Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

Developing a high-temperature solvent-free system for efficient biosynthesis of octyl ferulate

Shang-Ming Huang[a], Ping-Yu Wu[b], Jiann-Hwa Chen[b], Chia-Hung Kuo[c,*], Chwen-Jen Shieh[a,*]

[a]*Biotechnology Center, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan*

[b]*Graduate Institute of Molecular Biology, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan*

[c]*Department of Seafood Science, National Kaohsiung Marine University, 142 Haijhuan Road, Nanzih District, Kaohsiung 81143, Taiwan*

***Author to whom correspondence should be addressed.**

Dr. Chia-Hung Kuo (telephone +886-7-361-7141 ext. 3646; fax +886-7-364-0634; e-mail chkuo@webmail.nkmu.edu.tw)

Dr. Chwen-Jen Shieh (telephone +886-4-2284-0450 ext. 5121; fax +886-4-2286-1905; e-mail cjshieh@nchu.edu.tw)

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

ABSTRACT

Ferulic acid esters have been suggested as a group of natural chemicals with sunscreen function. The study aimed to utilize an environment-friendly enzymatic method to produce octyl ferulate by esterification of ferulic acid with octanol. The Box-Behnken design with response surface methodology (RSM) was adopted to evaluate the effects of synthesis variables, including reaction temperature (70–90 °C), enzyme amount (1,000–2,000 PLU) and stir speed (50–150 rpm), on the molar conversion of octyl ferulate. According to the joint test, both the reaction temperature and enzyme amount had great impacts on the molar conversion. RSM-developed second-order polynomial equation further showed great ability on data-fitting. Based on ridge max analysis, the optimum parameters for the biocatalyzed reaction were: 72 h reaction time, 92.2 °C reaction temperature, 1,831 PLU enzyme amount and 92.4 rpm stir speed, respectively. Finally, the molar conversion of octyl ferulate under optimum condition was verified to be 93.2 ± 1.5%. In conclusion, high yield of octyl ferulate synthesized by commercial immobilized lipase under elevated temperature conditions has been suggested, which our findings could broaden the utilization of the lipase and provide a biocatalytic approach, instead of the chemical method, for ferulic acid ester synthesis.

Keywords: ferulic acid esters, octyl ferulate, esterification, Box-Behnken design, response surface methodology, molar conversion, optimum condition

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

1. Introduction

Ferulic acid (FA) is one of the phenolic acids and abundantly exists in fruits and vegetables. FA has been demonstrated having many bioactivities such as antioxidation [1], excellent ultraviolet-absorbing activity [2,3], and potential health benefits against cardiovascular diseases, inflammatory diseases and cancers [4], etc. However, FA is a small and polar compound but only with limited solubility in oils. Due to the poor solubility of FA in a lipophilic medium, the applications of FA in food, cosmetics, and other nutraceutical industries were commonly limited owing to their low dissolution rate [5,6]. Thus, it is essential to improve the solubility of FA to increase its practicality as food antioxidants. A related study has been reported that the ester form of FA has better antioxidant activity than the acid form itself as well as the antioxidant activity of ferulate ester as compared to BHT [7]. To overcome the problem of poor solubility, pharmaceutical particle technology of chemical modifications has attracted much attention [8–13]. However, it is much difficult to synthesize such derivatives chemically, as FA is susceptible to oxidation under certain pH conditions [7,14,15]. Thus, enzymatic biosynthesis of FA lipids has been considered as an attractive alternative to conventional chemical processes [16]. However, environment-friendly preparation of hydrophobic derivatives of FA is still a challenge for researchers.

The hydrophilic feruloylated derivatives have been synthesized by the

esterification of FA with monosaccharides [17]. The water-soluble derivative of glyceryl ferulate biosynthesized by esterification of FA with glycerol using pectinase [18], and the solvent-free enzymatic transesterification of ethyl ferulate (EF) with triolein having a higher EF conversion of 77% [19], which were also reported. In additional, hydrophobic feruloylated derivatives can be produced by the esterification of FA with alcohols. The strategy of esterification of hydrophilic FA with lipophilic molecules, such as aliphatic alcohols, can be used to alter its solubility in a lipophilic medium. However, the reaction rate of FA condensation with alcoholic substrates is slow and might be that FA could conjugate with a carboxyl group and a bulky noncarboxylic region [20].

Lipases are the most commonly used among the biocatalysts in synthetic organic chemistry [21,22]. It has been extensively used to obtain chiral alcohols and carboxylic acids, due to their excellent chiral recognition [23]. The isoform B of the lipase from Candida Antarctica (CAL-B) is one of the most employed among them and possesses the high enantioselectivity for a broad range of substrates [24,25]. Novozym® 435 is a commercial enzyme prepared by immobilization of CAL-B onto a macroporous acrylic polymer resin. Novozym® 435 is a versatile biocatalyst, mainly used to perform the hydrolysis of oils and fats, under specific conditions, also capable of catalyzing esterification, transesterification, and interesterification reactions [26]. 3D-structures of

the Novozym® 435 have shown the presence of a short oligopeptide helix that may act as a lid, and it has been found to adopt different conformations as a function of detergent concentration, suggesting high mobility of the active environment [27]. The expression of catalytic activity requires that the enzyme active site should be flexible. Novozym® 435 is very active in organic solvents, which is needed to react under the condition of lower water activity in esterification process [28], suggesting that the enzyme could be adsorbed on this biocatalyst mainly by hydrophobic interactions [29,30]. Evidence that an increase in enzyme hydration is accompanied by an increase in the molecular mobility of enzymes [31,32]. Although Novozym® 435 had been used in the lipase-catalyzed synthesis of ferulate esters, the yield remained low (17%) after the reaction by several days [33]. Moreover, it has been reported that during the synthesis of ferulic esters by direct esterification with ferulic acid, the catalytic activity of the enzyme might be severely affected by the hydrophilicity of ferulic acid [34,35]. In fact, solvent-free reaction systems, an eco-friendly method minimizing the environmental pollution, could be applied in the lipase-catalyzed synthesis of ferulate esters, which might decrease the reaction time but increase the yield of synthesized esters [36,37]. Additionally, the solvent-free reaction system is a condition commonly with a simple mixture of substrates and offers some reaction advantages including maximization of substrate concentration, higher volumetric productiveness, less environmental hazard,

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

and cost savings in reactor design in large-scale process and chemical product separation/purification [38]. However, in the case of FA esterification, developing a solvent-free system operated at high temperature was required to increase the yield of FA esters.

As mentioned above, ferulic acid (FA) has been suggested as one of the natural nutraceuticals with biological function, but FA exhibits poor solubility and low stability in various solvent systems might limit its application. The aim of the present work was tending to develop a simple and environmentally-friendly biocatalytic method, instead of a chemical catalytic method, for the preparation of FA esters. In this study, the lipase-catalyzed esterification of FA with octanol to form octyl ferulate under solvent-free condition was conducted, followed by optimizing processes with an experimental design using RSM to evaluate the best experimental conditions. Finally, the thermodynamic and optimum effects on solvent-free reaction system of lipase-catalyzed biosynthesis of FA was evaluated.

2. Materials and methods

2.1. Materials

Immobilized lipase Novozym® 435 (10,000 U/g; propyl laurate units (PLU) from *Candida Antarctica* B (EC3.1.1.3) supported on a macroporous acrylic resin was

purchased from Novo Nordisk Bioindustrials Inc. (Copenhagen, Denmark). Ferulic acid (FA), octanol, hexanol, 2-methyl-2-butanol, methanol and acetic acid were purchased from the Sigma Chemical Co. (St. Louis, MO). A molecular sieve 4Å was purchased from Davison Chemical (Baltimore, MD, USA). All other reagents and chemicals, unless otherwise noted, were of analytical grade.

2.2. Enzymatic synthesis of octyl ferulate

All materials were dehydrated by a molecular sieve 4 Å for 24 h before use. Ferulic acid (20 mM) and Novozym® 435 were well mixed with octanol (1 mL) in the sealed 10 mL dark vials, and placed in a water bath for 72 h, under different experimental conditions of temperatures, enzyme amount and stir speed as **Table 1** design. The liquid samples were further analyzed by high-performance liquid chromatography (HPLC). Samples were centrifuged and diluted with hexanol/2-methyl-2-butanol (1:100). The analysis was done according to the procedure modified by Huang et al. [38]. The flow rate was 1.0 mL/min, and sampling was detected at the wavelength of 325 nm. The molar conversion was calculated based on the peak areas of the sample.

2.3. Experimental design

A 3-level-3-factor Box-Behnken requiring 15 experiments was employed in this study. To avoid bias, the 15 runs were performed in a random order. The variables and their levels selected for the study of octyl ferulate biosynthesis were: reaction

temperature (70–90°C), enzyme amount (1,000–2,000 PLU) and stir speed (50–150 rpm). All of the experiments were performed at a reaction time of 72 h. **Table 1** shows the independent factors (xi), levels and experimental design regarding coded and uncoded values.

2.4. Statistical analysis

Each data was analyzed by the response surface regression (RSREG) procedure with SAS software to fit the equation of second-order polynomial shown in Equation 1:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \qquad (1)$$

where Y is the molar conversion of octyl ferulate; β_0 is a constant, β_i, β_{ii} and β_{ij} are constant coefficients; and xi and xj are uncoded independent variables. The suffixes i and j are seen in the above equation with the three independent variables denoting: x_1 for reaction temperature, x_2 for enzyme amount and x_3 for stir speed. The option of a ridge max option in the SAS software was employed to compute the estimated ridge of maximum response when increasing the radius from the center of the original design.

3. Results and discussion

3.1. Primary experiment

Lipase-catalyzed synthesis of octyl ferulate from ferulic acid (20 mM), Novozym® 435 (1,500 PLU) and octanol were carried out in a water bath at a reaction time of 72 h. The octyl ferulate catalyzed by Novozym® 435, and the liquid samples analyzed by high-performance liquid chromatography (HPLC) are shown in **Figure 1**. Moreover, the effects of reaction temperature and reaction time on molar conversion of octyl ferulate are shown in **Figure 2**, illustrating that a higher molar conversion was obtained after the reaction time of 24 hours as well as by an increase of reaction temperature. Also, the higher the reaction temperature, the faster the reaction could be observed without deterioration of enantioselectivities [39]. It is a known fact that the reaction temperature is a crucial parameter in biocatalysis. However, the elevated temperature may result in inactive enzymes. From the report, enzymatic transesterification of ethyl ferulate (EF) and monostearin for feruloylated lipids production was investigated, which the optimum reaction temperature was 74°C [9]. A novel enzymatic route of feruloylated structured lipids synthesis by the transesterification of EF with castor oil in a solvent-free system was investigated. The study indicated that high EF conversion was obtained under the reaction temperature of 90°C [22]. The reaction temperature also has a considerable effect on the thermodynamic equilibrium of the reversible

reaction. Therefore, the experiments at three different temperatures (70, 80 and 90°C) were carried out to investigate the influence of temperature on biosynthesis reaction. As shown in **Figure 2**, the molar conversion increased with elevating reaction temperature. At a reaction temperature of 90°C, molar conversion reached the highest value of a molar conversion of 88.4%, and the fastest reaction rate (reflected by the slope of the linear part of the curves) was also observed. When the reaction time surpassed 72 h, the time course was leveled-off. The reason may be that the viscosity of the reaction mixture was gradually increased over reaction time, leading to a decrease in mutual solubility and inhibit the diffusion of substrates [40]. Thus, reducing mass transfer and unfavorable interactions between enzyme particles and substrates might occur in the process. However, longer reaction time could lead to a higher molar conversion. Thus, the following experimental design was conducted on the reaction time of 72 h.

3.2. Model fitting

In order to systematically understand the relationships between reaction temperature, enzyme amount and stir speed for the synthesis of octyl ferulate, a 3-level-3-factor Box-Behnken design (BBD) was applied to carry out a total of 15 treatments (experiments), followed by determining the experimental data for process optimization of octyl ferulate bioproduction, using statistical RSM modelling. The aim of this study

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

was the development and evaluation of a statistical approach to understanding the relationship between experimental variables and lipase-catalyzed responses well. Thus, the process could be optimized before the scaling-up procedure to save the production costs and time to obtain a high-quality product. Compared with the single-factor-at-a-time design, which has been adopted most often in literature, the RSM combined with a BBD employed in this study was more efficient in reducing experimental runs and time for investigating the optimized biosynthesis of octyl ferulate.

The experimental conditions and results of a 3-level, 3-factor BBD are shown in Table 1. The RSREG procedure for SAS was used to fit the second-order polynomial Equation (1) to the experimental data-percent mole conversions. Among these various treatments, the highest molar conversion (88.4 ± 3.1%) was treatment 8 (reaction temperature 90°C, enzyme amount 1,500 PLU and stirring speed 150 rpm), and the lowest molar conversion (42.4 ± 5.7%) was treatment 1 (reaction temperature of 70°C, enzyme amount of 1,000 PLU and stirring speed of 100 rpm). From the SAS output of RSREG, the second-order polynomial Equation (1) is given below:

$$Y = -299.1 + 5.6825X_1 + 0.6625X_2 + 0.22X_3 - 0.0031X_1X_2 - 0.0035X_1X_3$$

$$-0.0005X_2X_3 - 0.02025{X_1}^2 - 0.00059{X_2}^2 + 0.00091{X_3}^2 \quad (1)$$

Further, the analysis of variance (ANOVA) indicated that the second-order polynomial equation showed a highly significant correlation and was adequate to

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

represent the relationship between the experimental response (molar conversion) and significant variables, having a very small *p*-value (0.0001) as well as a satisfactory coefficient of determination ($R^2 = 0.9887$). Furthermore, the overall effects of the three synthesis variables on the molar conversion were further analyzed by a joint test. The results revealed that reaction temperature (X_1) and enzyme amount (X_2) were the most important factors, exerting a statistically significant overall effect ($p < 0.001$) on the responded molar conversion. The stirring speed (X_3) was insignificant statistically on the responded molar conversion.

3.3. Optimal synthesis conditions

The optimum biosynthesis of octyl ferulate was further determined by the ridge max analysis (**Figure 3**), indicating that the maximum molar conversion was 91.74 ± 2.3% at 72 h, temperature 92.2°C, enzyme amount 1,831 PLU and stir speed 92.4 rpm. **Figure 4** represents the correlation between the experimental values and the predicted values of the RSM model for average cutting speed. A verification experiment performed at the suggested optimum conditions could obtain a molar conversion of 93.2 ± 1.5%, which was close to the RSM-predicted molar conversion; thus, the predicted model was successfully established. In this study, the lipase-catalyzed biosynthesis of octyl ferulate could be carried out with an easy method.

Additionally, to confirm the enzyme reusability, the immobilized lipase for octyl

ferulate synthesis was also determined under optimum conditions. The lipase was recovered from the medium after the reaction, followed by being reused directly in the next batch. When the batch of immobilized lipase was reused five times, the molar conversion of octyl ferulate remained higher than 90% with very little loss of enzymatic activity (**Figure 5**). The data indicated that the immobilized lipase could keep enzymatic stability under the conditions of long-term octanol exposure and high reaction temperature (90°C). Thus, the result confirmed that the lipase could be effectively applied for the synthesis of octyl ferulate and that the enzymatic stability was high enough to permit reuse.

4. Conclusions

In this study, the esterification of ferulate with octanol catalyzed by lipase in a solvent-free medium was investigated. The immobilized Novozym® 435 could be well used to synthesize octyl ferulate. Both 3-level-3-factor BBD and RSM were employed successfully for the experimental design and data analysis. An environmentally friendly experimental model for the octyl ferulate synthesis was well built, and the optimal conditions for biosynthesis were respectively in a reaction time of 72 h, a reaction temperature of 92.2°C, enzyme amount of 1,831 PLU and stir speed of 92.4 rpm. Synthesis of octyl ferulate under this optimal condition was performed to obtain a molar conversion of 93.2 ± 1.5%. Notably, our results indicated that the high reaction

temperature significantly affected the efficiency of lipase-catalyzed ester synthesis under the solvent-free system. Overall, as compared to the chemical methods, our present study suggests that the lipase-catalyzed environmental-friendly synthesis of esters under solvent-free reaction system at higher reaction temperature without marked loss of enzymatic activity could offer a significant reference for industrial preparation process.

Acknowledgments

The authors are very appreciative of all the support from the Ministry of Science and Technology under the grant number of MOST-101-2313-B-005-040-MY3.

Conflicts of interest

There is no any conflict of interest regarding the publication of this article.

References

[1] Liu, J.; Wen, X.Y.; Lu, J. F.; Kan, J.; Jin, C.H. Free radical mediated grafting of chitosan with caffeic and ferulicacids: Structures and antioxidant activity. *Int. J. Biol. Macromol.* **2014**, *65*, 97–106.

[2] Choo, W.S.; Birch, E.J. Radical scavenging activity of lipophilized products from lipase-catalyzed transesterification of triolein with cinnamic and ferulic acids. *Lipids* **2009**, *44*, 145–152.

[3] Itagaki, S.; Kurokawa, T.; Nakata, C.; Saito, Y.; Oikawa, S.; Kobayashi, M. In vitro and in vivo antioxidant properties of ferulic acid: A comparative study with other natural oxidation inhibitors. *Food Chem.* **2009**, *114*, 466–471.

[4] Mancuso, C.; Santangelo, R. Ferulic acid: Pharmacological and toxicological aspects. *Food Chem. Toxicol.* **2014**, *65*, 185–195.

[5] Karboune, S.; St-Louis, R.; Kermasha, S. Enzymatic synthesis of structured phenolic lipids by acidolysis of flaxseed oil with selected phenolic acids. *J. Molec. Catal. B: Enzym.* **2008**, *52–53*, 96–105.

[6] Zheng, Y.; Branford-White, C.; Wu, X.M.; Wu, C.Y.; Xie, J.G.; Quan, J. Enzymatic synthesis of novel feruloylated lipids and their evaluation as antioxidants. *J. Amer. Oil Chem. Soc.* **2010**, *87*, 305–311.

[7] Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem*. **2002**, *50*, 2161–2168.

[8] Reddy, K.K.; Ravinder, T.; Kanjilal, S. Synthesis and evaluation of antioxidant and antifungal activities of novel ricinoleate-based lipoconjugates of phenolic acids. *Food Chem.* **2012**, *134*, 2201–2207.

[9] Sun, S.D.; Song, F.F.; Bi, Y.L.; Yang, G.L.; Liu, W. Solvent-free enzymatic transesterification of ethyl ferulate and monostearin: Optimized by response surface methodology. *J. Biotechnol.* **2012**, *164*, 340–345.

[10] Xin, J.; Zhang, L.; Chen, L.; Zheng, Y.; Wu, X.; Xia, C. Lipase-catalyzed synthesis of ferulyl oleins in solvent-free medium. *Food Chem.* **2009**, *112*, 640–645.

[11] Yang, Z.; Guo, Z.; Xu, X. Enzymatic lipophilisation of phenolic acids through esterification with fatty alcohols in organic solvents. *Food Chem.* **2012**, *132*, 1311–1315.

[12] Yang, H.; Mu, Y.; Chen, H.; Xiu, Z.; Yang, T. Enzymatic synthesis of feruloylated lysophospholipid in a selected organic solvent medium. *Food Chem.* **2013**, *141*, 3317–3322.

[13] Fang, X.; Shima, M.; Kadota, M.; Tsuno, T.; Adachi, S. Suppressive effect of alkyl ferulate on the oxidation of linoleic acid. *Biosci. Biotech. Biochem.* **2006**, *70*, 457–461.

[14] Yang, X.Z.; Diao, X.J.; Yang, W.H.; Li, F.; He, G.W.; Gon, G.Q.; Xu, Y.G. Design, synthesis and antithrombotic evaluation of novel dabigatran prodrugs containing methyl ferulate, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2089–2092.

[15] Giuliani, S.; Piana, C.; Setti, L.; Hochkoeppler, A.; Pifferi, P.G.; Williamson, G.; Faulds, C.B. Synthesis of pentylferulate by a feruloyl esterase from Aspergillus niger using water-in-oil microemulsions. *Biotechnol. Lett.* **2001**, *23*, 325–330.

[16] Saija, A.; Tomaino, A.; Cascio, R.L.; Trombetta, D.; Proteggente, A.; Pasquale, A. D.; Uccella, N.; Bonina, F. Ferulic and caffeic acids as potential protective agents against photooxidative skin damage. *J. Sci. Food Agric.* **1999**, *79*, 476–480.

[17] Stamatis, H.; Sereti, V.; Kolisis, F.N. Enzymatic synthesis of hydrophilic and hydrophobic derivatives of natural phenolic acids in organic media. *J. Molec. Catal. B: Enzym.* **2001**, *11*, 323–328.

[18] Matsuo, T.; Kobayashi, T.; Kimura, Y.; Tsuchiyama, M.; Tsuchiyama, M.; Oh, T.; Sakamoto, T.; Adachi, S. Synthesis of glyceryl ferulate by immobilized ferulic acid esterase. *Biotechnol. Lett.* **2008**, *30*, 2151–2156.

[19] Compton, D.L.; Laszlo, J.A.; Berhow, M.A. Lipase-catalyzed synthesis of ferulate esters. *J. Amer. Oil Chem. Soc.* **2000**, *77*, 513–519.

[20] Kobayashi, T.; Adachi, S.; Matsuno, R. Lipase-catalyzed condensation of pmethoxyphenethyl alcohol and carboxylic acids with different steric and electrical properties in acetonitrile. *Biotechnol. Lett.* **2003**, *25*, 3–7.

[21] Zagozda, M.; Plenkiewicz, J. Biotransformations of 2,3-epoxy-3-arylpropanenitriles by Debaryomyces hansenii and Mortierella isabellina cells. *Tetrahedron Asymmetr.* **2008**, *19*, 1454–1459.

[22] Sun, S.D.; Zhu, S.; Bi, Y.L. Solvent-free enzymatic synthesis of feruloylated structured lipids by the transesterification of ethyl ferulate with castor oil. *Food Chem.* **2014**, *158*, 292–295.

[23] Bornscheuer, U.T.; Kazlauskas, R.J. Hydrolases in Organic Synthesis, Wiley-VCH: Weinheim, 2006; pp. 61–183.

[24] Xu, D.; Li, Z.; Ma, S. Novozym-435-catalyzed enzymatic separation of racemic propargylic alcohols. A facile route to optically active terminal aryl propargylic alcohols. *Tetrahedron Lett.* **2003**, *44*, 6343–6346.

[25] Jacobsen, E.E.; van Hellemond, E.; Moen, A.R.; Prado, L.C.V.; Anthonsen, T. Enhanced selectivity in Novozym 435 catalyzed kinetic resolution of secondary alcohols and butanoates caused by the (R)-alcohols. *Tetrahedron Lett.* **2003**, *44*, 8453–8455.

[26] Rajendran, A.; Palanisamy, A.; Thangavelu, V. Lipase catalyzed ester synthesis for food processing industries. *Braz. Arch. Biol. Technol.* **2009**, *52*, 207–219.

[27] Uppenberg, J.; Hansen, M.T.; Patkar, S.; Jones, T.A. The sequence, crystal structure determination and refinement of two crystal forms of lipase B from *Candida Antarctica*. *Structure* **1994**, *2*, 293–308.

[28] Jaeger, K.E.; Eggert, T. Lipases for biotechnology. *Curr. Opin. Biotechnol.* **2002**, *13*, 390–397.

[29] Mei, Y., Miller, L., Gao, W., Gross, R. A. Imaging the distribution and secondary structure of immobilized enzymes using infrared microspectroscopy. *Biomacromolecules* **2003**, *4*, 70–74.

[30] Cabrera, Z.; Fernandez-Lorente, G.; Fernandez-Lafuente, R.; Palomo, J.M.; Guisan, J.M.Novozym 435 displays very different selectivity compared to lipase from *Candida Antarctica* B adsorbed on other hydrophobic supports. *J. Mol. Catal. B: Enzym.* **2009**, *57*, 171–176.

[31] Burke, P.A.; Griffin, R.G.; Klibanov, A.M. Solid-state nuclear magnetic resonance investigation of solvent dependence of tyrosyl ring motion in an enzyme. *Biotechnol. Bioeng.* **1993**, *42*, 87–94.

[32] Affleck, R.; Xu, Z.F.; Suzawa, V.; Focht, K.; Clark, D.S.; Dordick, J.S. Enzymatic catalysis and dynamics in low-water environments. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 1100–1104.

[33] Compton, D. L.; Laszlo, J. A.; Berhow, M. A. Lipase-catalyzed synthesis of ferulate esters. *J. Amer. Oil Chem. Soc*. **2000**, *77*, 513–519.

[34] Iwai, M.; Okumura, S.; Tsujisaka, Y. Synthesis of terpene alcohol esters by lipase. *Biol. Chem*. **1980**, *44*, 2731–2732.

[35] Langrand, G.; Rondot, N.; Triantaphylides, C.; Baratti, J. Short chain flavour esters synthesis by microbial lipases. *Biotechnol. Lett*. **1990**, *12*, 581–586.

[36] Sun, S.; Song, F.; Bi, Y.; Yang, G.; Liu, W. Solvent-free enzymatic transesterification of ethyl ferulate and monostearin: optimized by response surface methodology. *J. Biotechnol*. **2012**, *164*, 340–345.

[37] Sun, S.; Zhu, S.; Bi, Y. Solvent-free enzymatic synthesis of feruloylated structured lipids by the transesterification of ethyl ferulate with castor oil. *Food Chem*. **2014**, *158*, 292–295.

[38] Huang, K. C; Li, Y.; Kuo, C. H.; Twu,Y. K.; Shieh, C. J. Highly efficient Synthesis of an emerging lipophilic antioxidant: 2-ethylhexyl ferulate. *Molecules* **2016**, *21*, 478–489.

[39] Bezerra, M.A.; Santelli, R.E.; Oliveira, E.P.; Villar, L.S.; Escaleira, L.A. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta.* **2008**, *76*, 965–977.

[40] Jeon, N.Y.; Ko, S.J.; Won, K.H.; Kang, H.Y.; Kim, B.T.; Lee, Y.S.; Lee, H. Synthesis of alkyl (*R*)-lactates and alkyl (*S,S*)-O-lactyllactates by alcoholysis of rac-lactide using Novozym 435. *Tetrahedron Lett.* **2006**, *47*, 6517–6520.

Figure captions

Figure 1. Scheme of octyl ferulate biosynthesis and HPLC chromatogram of ferulic acid and octyl ferulate. Peak 1 of HPLC chromatogram is ferulic acid, and peak 2 is octyl ferulate.

Figure 2. Effects of reaction time and temperature on Novozym® 435-catalyzed synthesis of octyl ferulate. The reaction was carried out at a reaction temperature of 70-90 °C and enzyme amount of 1,500 PLU in a water bath.

Figure 3. Response surface plots are showing the relationships between the molar conversion of octyl ferulate synthesis and reaction parameters (reaction temperature and enzyme amount).

Figure 4. Comparison of the experimental data with those predicted by the RSM model.

Figure 5. Reusable cycles of Novozym® 435 in the synthetic process of octyl ferulate under indicated optimal condition.

Peer-reviewed version available at Catalysts 2018, 8, 338; doi:10.3390/catal8080338

Table 1

Box-Behnken design experiments and observed data of molar conversion.

Treatment No.[a]	Experimental factors			Molar Conversion[b] (%)
	Reaction Temperature (°C)	Enzyme Amount (PLU)	Stir Speed (rpm)	
	X_1	X_2	X_3	
1	-1(70)	-1(1000)	0(100)	42.4 ± 5.7
2	1(90)	-1(1000)	0(100)	75.2 ± 0.7
3	-1(70)	1(2000)	0(100)	61.7 ± 3.6
4	1(90)	1(2000)	0(100)	88.3 ± 0.5
5	-1(70)	0(1500)	-1(50)	49.4 ± 1.1
6	1(90)	0(1500)	-1(50)	88.3 ± 0.1
7	-1(70)	0(1500)	1(150)	56.5 ± 0.7
8	1(90)	0(1500)	1(150)	88.4 ± 3.1
9	0(80)	-1(1000)	-1(50)	56.4 ± 0.1
10	0(80)	1(2000)	-1(50)	80.2 ± 3.8
11	0(80)	-1(1000)	1(150)	64.7 ± 4.9
12	0(80)	1(2000)	1(150)	83.5 ± 1.1
13	0(80)	0(1500)	0(100)	71.1 ± 1.4
14	0(80)	0(1500)	0(100)	70.2 ± 2.8
15	0(80)	0(1500)	0(100)	70.2 ± 3.5

[a]The treatments were run in a random order.

[b]Molar conversion for octyl ferulate showing means ± SD of duplicated experiments.

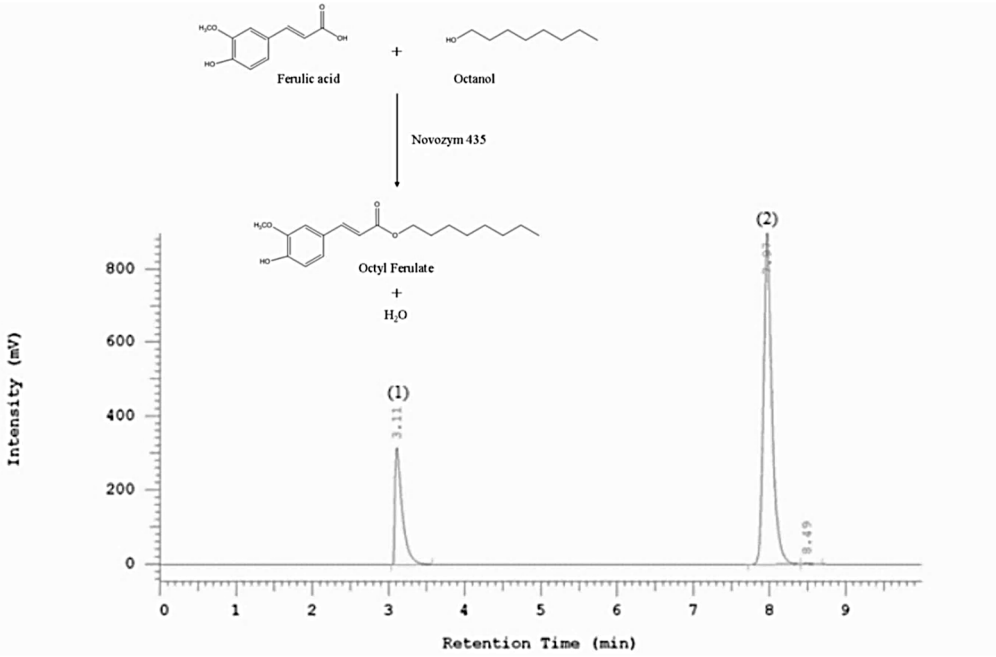

Figure 1

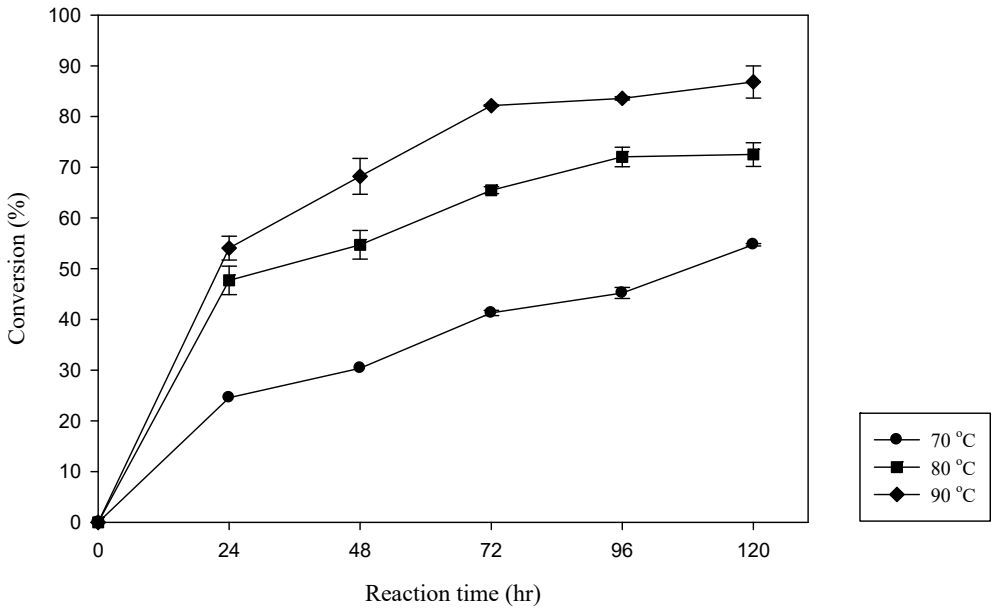

Figure 2

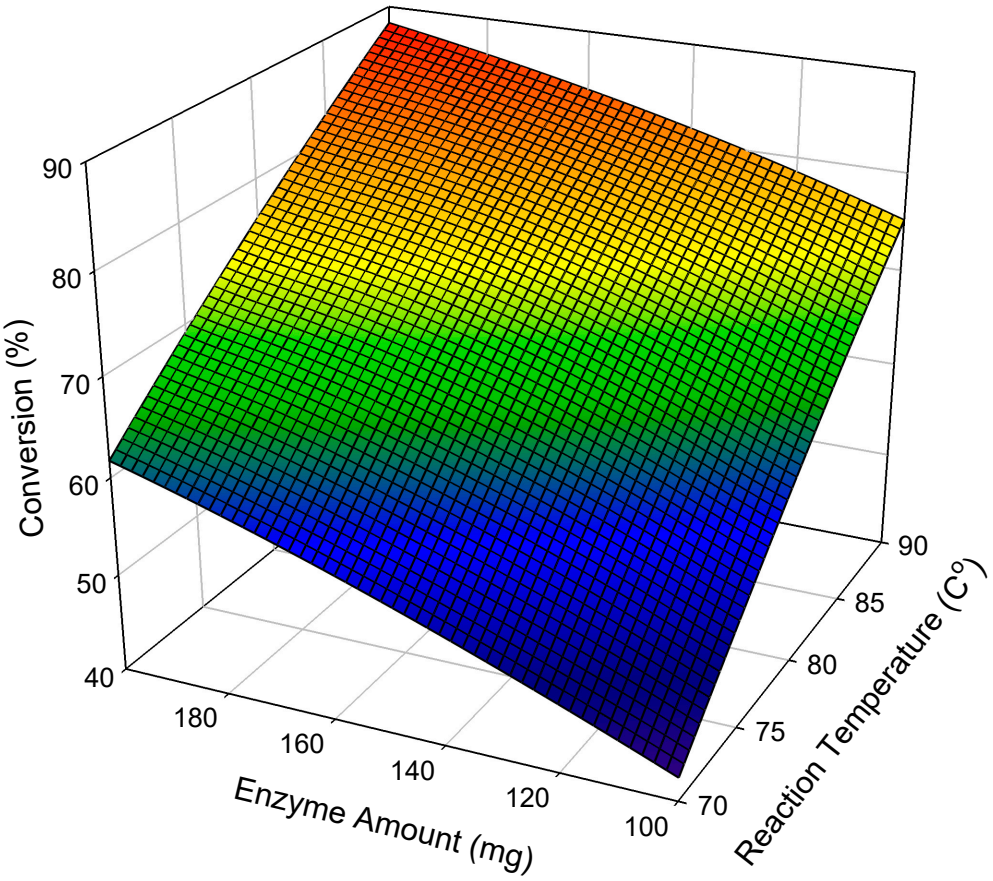

Figure 3

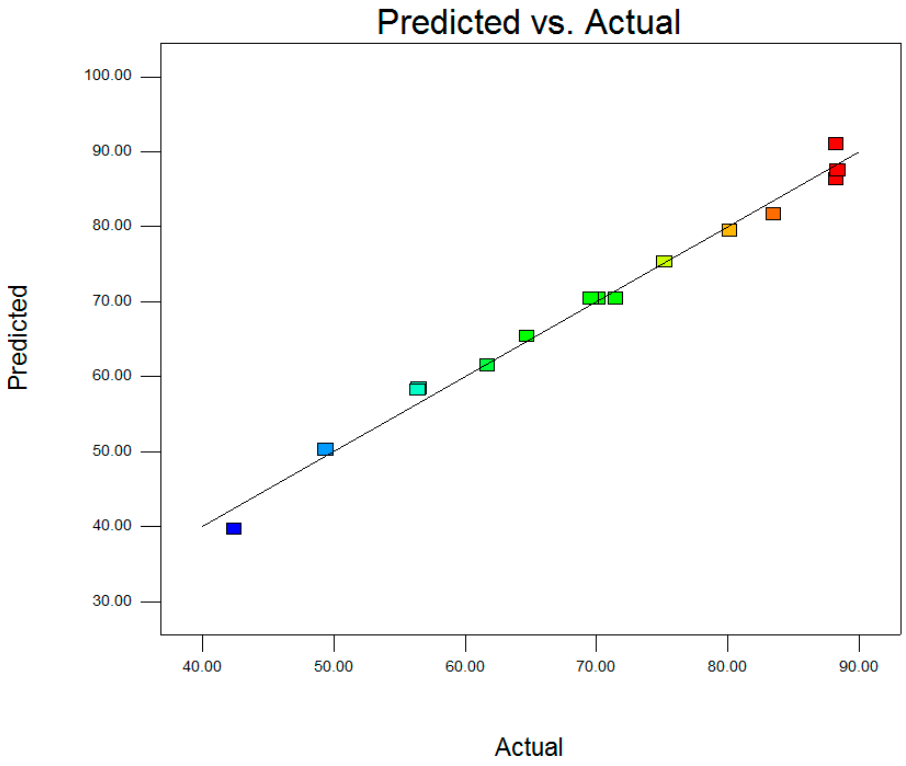

Figure 4

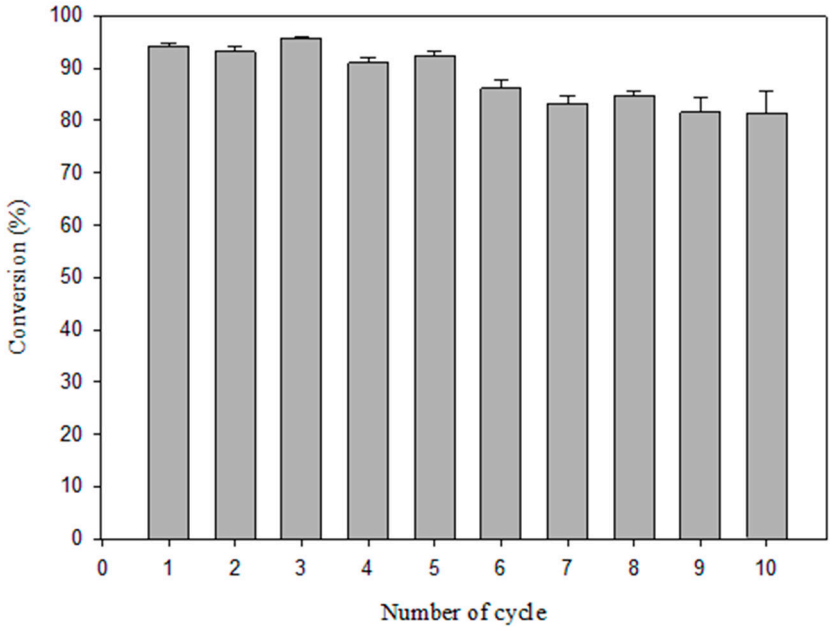

Figure 5