Biocatalysis of Rutin Hexadecanedioate Derivatives: Effect of Operating Conditions on Acylation Performance and Selectivity

Manel Slimane, Aude Cordin, Mohamed Ghoul and
Chebil Latifa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67621

Abstract

Rutin was enzymatically acylated with hexadecanedioic acid, in *tert*-amyl alcohol, by an immobilized lipase from *Candida antartica* "Novozym 435". The effect of different techniques of water removal, temperature, concentration of rutin and diacid/rutin molar ratio was investigated. The obtained results indicated that drying the media by adding the molecular sieves in the outer loop of the reactor was the most efficient method leading to water content lower than 200 ppm. The highest performances (conversion yield and initial rate) were reached at 90°C, 131 mM of rutin, and 118 mM of acid. Depending on the water content and the diacid/rutin molar ratio, only rutin 4‴-hexadecanedioate or both rutin 4‴-hexadecanedioate and dirutin 4‴, 4‴-hexadecanedioate were synthesized.

Keywords: biocatalysis, lipase, rutin, hexadecanedioic acid, water activity

1. Introduction

Flavonoids are benzo-γ-pyrone derivatives widely distributed in plants kingdom [1–4]. They have been shown to possess a wide range of biological activities including antiviral, anti-allergic, anti-inflammatory, anti-tumor properties [5, 6]. These properties of flavonoids are mainly due to their antioxidant activities based on their ability to act as hydrogen or electron donors [7]. The magnitude of this activity depends on their structure. Flavonoids with high biological activities are used in food preparations, cosmetics or pharmaceuticals [8–10]. However, their integration into several preparations is affected by their poor solubility in very hydrophilic and very hydrophobic solvents [11, 12]. In order to overcome this drawback, different ways of functionalization

INTECH
open science | open minds

are described in the literature. One of them is the acylation reaction, with the aim to enhance both the solubility in hydrophobic media and the biological activities of flavonoids [13]. This reaction can be carried out via enzymatic or chemical route. Enzymatic reactions are preferred to chemical ones due to their regio-selectivity toward polyhydroxylated compounds like rutin [14, 15]. Rutin is a flavonol glycoside and one of the most studied flavonoids in the literature. Several papers dealt with improvement of the solubility and the biological activities of rutin by enzymatic acylation under a wide range of operating conditions. These studies showed that the performance (conversion yield, regioselectivity, etc.) of this reaction was affected by several factors (reaction media, solubility and nature of the substrates, operating conditions, enzyme concentration, etc.). Lue et al. [16] reported that the use of ionic liquids as acylation medium enhances the solubility of substrates and gives high conversion yields of rutin. Zheng et al. [17] observed better rutin conversion rates when acylation was conducted under ultrasound radiations. This improvement was attributed by these authors to the enhancement of lipase activity. Water content of the medium has a strong effect on acylation reaction. It can affect both the solubility of the substrates and the activity of the biocatalyst. In the case of rutin, Ardhaoui et al. [18] reported that the highest rutin conversion yield was reached with water content less than 200 ppm (76%).

The most used enzyme for rutin acylation reactions is lipase B from *Candida antarctica*. This enzyme has shown high performances in terms of conversion yield and enantioselectivity [19].

The chain length or the nature of the acyl donor can also affect the conversion yield of rutin and the regioselectivity of the reaction [16, 18, 20, 21]. Conversion yields arise from 48 to 87% with the length of carbon chain [18]. Dicarboxylic acids, another class of fatty acids, can be used for the acylation of flavonoids. These compounds have two advantages compared to monocarboxylic acids. In one hand, they exhibit more flexibility due to their second carboxyl group. In the second hand, according to their structure, they have bacteriostatic and bactericidal properties against a variety of aerobic and anaerobic bacteria [22]. However, few data are available concerning their use as acyl donors with flavonoids. Only Theodosiou et al. [23] and Ardhaoui et al. [18] reported the enzymatic modification of flavonoids with diacids without any optimization of operating conditions of this reaction. The aim of the present work was to study the enzymatic synthesis of rutin hexadecanedioate by Novozym 435 in organic medium. The effect of several factors such as drying techniques, temperature, rutin concentration, molar ratio rutin/hexadecanedioic acid, concentration of biocatalyst, and its reuse were investigated. The behavior of initial rate, productivity, conversion rates, and the regioselectivity of the reaction were quantified and discussed.

2. Materials and methods

2.1. Enzyme and chemicals

Immobilized lipase B from *Candida antarctica* (Novozym 435, 7000 PLU/mg: propyl laurate units synthesized per gram of catalyst) was purchased from Novozymes AS, Bagsvaerd, Denmark. *tert*-amyl alcohol was supplied by Merck (France). Rutin was furnished by Sigma-Aldrich (France). Hexadecanedioic acid (Cathay Biotechnology, China) was used as acyl donor. The molecular sieves 4Å, used to dry solvents and reactions media, were provided by Acros organis (France).

2.2. Rutin ester synthesis

The enzymatic syntheses of rutin hexadecanedioate were carried out in a stirring batch reactor (250 ml) from "Pilotes Systèmes" (France) or Wheaton® reactors (USA). Agitation speed was varied from 300 to 500 rpm. Rutin (65–196 mM) was dissolved in 250 ml of dried *tert*-amyl alcohol at different temperatures (60, 80, and 90°C). The hexadecanedioic acid concentration was adjusted to obtain a di-acid/rutin molar ratio in the range of 0.05–20 in the solution. Esterification reactions were started by the addition of Novozym 435 (10, 30, and 50 g/L). The reaction was stopped after 50–72 h by removing the biocatalyst. Blank samples, containing all components except the enzyme preparation, were carried out in tandem with the enzymatic trials.

Different drying techniques were used: (i) drying with molecular sieves added to the bulk medium or (ii) introduced in an external loop. In this last case, solvent and water were evaporated under vacuum, crossing in a fist part molecular sieves as vapor phase then as liquid phase (**Figure 1a**). Another configuration of external loop consists in the water removal only in liquid phase (**Figure 1b**).

Water content was 800–1000, 400–550, and 200–300 ppm, respectively with molecular sieves added to the bulk medium, the use of drying in vapor and liquid phases and drying only through liquid phase.

2.3. Analytical procedure

2.3.1. Karl Fisher analysis

The water content of the reaction medium was determined by a coulometric Karl Fisher apparatus (KF 737II coulometer) Metrohm (France). The reagent was Hydranal-Coulomat AG-H (Riedel-de-Haën, France).

2.3.2. High-performance liquid chromatography analysis

The substrate and product concentrations were determined by high-pressure liquid chromatography in external calibration. Analysis were carried out at 55°C in a system (Alliance 2690 Waters) composed of a column (Symmetry® C18, 4.6 × 250 mm, 5 µm, Waters, France), a UV detector (250 and 350 nm, Waters 2487, France) and a ELSD (Evaporative Light Scattering detector, Altech 2000, France). The various compounds were separated using water (0.1% acetic acid)/methanol (0.1% acetic acid) solutions: 0 min (70/30), 5 min (0/100), 10 min (0/100), 12 min (70/30), 15 min (70/30).

2.3.3. Purification and determination of the chemical structure of rutin esters

Rutin esters were purified by liquid–liquid extraction. The residual flavonoid was removed at 60°C under agitation during 45 min with a water/heptane solution (2/3, v/v), while, the flavonoid esters were separated from the acyl donor by using acetonitrile (50 mL) at 40°C during 20 min of agitation. The flavonoid esters solution was concentrated by solvent evaporation under reduced pressure for injection in a preparative HPLC (Waters, France). A column RP18
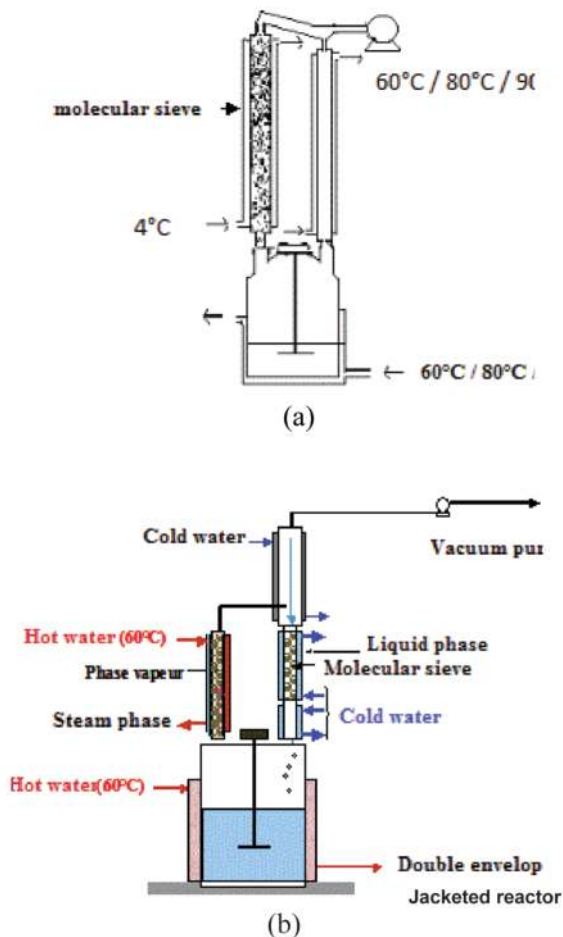
Figure 1. Drying techniques used to remove water from the reaction media. (a) Drying the media by using molecular sieves in the outer loop of the reactor in liquid phase. (b) Drying the media by using molecular sieves in the outer loop of the reactor in vapor and liquid phase.

(30 × 100 mm, 5 µm, Waters XTerra®, France) and a UV detector (350 nm, Waters 2487, France) were used for separate and analyze esters. A gradient of water and acetonitrile with 0.1% ace-tic acid at a flow rate of 18 mL/min was applied: 0 min (70/30), 10 min (20/80), 12 min (20/80), 13 min (70/30), 15 min (70/30). The medium was diluted 2.5 times in the starting phase (water with 0.1% acetic acid/acetonitrile with 0.1% acetic acid, 70/30) and 850 µl of this solution were injected for each batch. Eighteen batches were produced. Rutin hexadecanedioate and dirutin hexadecanedioate are obtained with a purity ≥95%. This purification method was adapted from Ardhaoui et al. [18].

The chemical structures of the purified dirutin hexadecanedioate and rutin hexadecanedioate were determined by 1H NMR and 13C NMR in DMSO-d6 using a Brücker AM 400 at 400 MHz and at 100 MHz, respectively.

2.4. Determination of conversion rate, initial rate, productivity, and selectivity

2.4.1. Conversion rate

The conversion rate of rutin and acid was calculated from concentrations given by HPLC analysis.

$$\text{Conversion (\%)} = ([S]i - [S]f) * 100/[S]i \tag{1}$$

[S]i: initial substrate concentration (mmol/L)

[S]f: final substrate concentration (mmol/L)

2.4.2. Initial rate of monorutin hexadecanedioate formation

The initial rate was calculated during the first three hours of the synthesis reaction of rutin hexadecanedioate by taking the slope of the kinetic linear portion.

2.4.3. Productivity

The productivity is given by the following expression:

$$\text{Productivity } (g/(L/h)) = \text{Mass of ester formed divided by the working}$$
$$\text{volume of the reactor and by the duration of the reaction} \tag{2}$$

2.4.4. Selectivity

The selectivity is given by the following expression:

$$\text{Selectivity (\%)} = [\text{monorutin}] \, t * 100/([\text{monorutin}] \, t + [\text{dirutin}] \, t) \tag{3}$$

Where, [monorutin] t and [dirutin] t are molar concentrations at t (time) of mono and dirutin esters.

3. Results and discussion

3.1. Influence of water removal techniques

The presence of a minimum amount of water is necessary for maintaining the catalytic activity of lipase. But, high concentration of water can favor the hydrolysis reaction.

Different water removal techniques were investigated. In the presence of molecular sieves, two alternatives have been tested. The molecular sieves were introduced either in the bulk medium or in the outer loop of the reactor. The performance of these reactions carried out with or without water removal were evaluated and compared.

The results are reported in **Figure 3a–c**. The reaction carried out with high water content leads to the lowest conversion yield, initial rate, and productivity while, reactions conducted with water removal are characterized by high conversion yield, initial rate, and productivity. The highest conversion rates of rutin were almost similar for the two configurations of outer loop, 79 and 68%, respectively. In the presence of high water content, only monorutin formation was observed, while at low water content both mono- and di-rutin esters were synthesized. HPLC and NMR analyses showed that the synthesized mono and dirutin esters are respectively rutin 4‴-hexadecanedioate and dirutin 4‴, 4‴-hexadecanedioate (**Figure 2**).

The improved performance of the acylation reaction with a water removal system has already been described by several authors [24–26], but its influence on the selectivity of the reaction has never been mentioned before. The water removal by the two outer loop configurations gave similar results and presents the advantage of avoiding the abrasion of the enzyme.

Due to its implementation facility, the water removal by vapor and liquid phase (**Figure 3b**) was selected as a standard method to investigate the effect of the other factors on this reaction.

3.2. Effect of the temperature on the rutin solubility and esters synthesis

3.2.1. Effect on the solubility

The solubility of substrates is one of the main factors that affect the performance of the acylation reaction. This solubility is drastically influenced by the temperature. For this reason, the effect of the temperature (60, 80, and 90°C) on the solubility of rutin in *tert*-amyl alcohol was evaluated in the first step. In all cases, solubility increases with temperature. At the equilibrium
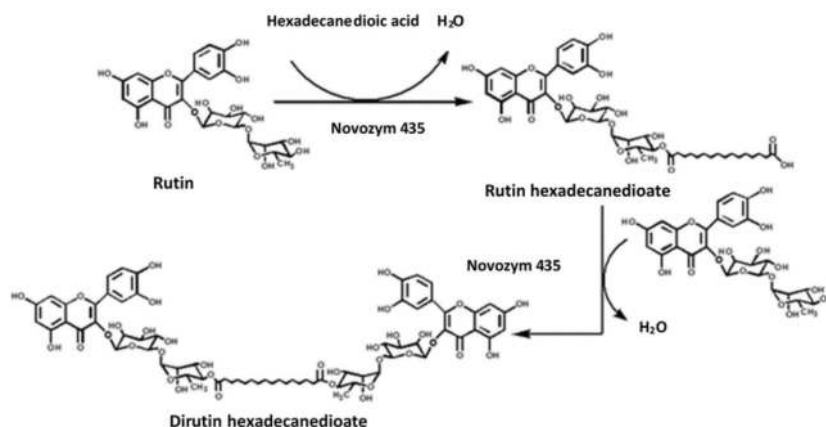

Figure 2. Synthesis of rutin hexadecanedioate and dirutin hexadecanedioate.
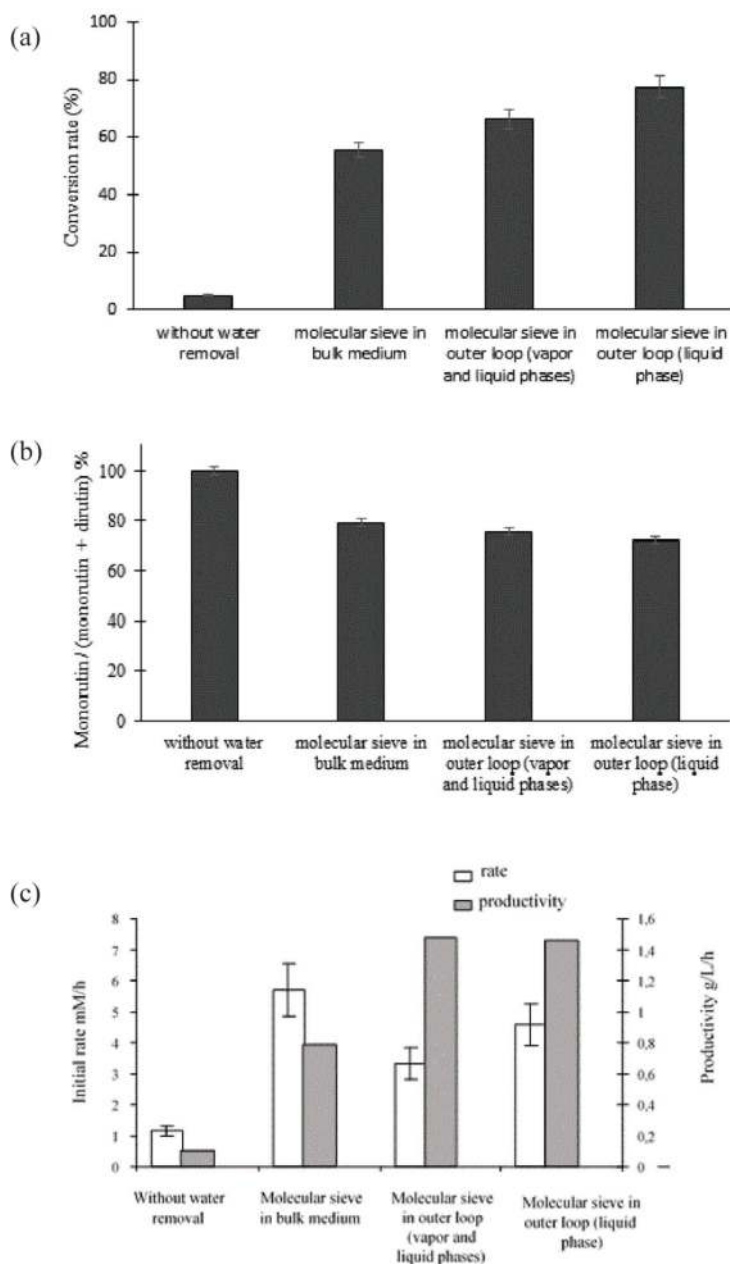
Figure 3. Effect of drying techniques on the esterification of rutin. Reactions carried out with 131 mM of rutin, 117.9 mM of acid, 30 g/L of Novozym 435 at 90°C. (a) Conversion rate of rutin at 50 h. (b) Selectivity of the reaction at 50 h. (c) Initial rate of rutin monoester formation and productivity at 50 h.

(10 h), the concentrations of rutin in the bulk medium are 42.3 ± 2.1 mM, 81.5 ± 4.0 mM, and 102.2 ± 5.1 mM, respectively for 60, 80, and 90°C. Fatty acids are totally soluble in *tert*-amyl alcohol at the studied concentrations and temperatures.

3.2.2. Effect on rutin esters synthesis

To investigate the effect of temperature on conversion rate, initial rate, and productivity, three set points of temperature (60, 80, and 90°C) were studied. Meanwhile, the other factors were kept constant at 65 mM of rutin, 58.5 mM of diacid, and 30 g/L of Novozym 435. NMR analysis showed that the two formed products are rutin 4‴-hexadecanedioate and dirutin 4‴, 4‴-hexadecanedioate (**Figure 2**).

Theodosiou et al. [23] reported similar results during their studies of enzymatic acylation of silybin by different dicarboxilic acids. They observed that at 50°C and after 96 h of incubation, both mono- and di-esters of silybin were synthetized but they identified only the monoester by NMR.

The behavior of the kinetics of rutin esters formation and the productivity of the reaction are summarized in **Figure 4a** and **b**. The highest performances were obtained at 90°C with 38.4 mM of total esters and a productivity of 0.78 g/L/h at 50 h, while the lowest values were found at 60°C with 20.8 mM and 0.38 g/L/h, respectively. Using esculin and palmitic acid as substrates, Lue et al. [16] reported similar effect of temperature. The observed results could be explained by the increase of rutin solubility, the decrease of the medium viscosity, and thus the increase of the mass transfer rate at high temperature. The increase of the temperature favors also the water removal and then the shift of the equilibrium to the product formation. These results suggested that the temperature has to be maintained as high as possible (≥80°C).

3.3. Effect of rutin concentration

During the acylation reaction of rutin by hexadecanedioate acid, three concentrations of rutin were tested (65, 131, and 196 mM) in the presence of hexadecanedioate acid (58.5, 117.9, and 176.4 mM, respectively), and 30 g/L of Novozym 435, in *tert*-amyl-alcohol at 90°C.

Figure 5 reports the obtained results concerning conversion yields (**Figure 5a**), selectivity, productivity (**Figure 5b**), and initial rate of rutin acylation (**Figure 5c**).

It appears that the initial rates of rutin acylation as well as the productivity increase with substrate concentrations (from 2.88 to 5.61 mM/h and from 0.78 to 2.56 g/(L/h), respectively), while conversion yields of both substrates (70% of rutin and 66% of hexadecanedioic acid) and selectivity of the reaction (76%) remain almost unchanged.

At 90°C, whatever the initial concentration, the rutin is totally soluble. Therefore, the behavior of the conversion rate and selectivity is rather due to the effect of molar ratio and not due to the solubility.

3.4. Effect of diacid/rutin molar ratio

The hydrophobicity of the reaction medium varies depending on the diacid/rutin molar ratio. Consequently, the selectivity of the reaction could be affected. This assumption was checked
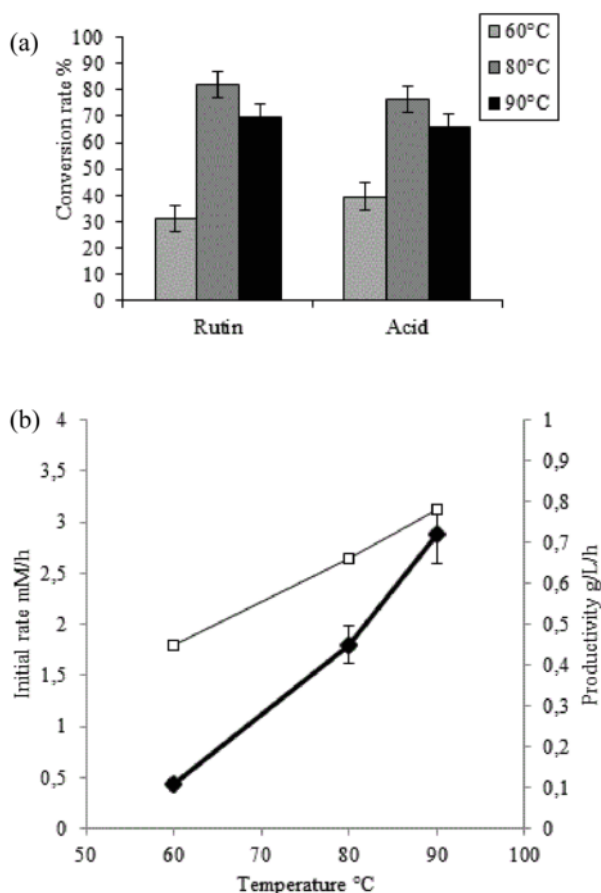
Figure 4. Effect of temperature on the performance of rutin hexadecanedioate synthesis. Reactions carried out with 65 mM of rutin, 58.5 mM of acid, and 30 g/L of Novozym 435 at 60, 80, and 90°C. (a) Effect of temperature on the conversion rate of rutin and hexadecanedioic acid at 50 h. (b) Effect of temperature on the initial rate (■) and productivity (□) at 50 h.

during a preliminary study with a diacid/rutin molar ratio of 0.05, 0.9, and 20. The obtained results showed that only rutin monoester was synthesized in the presence of a diacid/rutin molar ratio of 20 and both mono- and di-esters are produced with the two other lower ratios. In all cases, the rutin diester formation appears only after 5 h of incubation while the acid was completely depleted from the medium after 30–40 hours independently to molar ratio values. After the depletion of the fatty acid concentration, the monorutin ester became a substrate for the dirutin ester synthesis. At the end of the reaction (50 h), a plateau was reached (**Figure 6a–c**). These results suggested that rutin and diacid are better substrates to the enzyme than monorutin ester.

The effect of the molar ratio was investigated in several works. Similar results were observed by Ma et al. [27] during the acylation of isoorientin and isovitexin by Novozym 435.
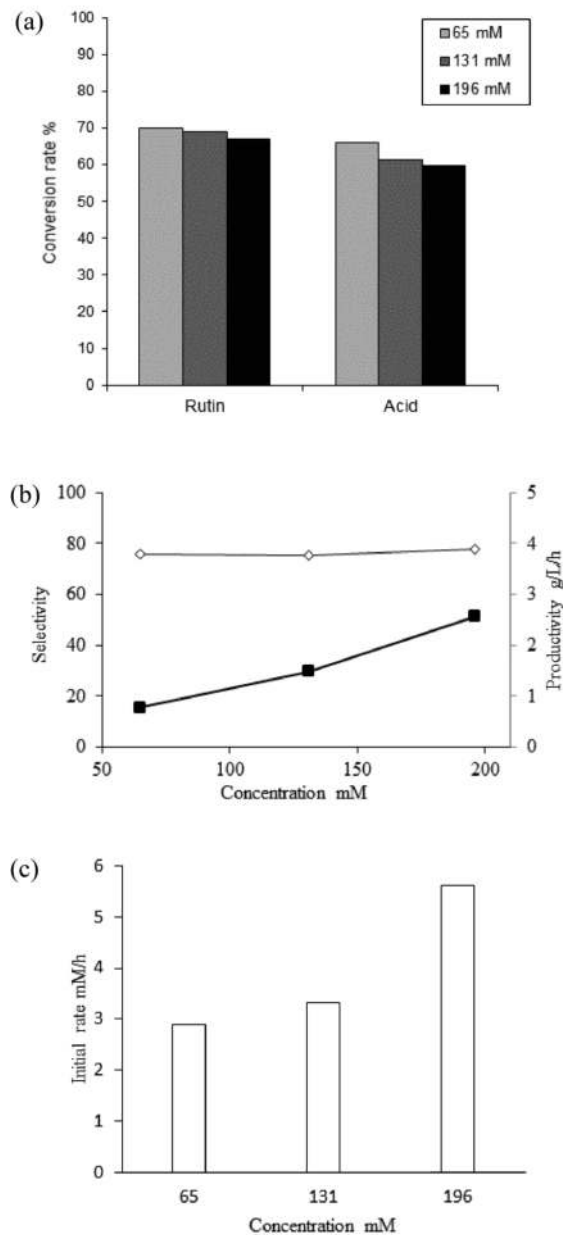
Figure 5. Effect of rutin concentration on the performance of rutin acylation. Reactions performed at 65, 131, 196 mM of rutin with 58.5, 117.9 and 176.4 mM, respectively of acid with 30 g/L of Novozym 435 at 90°C. (a) Rutin and hexadecanedioic acid conversion rate at 50 h. (b) Selectivity (□) and productivity at (■) 50 h. (c) Initial rate of rutin acylation.
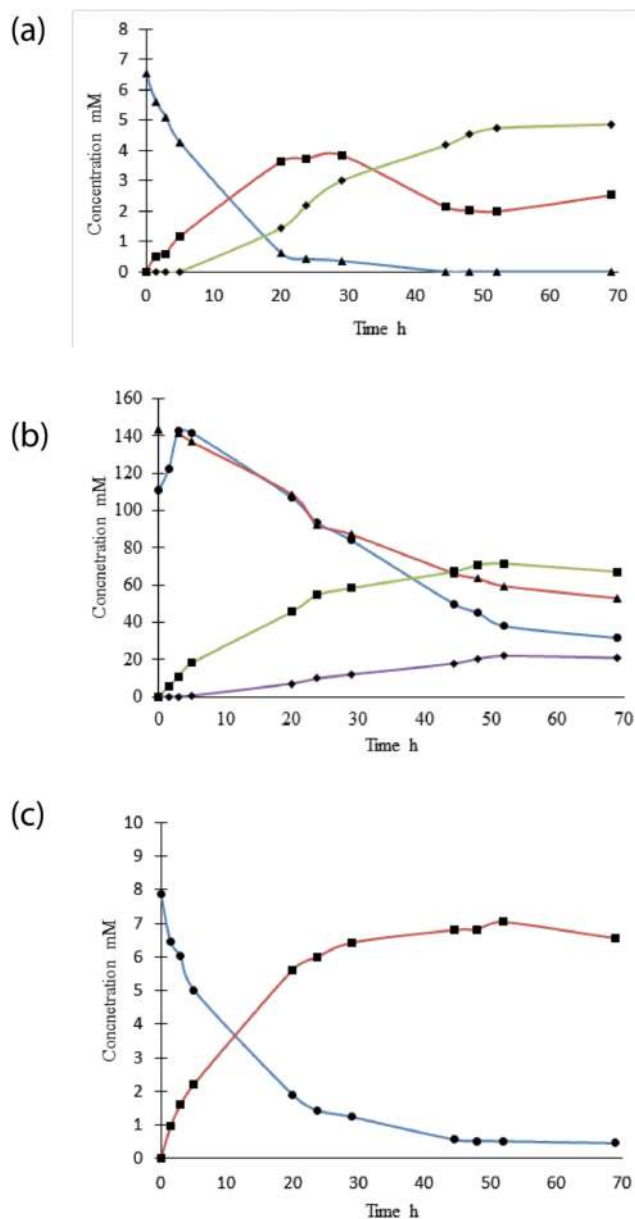
Figure 6. Kinetics of rutin esterification using different initial rutin concentrations. (a) Kinetics of rutin (131 mM) esterification reaction with hexadecanedioic acid (0.05 eq) by Novozym 435 (30 g/L) at 90°C, acid (▲), rutin hexadecanedioate (■) dirutin hexadecanedioate (◆). (b) Kinetics of rutin (131 mM) esterification reaction with hexadecanedioic acid (0.9 eq) by Novozym 435 (30 g/L) at 90°C, rutin (●) acid, (▲) rutin hexadecanedioate (■), dirutin hexadecanedioate (◆). (c) Kinetics of rutin (65 mM) esterification reaction with hexadecanedioic acid (20 eq) by Novozym 435 (30 g/L) at 90°C, rutin (●), rutin hexadecanedioate (■).

4. Conclusion

The effects of several factors on the acylation reaction of rutin by hexadecanedioate acid were investigated. The reactions conducted at 80 - 90°C showed high performances. The increase of rutin concentration from 65 mM to 196 mM led to the formation of mono ester (rutin 4‴-hexadecanedioate) and diester (dirutin 4‴, 4‴-hexadecanedioate). The molar ratio diacid/rutin (0.05 to 20) affects the selectivity of the reaction. In fact, in presence of excess of rutin both monoester and diester were synthesized. However, with diacid excess only the mono-ester was obtained. The results showed also that the water content of the media is a crucial factor. Drying the media by adding the molecular sieves in the outer loop of the reactor was the most efficient technique leading to water content lower than 200 ppm. In these conditions, the highest performances (conversion yield, initial rate) were reached at 90°C, 131 mM of rutin, 118 mM of acid, and 20 g/L of biocatalyst. At the equilibrium (50 hours), conversion yields of acid and rutin were respectively 73 and 74%, initial rate of monorutin ester formation was 2.20 mM/h and productivity was 1.44 g/(L/h). Depending on the water content and the diacid/rutin molar ratio, only mono- or both mono- and di-rutin esters were synthesized. For water content higher than 800 ppm, only rutin 4‴-hexadecanedioate was produced. At lower water content (<400 ppm), both rutin 4‴-hexadecanedioate and dirutin 4‴, 4‴-hexadecanedioate were observed. Higher values of diacid/rutin molar ratio favor the formation of monorutin ester.

Depending on the target application, the level of water content and molar ratio can be used to modulate the production of only mono- or both mono- and di-ester. The acylation reaction took place only on the glycosidic part, which is the main quality to preserve or enhance the biologic activity of flavonoids. In a further study, physicochemical and biological activity of synthesized esters will be evaluated.

Author details

Manel Slimane[1], Aude Cordin[2], Mohamed Ghoul[1] and Chebil Latifa[1]*

*Address all correspondence to: latifa.chebil@univ-lorraine.fr

1 Reactions and Process Engineering Laboratory, University of Lorraine, Vandœuvre-lès-Nancy, France

2 Compiègne University of Technology, Research Center, Compiegne Cedex, France

References

[1] Arora, A.; Nair, M.G.; Strasburg, G.M. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radic. Biol. Med. 1998; *24*, 1355-1363. doi: 10.1016/S0891-5849(97)00458-9

[2] Pietta, P.G. Flavonoids as antioxidants. J. Nat. Prod. 2000; *63*, 1035-1042. doi:10.1021/np9904509

[3] Rice-Evans, C.A.; Bagchi, D. Nutritional proanthocyanidins, flavonoids, and related phenols. Antioxid. Redox Signal. 2001; *3*, 939-940. doi:10.1089/152308601317203486

[4] Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. J. Agric. Food Chem. 2001; *49*, 2774-2779. doi:10.1021/jf001413m

[5] Di Carlo, G.; Mascolo, N.; Izzo, A.A.; Capasso, F. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999; *65*, 337-353. doi:10.1016/S0024-3205(99)00120-4

[6] Middleton, E.; Kandaswami, C.; Theoharides, T.C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 2000; *52*, 673-751

[7] Le, K.; Chiu, F.; Ng, K. Identification and quantification of antioxidants in Fructus lycii. Food Chem. 2007; *105*, 353-363. doi:10.1016/j.foodchem.2006.11.063

[8] Foti, M.; Piattelli, M.; Baratta, M.T.; Ruberto, G. Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system. Structure-activity relationship. J. Agric. Food Chem. 1996; *44*, 497-501. doi:10.1021/jf950378u

[9] Bohm, H.; Boeing, H.; Hempel, J.; Raab, B.; Kroke, A. Flavonols, flavones and anthocyanins as native antioxidants of food and their possible role in the prevention of chronic diseases. Z. Ernahrung. 1998; *37*, 147-163

[10] Mihara, R.; Barry, K.M.; Mohammed, C.L.; Mitsunaga, T. Comparison of antifungal and antioxidant activities of *Acacia mangium* and *A. auriculiformis* heartwood extracts. J. Chem. Ecol. 2005; *31*, 789-804. doi:10.1007/s10886-005-3544-x

[11] Kuleshova, L.N.; Hofmann, D.W.M.; Boese, R. Lattice energy calculation: a quick tool for screening of cocrystals and estimation of relative solubility. Case of flavonoids. Chem. Phys. Lett. 2013; *564*, 26-32. doi:10.1016/j.cplett.2013.02.008

[12] Chebil, L.; Humeau, C.; Anthoni, J.; Dehez, F.; Engasser, J.-M.; Ghoul, M. Solubility of flavonoids in organic solvents. J. Chem. Eng. Data. 2007; *52*, 1552-1556. doi:10.1021/je7001094

[13] Viskupicova, J; Ondrejovic, M; Maliar, T. Enzyme-mediated preparation of flavonoid esters and their applications. In: Prof. Deniz Ekinci (Ed.), Biochemistry. InTech; 2012. doi:10.5772/34174

[14] Ardhaoui, M.; Falcimaigne, A.; Ognier, S.; Engasser, J.; Moussou, P.; Pauly, G.; Ghoul, M. Effect of acyl donor chain length and substitutions pattern on the enzymatic acylation of flavonoids. J. Biotechnol. 2004a; *110*, 265-272. doi:10.1016/j.jbiotec.2004.03.003

[15] Chebil, L.; Humeau, C.; Falcimaigne, A.; Engasser, J.-M.; Ghoul, M. Enzymatic acylation of flavonoids. Process Biochem. 2006; *41*, 2237-2251. doi:10.1016/j.procbio.2006.05.027

[16] Lue, B.-M.; Guo, Z.; Xu, X. Effect of room temperature ionic liquid structure on the enzymatic acylation of flavonoids. Process Biochem. 2010; *45*, 1375-1382. doi:10.1016/j.procbio.2010.05.024

[17] Zheng, M.-M.; Wang, L.; Huang, F.-H.; Guo, P.-M.; Wei, F.; Deng, Q.-C.; Zheng, C.; Wan, C.-Y. Ultrasound irradiation promoted lipase-catalyzed synthesis of flavonoid esters with unsaturated fatty acids. J. Mol. Catal. B-Enzym. 2013; *95*, 82-88. doi:10.1016/j.molcatb.2013.05.028

[18] Ardhaoui, M.; Falcimaigne, A.; Engasser, J.-M.; Moussou, P.; Pauly, G.; Ghoul, M. Acylation of natural flavonoids using lipase of *Candida antarctica* as biocatalyst. J. Mol. Catal. B-Enzym. 2004; *29*, 63-67. doi:10.1016/j.molcatb.2004.02.013

[19] De los Rios, A.P.; van Rantwijk, F.; Sheldon, R.A. Effective resolution of 1-phenyl ethanol by *Candida antarctica* lipase B catalysed acylation with vinyl acetate in protic ionic liquids (PILs). Green Chem. 2012; *14*, 1584-1588. doi:10.1039/c2gc35196j

[20] Huo, Q.; Gu, X.; Lin, Q.; Sun, Y.; Xu, C.; Xiao, L. Selective esterification and antioxidant activity of rutin. Asian J. Chem. 2011; *23*, 3413-3416

[21] Viskupicova, J.; Danihelova, M.; Ondrejovic, M.; Liptaj, T.; Sturdik, E. Lipophilic rutin derivatives for antioxidant protection of oil-based foods. Food Chem. 2010; *123*, 45-50. doi:10.1016/j.foodchem.2010.03.125

[22] Jung, H.W.; Tschaplinski, T.J.; Wang, L.; Glazebrook, J.; Greenberg, J.T. Priming in systemic plant immunity. Science. 2009; *324*, 89-91. doi:10.1126/science.1170025

[23] Theodosiou, E.; Loutrari, H.; Stamatis, H.; Roussos, C.; Kolisis, F.N. Biocatalytic synthesis and antitumor activities of novel silybin acylated derivatives with dicarboxylic acids. New Biotech. 2011; *28*, 342-348. doi:10.1016/j.nbt.2011.01.006

[24] Gayot, S.; Santarelli, X.; Coulon, D. Modification of flavonoid using lipase in non-conventional media: effect of the water content. J. Biotechnol. 2003; *101*, 29-36. doi:10.1016/S0168-1656(02)00286-9

[25] Kontogianni, A.; Skouridou, V.; Sereti, V.; Stamatis, H.; Kolisis, F.N. Regioselective acylation of flavonoids catalyzed by lipase in low toxicity media. Eur. J. Lipid Sci. Technol. 2001; *103*, 655-660. doi:10.1002/1438-9312(200110)103:10<655::AID-EJLT655>3.3.CO;2-O

[26] Kontogianni, A.; Skouridou, V.; Sereti, V.; Stamatis, H.; Kolisis, F.N. Lipase-catalyzed esterification of rutin and naringin with fatty acids of medium carbon chain. J. Mol. Catal. B-Enzym. 2003; *21*, 59-62. doi:10.1016/S1381-1177(02)00139-X

[27] Ma, X.; Yan, R.; Yu, S.; Lu, Y.; Li, Z.; Lu, H. Enzymatic acylation of isoorientin and isovitexin from bamboo-leaf extracts with fatty acids and antiradical activity of the acylated derivatives. J. Agric. Food Chem. 2012; *60*, 10844-10849. doi:10.1021/jf303595e