic relationship with FFA, S, and TG, where these should be as high as possible to improve yield.

CONCLUSION

Enzymatic reactions to produce steryl ester from deodorizer distillates were successfully performed with an acceptable yield (>80%), using less expensive and industrially available sources of free fatty acids and sterols, such as the mixture of deodorizer distillates with different compositions and refined oil rich in triglycerides. The mathematical model developed in a previous work[10] was shown to be able to predict the profile of compounds during the reaction, in conditions of a wide variability of the composition of the reaction mixture. The applicability of the model is only restricted if the initial concentration of FFA is higher than 0.34 kg/kg. In this case, an additional effect not captured by the model may occur.

In order to obtain high yields of steryl ester production, there are other constraints concerning the composition of the initial reaction mixture. A sensitivity analysis performed for the model indicates that the production of steryl esters is affected mainly by the initial concentration of sterols (S), followed by the concentrations of free fatty acids (FFA) and steryl esters (SE), and, finally, by the triglycerides (TG) content. Consequently, guidelines to obtain high yields were only focused on these parameters. It was established that the initial concentration of SE should be as low as possible, and the initial reaction mixture should contain a high content of S, TG, and FFA, but the initial concentration of FFA should not be higher than 0.34 kg/kg; otherwise, an inhibition by excess of substrate can occur. This inhibition can be mitigated using a reaction medium rich in triglycerides as an alternative source of FFA.

ASSOCIATED CONTENT

Supporting Information

Table S1, kinetic constants of reactions I–IV; Table S2, characterization of deodorizer distillates; Table S3, proportion

of sunflower deodorizer distillate and source of free fatty acids used for preparation of the reaction mixtures; Table S4, initial concentrations of reactant compounds and respective modeling range. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Gunawan, S.; Ju, Y.-H. Vegetable Oil Deodorizer Distillate: Characterization, Utilization and Analysis. *Sep. Purif. Rev.* **2009**, *38*, 207−241.

(2) Newkirk, D. *Studies on Vegetable Oil Deodorizer Distillates with Respect to Lipid and Pesticide Content*; American University: Washington, DC, 1982.

(3) Chaudry, M.; Nelson, A.; Perkins, E. Distribution of aldrin and dieldrin in soybeans, oil, and by-products during processing. *J. Am. Oil Chem. Soc.* **1976**, *53*, 695−697.

(4) Torres, C. F.; Fornari, T.; Torrelo, G.; Señoráns, F. J.; Reglero, G. Production of phytosterol esters from soybean oil deodorizer distillates. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 459−463.

(5) Pagani, M. A.; Baltanás, M. A. Production of natural antioxidants from vegetable oil deodorizer distillates: effect of catalytic hydrogenation. *Bioresour. Technol.* **2010**, *101*, 1369−1376.

(6) Dumont, M.-J.; Narine, S. S. Soapstock and deodorizer distillates from North American vegetable oils: Review on their characterization, extraction and utilization. *Food Res. Int.* **2007**, *40*, 957−974.

(7) Shimada, Y.; Nakai, S.; Suenaga, M.; Sugihara, A.; Kitano, M.; Tominaga, Y. Facile purification of tocopherols from soybean oil deodorizer distillate in high yield using lipase. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1009−1013.

(8) EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA).. Scientific Opinion on the substantiation of a health claim related to 3 g/day plant sterols/stanols and lowering blood LDL-cholesterol and reduced risk of coronary heart disease pursuant to Article 19 of Regulation (EC) No 1924/2006. *EFSA J.* **2012**, *10*, 2693.

(9) Moreau, R. A.; Whitaker, B. D.; Hicks, K. B. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Prog. Lipid Res.* **2002**, *41*, 457−500.

(10) Teixeira, A. R. S.; Santos, J. L. C.; Crespo, J. G. Production of Steryl Esters from Vegetable Oil Deodorizer Distillates by Enzymatic Esterification. *Ind. Eng. Chem. Res.* **2011**, *50*, 2865−2875.

(11) Torres, C. F.; Torrelo, G.; Señoráns, F. J.; Reglero, G. A two steps enzymatic procedure to obtain sterol esters, tocopherols and fatty acid ethyl esters from soybean oil deodorizer distillate. *Process Biochem.* **2007**, *42*, 1335−1341.

(12) Villeneuve, P.; Turon, F.; Caro, Y.; Escoffier, R.; Baréa, B.; Barouh, B.; Lago, R.; Piombo, G.; Pina, M. Lipase-catalyzed synthesis of canola phytosterols oleate esters as cholesterol lowering agents. *Enzyme Microb. Technol.* **2005**, *37*, 150−155.

(13) Nagao, T.; Hirota, Y.; Watanabe, Y.; Kobayashi, T.; Kishimoto, N.; Fujita, T.; Kitano, M.; Shimada, Y. Recovery of sterols as fatty acid steryl esters from waste material after purification of tocopherols. *Lipids* **2004**, *39*, 789−794.

(14) Ghazani, S.; Marangoni, A. G. Minor Components in Canola Oil and Effects of Refining on These Constituents: A Review. *J. Am. Oil Chem. Soc.* **2013**, *90*, 923−932.

(15) Bondioli, P.; Mariani, C.; Lanzani, A.; Fedeli, E.; Muller, A. Squalene recovery from olive oil deodorizer distillates. *J. Am. Oil Chem. Soc.* **1993**, *70*, 763−766.

(16) Akgün, N. A. Separation of squalene from olive oil deodorizer distillate using supercritical fluids. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1558−1565.

(17) Gunawan, S.; Fabian, C.; Ju, Y.-H. Isolation and Purification of Fatty Acid Steryl Esters from Soybean Oil Deodorizer Distillate. *Ind. Eng. Chem. Res.* **2008**, *47*, 7013−7018.

(18) Verleyen, T.; Verhe, R.; Garcia, L.; Dewettinck, K.; Huyghebaert, A.; De Greyt, W. Gas chromatographic characterization of vegetable oil deodorization distillate. *J. Chromatogr., A* **2001**, *921*, 277−285.

(19) MatLab. *SimBiology Users Guide R2013a*; The MathWorks: Natick, MA, 2013.