Electronic Journal of Biotechnology 68 (2024) 47–56

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

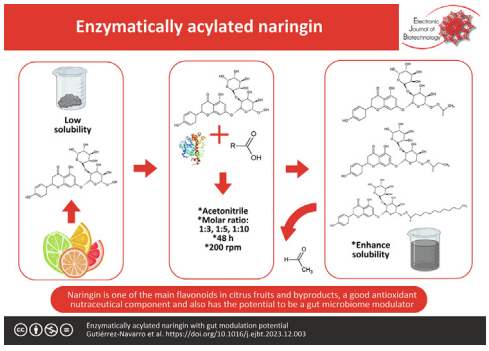
Research article

Enzymatically acylated naringin with gut modulation potential ☆

Elisa Gutiérrez-Navarro [a,b,c,1], José Daniel Padilla-de la Rosa [b,1], Adriana Macías [a], Josué Solís [a,*], Georgina Sandoval [c,*]

[a] Laboratorio de Microbiología Industrial, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Guadalajara, Jalisco, Mexico
[b] Unidad de Tecnología Alimentaria, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C., Zapopan, Jalisco, Mexico
[c] Laboratorio LIBBA, Unidad de Biotecnología Industrial, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C, Guadalajara, Jalisco, Mexico

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 August 2023
Accepted 21 December 2023
Available online 2 February 2024

Keywords:
Acyl donors
Acylation process
Antioxidant
Enzymatic acylation
Flavanone
Flavonoids
Gut modulator
Lipase
Naringin
Nutraceutical
Solubility

ABSTRACT

Background: Naringin is one of the main flavonoids in citrus fruits and byproducts. This flavanone has been shown to be a good antioxidant nutraceutical component, and it also has potential as a gut microbiome modulator, although its applications in final formulations represent a challenge due to its low solubility, both in water and in organic solvents. This work addresses this problem by functionalizing naringin through enzymatic acylation.

Results: The enzymatic acylation catalyzed by the lipase Novozym® 435 and using acyl donors of different chain lengths, acetate (C2), propionate (C3), and laurate (C12), yielded in conversions of 95% at 24 h and 100% at 48 h, generating a monoacylated product. Both the aqueous and solvent solubility of acylated naringin products were improved while maintaining or even increasing their antioxidant activity.

Conclusions: This acylation process significantly enhanced both the water and solvent solubility of the acylated naringin products while preserving or even enhancing their antioxidant activity. In addition to the gut-modulating properties of flavonoids, acylating them with short- and medium-chain fatty acids could enhance their potential applications in the emerging field of research dedicated to understanding and modulating gut health.

How to cite: Gutiérrez-Navarro E, Padilla-de la Rosa JD, Macías A, et al. Enzymatically acylated naringin with gut modulation potential. Electron J Biotechnol 2024; 68. https://doi.org/10.1016/j.ejbt.2023.12.003.

☆ Audio abstract available in Supplementary material.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

* Corresponding authors.
E-mail addresses: raymundo.solis@academicos.udg.mx (J. Solís), gsandoval@confluencia.net, gsandoval@ciatej.mx (G. Sandoval).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejbt.2023.12.003
0717-3458/

1. Introduction

Flavonoids are polyphenolic compounds that humans consume through vegetables and fruits. There are a variety of more than six thousand compounds that are part of different biochemical and physiological processes [1,2]. They are characterized as crystallized substances of white–yellowish colors, poorly soluble in both polar and apolar compounds, easily oxidizable and of low toxicity with antioxidant, diuretic, and anti-inflammatory effects. In citrus fruits, more than 60 types of flavonoids have been identified [3].

Recent research has demonstrated the anti-inflammatory and antioxidant capacity of these compounds [4,5], they have potential as dietary supplements for conditions such as obesity, diabetes, hypertension, and metabolic syndrome [6,7]. Citrus flavonoids have additional benefits, including improved endothelial function, reduced blood pressure, and enhanced antioxidant and anti-inflammatory effects on intestinal cells.

Naringin, a naturally present citrus fruit flavonoid. [8], influences the modulation of the intestinal microbiota [9], improving intestinal function and health [10]. With its structure containing multiple hydroxyl groups, naringin exhibits high antioxidant activity and has an appealing aroma for food flavorings. It has been found to inhibit cell proliferation, migration, and invasion in colon cancer cells [11]. Furthermore, naringin demonstrates strong anti-inflammatory effects. Recent rat studies have shown its neuroprotective properties and its ability to suppress the COX-2 enzyme, crucial for the formation of inflammatory compounds associated with Alzheimer's and Huntington's diseases [12]. These findings indicate that naringin promotes the production of neurotropic factors, inhibits apoptosis and inflammation, and improves synaptic function. It shows promise as an effective treatment for neurodegenerative diseases and memory deficits [13].

The beneficial effects of naringin have been proven in several studies, and some of its biological activities go beyond its neuroprotective capacity, which is applicable not only to Alzheimer's but also to several neurological disorders [14]; it has a hepatoprotective effect that is expressed through the regulation of fatty acid metabolism [15], in addition to being antiasthmatic since it reduces the production of mucus and inflammation of the bronchial tubes [16]. Its positive effect has also been related to the improvement of osteoporosis, arthritis, and osteoarthritis, increasing osteogenesis and preventing the accumulation of collagen in arthritic joints [17]. Its carcinogenic effects have not only been tested on colon cancer cells but also on cervical and ovarian cancer cells [18].

Flavonoids are well-recognized for their inherent low solubility in both oil and aqueous phases, a characteristic that significantly curtails their stability and, in turn, their bioavailability and adaptability for use in various formulations such as dietary supplements, pharmaceuticals, functional foods, and cosmetic products. Bioavailability refers to the proportion of a substance that reaches the systemic circulation, following a particular route of administration, which, in the case of dietary flavonoids, is oral. It has been confirmed in several studies that the bioavailability of flavonoids is mostly <1% of the administered dose [19]. Flavonoid glycosides are converted, or modified, to their deglycosylated, hydroxylated, denatured, sulfated, or glucuronidated forms during absorption and metabolism. These variations and modifications are related to the diversity in their bioavailability and physiological functions [20].

The absorption of flavonoids can be divided according to the magnitude of absorption into initial, main, and marginal absorption (Fig. 1). Initial absorption begins in the stomach, where nonenzymatic hydrolysis takes place, producing aglycones that are transported by passive diffusion; the small intestine is the major site for flavonoid absorption. Endogenic β-glucosidases are involved at this stage to release aglycones from flavonoids. Free aglycones are more hydrophobic and smaller than glycosides and thus are more likely to passively penetrate the ephitelial layer. In contrast, intact glycosides are also absorbed by the small intestine either by inefficient passive diffusion or by the sodium-dependent glucose transporter (SGLT1). In the colon, studies have shown that the time required for flavones to reach concentration is 5 to 7 h after ingestion. This indicates that 7-*O* rutinosides are not deglycosylated and absorbed in the intestine but pass to the colon, where they are released by the action of bacterial enzymes such as *R*-rhamnosidase and glucosidases to be subsequently glucurinated during transport through colonocytes in the intestinal wall toward the circulatory system [21].

The modulation of the microbiota by the effect of anthocyanins has been demonstrated; the results of some preliminary clinical studies [22,23] have shown the gut effect of flavonoids, specifically naringin and hesperidin. These natural compounds were investigated in a controlled trial, which revealed their ability to selectively stimulate the growth and activity of beneficial gut bacteria. The study demonstrated that naringin and hesperidin act as gut modulators by promoting the proliferation of specific strains, enhancing the overall diversity and balance of the gut microbiota. These findings suggest that flavonoids, such as naringin and hesperidin, may have potential therapeutic applications for improving gut health and overall well-being. Notably, there is a lack of specific studies in the scientific literature that have focused on the modulation of intestinal microbiota due to the presence of flavonoids in agro-industrial byproducts, such as those found in citrus fruits. It is possible to increase its solubility and bioavailability by esterification with short-chain fatty acids (acetate, propionate and laurate) to generate metabolites capable of being a substrate in the colon, which, when hydrolyzed, release short-chain fatty acids capable of modulating the ratio of Bacteroides and Firmicutes, as well as their beneficial effects in humans.

Short-chain fatty acids such as acetic and propionic acid can be produced by the microbiota as secondary metabolites, while propionic acid has important effects on human health, such as reducing fatty acids in liver and plasma, has been shown to generate a satiety effect reducing food intake and showing an anti-inflammatory effect in the colon, as well as helping in the prevention of obesity and diabetes, also improving insulin sensitivity [24]. Acetic acid is the most abundant short-chain fatty acid in the colon produced by the microbiota. This fatty acid has multiple metabolic activities in the human body, ranging from increasing oxidative capacity in the liver to activating receptors for blood pressure regulation [25]. Recent studies have shown that acetic acid plays an important role in the regulation of body weight and insulin sensitivity through lipid metabolism. At present, efforts have been made to supplement the human diet with products containing acetic acid because its production by the microbiota is not sufficient [26]. Both propionic and acetic acid can increase fatty acid oxidation, inhibit fatty acid synthesis, increase heat production, and reduce fat storage. These fatty acids can be consumed as dietary supplements; however, their major production is through microbial fermentation, which in turn allows the regulation of the intestinal microbiota that promotes eubiosis [27].

Lauric acid is a medium-chain fatty acid with anticancer activity that has been tested *in vitro*, *in vivo* and *ex vivo* studies, the results of which concluded that this fatty acid has a high potential for use in the pharmaceutical industry since it induces cell death of cancerous cells in the colon [28], in addition to having antimicrobial activity [29] that can modulate the intestinal microbiota. Therefore lauric acid is an attractive acyl donor for enzymatic acylation.

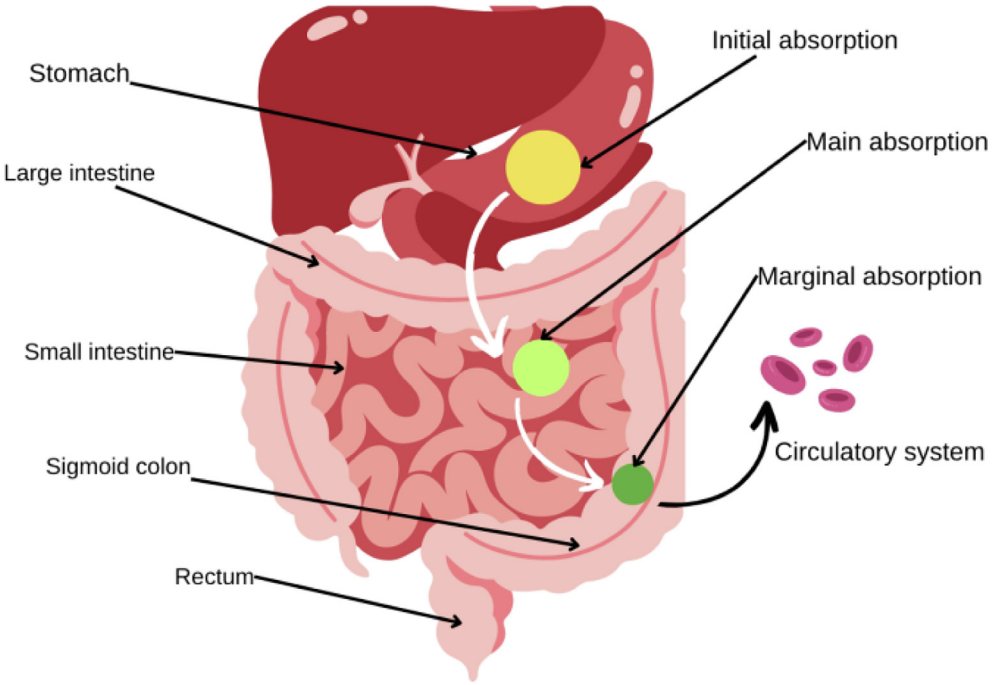


Fig. 1. Main routes of the absorption process of flavonoids.

Enzymatic acylation consists of the substitution of a hydroxyl group of the aromatic ring by an acyl group, and the catalyst to be used will depend on the compound to be acylated; the reaction by elimination of OH groups reduces the antioxidant activity of flavonoids; however, it makes them more soluble in polar media [30]. Lipase B from *Candida antarctica* (CALB) has been considered a highly efficient and versatile enzyme in the enzymatic acylation of flavonoids from some years, specially with long-chain acyl donnors [31]. However, the flavonoid acylation with short-chain length is scarcely reported. Therefore, this work aims to functionalize the structure of naringin by acylation with two short- and one medium-chain length, as shown in Fig. 2, to improve its solubility in water and in organic solvents and their gut modulation properties.

2. Materials and methods

2.1. Materials and reagents

Naringin (98% purity), as well as vinyl (vinyl propionate, vinyl laurate, vinyl acetate) and HPLC grade solvents (acetonitrile, methanol, ethanol, and acetone), were acquired from Sigma–Aldrich (Mexico). Glacial acetic acid was purchased from JT Baker (Mexico), and immobilized lipase B from *Candida antarctica*, Novozym® 435 (N435) was acquired from Novozymes through a local broker.

2.2. Lipase activity

The lipase activity of N435 lipase before and after acylation was evaluated to compare the loss of lipase activity. Lipase activity was measured by the ability of the N435 enzyme to hydrolyze the *p*NPB (p-Nitrophenyl butyrate) substrate and detect the release of the product *p*-nitrophenol, according to the conditions mentioned by Reyes-Reyes et al. [32].

2.3. Enzymatic acylation

For enzymatic acylation, the protocols reviewed by Chebil et al. [31] were used as a basis and further improved. Acylation of naringin (8.61 mM, solubility limit in the solvent) was carried out in acetonitrile as reaction solvent, at 50°C and 200 rpm with three esterified fatty acids, vinyl acetate (C2), vinyl propionate (C3), and vinyl laurate (C12). Regarding the molar ratio for the naringin/acylating agent reaction, 3 different molar ratios were tested (1:10, 1:5, 1:3). The experiments were carried out in triplicate using a control without enzymes.

This is a factorial design that allows for the systematic investigation of how different factors and their interactions influence the outcome of the acylation process.

2.4. HPLC analysis

For the detection of naringin and naringin/acylating agent, a specially developed isocratic HPLC method was generated using HPLC and a diode array detector (PDA). In all cases, a Phenomenex® Phenyl C-6 column was used at 40°C and read with a diode array detector at a wavelength of 280 nm for phenolic compounds and 210 nm for fatty acids. The isocratic flow mobile phase was acidified methanol (0.1% acetic acid) and acidified Milli-Q water (0.1% acetic acid) 60:40 at a flow rate of 1 mL/min during the determination of naringin acetate, 70:30 at a flow rate of 1 mL/min for propionate, and 90:10 at a flow rate of 0.7 mL/min for laurate.

The conversion was calculated by quantifying the consumption of naringin. No further purification was performed, except enzyme separation and solvent evaporation before analysis.

2.5. Product confirmation

2.5.1. FTIR spectrometry

After confirming the purity of the compounds derived from the acylation of naringin and removing the enzyme, we proceeded to concentrate the products using a rotary evaporator to remove the solvent. Subsequently, 2 mg of each sample was taken to confirm

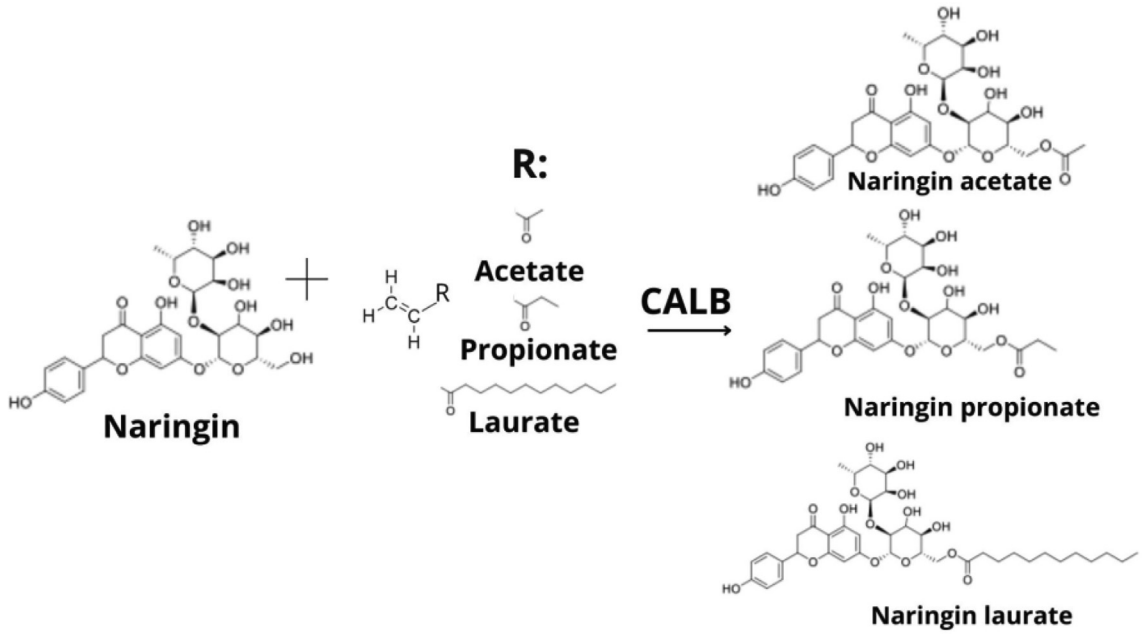


Fig. 2. Naringin acylation.

the presence of the ester bond. For this purpose, FT-IR spectra were obtained using a Bruker FTIR instrument with ATR in transmission mode, covering a range of 400 to 4000 cm^{-1}.

2.5.2. MS/MS spectrometry

The mass confirmation of acylated naringin was meticulously carried out using a state-of-the-art Waters quadrupole instrument. The mass spectra were meticulously recorded in the negative ion mode, scanning an extensive range from 50 m/z to 2000 m/z. To ensure utmost accuracy, crucial parameters such as the capillary voltage (2.60 kV), cone voltage (40–44 V), source temperature (149–150°C), desolvation temperature (344–350°C), cone gas flow rate (50–97 L/h), and desolvation gas flow rate (650 L/h) were scrupulously controlled. These finely tuned settings played a pivotal role in facilitating the precise identification and comprehensive characterization of acylated naringin and its related compounds. Mass confirmation of acylated naringin was performed in a Waters quadrupole.

2.6. Solubility

The solubility assessment played a pivotal role in this investigation, yielding significant insights into the solubility profiles of both naringin and its acylated derivatives. The preparation of supersaturated solutions was meticulously executed across a spectrum of solvents, including water, acetonitrile, acetone, ethanol, and methanol. To generate these solutions, precisely measured quantities of naringin (60 mg) and acylated compounds (450 mg) were introduced into 1 mL of each respective solvent. Following a sequence of sonication and sedimentation, the resultant supernatants were systematically collected, subjected to filtration, and appropriately diluted in the mobile phase for subsequent HPLC analysis, following established methodologies. This dataset assumes a paramount role in comprehending the solubility dynamics of these compounds across an array of solvents, and its implications extend to diverse domains including formulation development, pharmacokinetics, and a multitude of industrial applications.

This selection of solvents thoughtfully encompasses a diverse range of polarities and intrinsic properties commonly encountered in laboratory research as well as industrial settings. The inherent variance in solvent polarity significantly influences the solubility characteristics and intricate interactions of the tested compounds within each solvent medium.

2.7. LogP

We calculated the theoretical Log*P* values for each naringin acylate using the ACD/Log*P*™ computational program from ACD/Labs®. This program relies on a classical algorithm supported by over 12,000 experimental Log*P* values. It utilizes the principle of isolating carbons to perform the calculations. The structures of the acylates were drawn using ChemSketch™ and entered into the program in SK2 format. The Log*P* value is a measure indicating a compound's ability to dissolve in different media, specifically, its partitioning between an organic phase and an aqueous phase. A positive Log*P* value indicates a greater affinity of the compound for the organic phase (lipophilic), while a negative value indicates a greater affinity for the aqueous phase (hydrophilic) [30].

2.8. Antioxidant activity

2.8.1. DPPH

DPPH for antioxidant activity estimation was performed according to Milisavljević et al. [33]. Methanol was used as the solvent and as a blank, and Trolox was used as a positive control.

2.8.2. β–Carotene-linoleic acid method

The β-carotene method from Loucif et al. [34] was used to determine the antioxidant activity of naringin and its acylated products. Trolox was used as a reference.

2.9. Oxidative stability

To assess the antioxidant effect of naringin and its acylated products on olive oil, the oxidative stability was obtained according to the Rancimat method [35], using the 893 Professional Bio-

diesel Rancimat. A total of 7.5 g of each sample was placed in a test tube, bubbling air at 10 L/h and heating the tube at 110°C. The conductivity was measured, and the oxidation time was expressed in minutes.

2.10. Statistical analysis

Statistical analysis of three independent experiments was carried out by analysis of variance tests using Excel software version 16.64 of Microsoft 365.

3. Results

3.1. Acylation

Three activated acyl donors, acetate (C2), propionate (C3), and laurate (C12) vinyl esters, were used. The optimized reaction conditions were as follows: 1.6 g/L of the enzyme was used at 50°C with 200 rpm agitation in an incubator/stirrer Enviro-genie® for 48 h, and the reaction solvent was acetonitrile. Kinetics was followed by an isocratic HPLC method developed for this work (**Section 2.3**), and confirmation of product identity was obtained by FTIR and MS/MS. Only a main monoacylated product was observed in HPLC analysis (Fig. S1, Fig. S2, Fig. S3), which was also corroborated by FTIR (Fig. S4, Fig. S5, Fig. S6), where the stretches of OH, C=C, and C=O functional groups are observed).

Mass spectrometry confirmed what was seen by HPLC and FTIR. In S7, S8, S9, it is possible to observe the molecular ions of naringin and their acylated products with acetate (Fig. S7, m/z 621.1), propionate (Fig. S8, m/z 635.6), and laurate (Fig. S9, m/z 761.5). The mass of fragments of naringin were also observed, for instance, at m/z 271.1, and two rearrangements of m/z 150.9 and 459.3, as well as some adducts. These fragmentations provide a fingerprint and confirmation that the molecule is indeed an acylation of naringin with the acylates [36]. Given the high conversion obtained (Fig. 2, Fig. 3, Fig. 4), no further purification was performed, except enzyme separation and solvent evaporation before analysis.

For naringin acetate, we found that the acylation reaction at the three molar ratios reached a maximum of 99.3% conversion in 24 h, while at 48 h, it reached 100% conversion (Fig. 3). However, the reaction rate was faster for the 1:5 molar ratio, although at 48 h, there was no significant difference in conversion between them when performing an ANOVA test, where the p value was above 0.05, being 0.07, 0.8, and 0.1, respectively. The same was true for naringin propionate and laurate with the ANOVA results, where the p value was above 0.05 for all molar ratios. The only difference was for laurate, where the kinetics were faster for the 1:3 molar ratio (Fig. 4, Fig. 5), and for laurate, only 90% conversion was reached at 48 h (further reaction time did not increase the conversion).

3.2. Lipase activity

The initial lipase activity of the sample was 2058 U/g, while the lipase activity of the recovered enzyme was 1974 U/g. This indicates that the recovered N435 lipase activity decreased by approximately 4% compared to the initial lipase activity.

3.3. Solubility

The solubility of naringin before and after acylation was determined by HPLC and is shown in Table 1, Table 2 and Table 3. There was a significant difference in all solubility tests ($P < 0.05$).

It is evident that the acylated compounds (naringin acetate, naringin propionate, and naringin laurate) exhibited significantly higher solubility compared to naringin alone. For example, in Table 1, the solubility of naringin acetate was approximately 4 times greater than that of naringin in water, and the enhancement factors were even more pronounced in other solvents. Similarly,

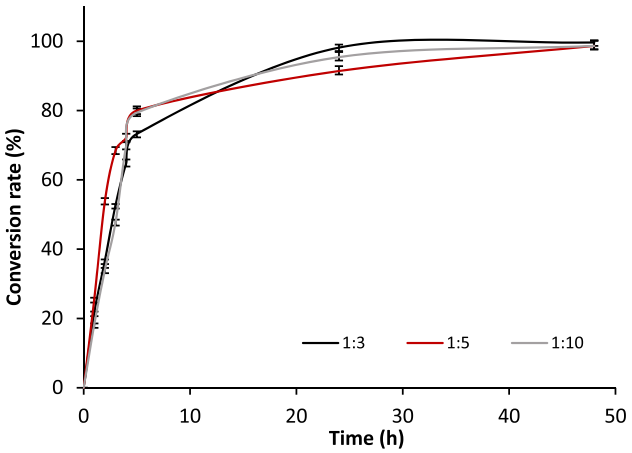


Fig. 3. Conversion of naringin in naringin acetate synthesis at different times and acyl donor ratios (naringin: acyl donor).

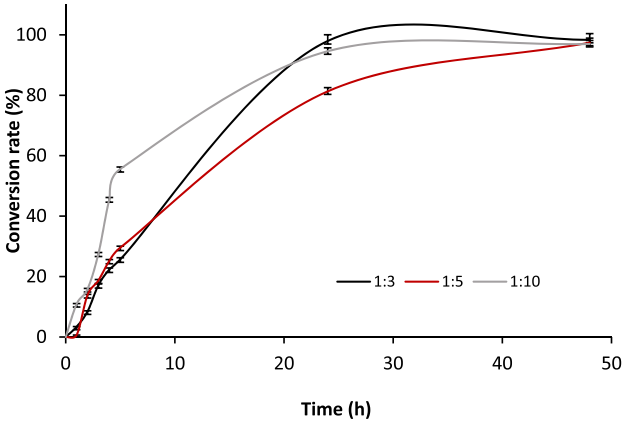


Fig. 4. Conversion of naringin in naringin propionate synthesis at different times and acyl donor ratios (naringin: acyl donor).

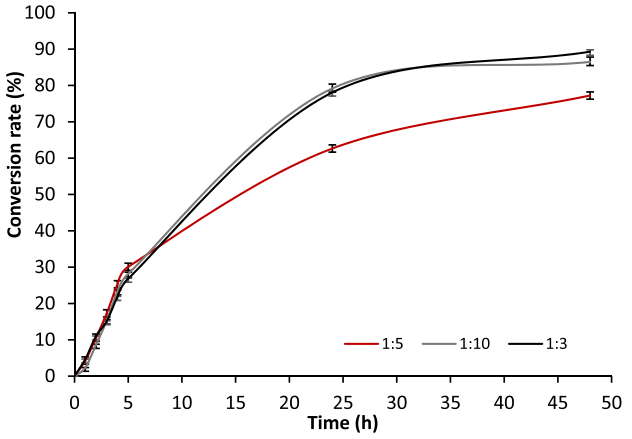


Fig. 5. Conversion of naringin in naringin laurate synthesis at different times and acyl donor ratios (naringin: acyl donor).

Table 1
Solubility in mg/mL of naringin vs. naringin acetate. In addition, its solubility enhancement factor.

Solvent	Naringin	Naringin acetate	Solubility enhancement factor
Water	2.94	11.9	4
Ethanol	29.4	61.6	2
Methanol	3.90	53.8	14
Acetonitrile	4.94	55.0	11
Acetone	4.93	38.6	8

Table 2
Solubility in mg/mL of naringin vs. naringin propionate. In addition, its solubility enhancement factor.

Solvent	Naringin	Naringin propionate	Solubility enhancement factor
Water	2.94	2.3	1
Ethanol	29.4	284.9	10
Methanol	3.90	224.7	58
Acetonitrile	4.94	330.6	67
Acetone	4.93	236.7	48

Table 3
Solubility in mg/mL of naringin vs. naringin laurate. In addition, its solubility enhancement factor.

Solvent	Naringin	Naringin laurate	Solubility enhancement factor
Water	2.94	1.0	1
Ethanol	29.4	332.9	11
Methanol	3.90	391.8	100
Acetonitrile	4.94	109.0	22
Acetone	4.93	404.8	82

Table 2 and Table 3 show substantial increases in solubility for naringin propionate and naringin laurate in various solvents.

Enzymatic acylation of naringin with short- and medium-chain fatty acids induces structural changes that enhance its solubility. This modification increases the molecule's lipophilicity, making it more compatible with organic solvents and fats. Simultaneously, it reduces the number of hydroxyl groups, diminishing hydrogen bonding tendencies and boosting water solubility. The addition of the fatty acid chain also alters the compound's molecular weight, affecting its solubility in various solvents. Moreover, the fatty acid chains can interact effectively with other molecules and solvents, promoting solubility in different environments. These structural alterations, corroborated by FTIR analyses, significantly enhance naringin's solubility, holding promise for applications in pharmacology and the food industry.

3.4. LogP

Regarding the LogP values in Table 4, the acylated compounds (naringin acetate, naringin propionate, and naringin laurate) were generally higher compared to naringin alone. This indicates that the acylated compounds have a greater tendency to dissolve in organic solvents and exhibit enhanced lipophilicity.

3.5. Antioxidant activity

3.5.1. DPPH radical scavenging

The antioxidant activity measured by DPPH showed a weaker absorbance when DPPH met the flavonoids, and the naringin ability of free-radical scavenging was approximately 80% of the Trolox equivalent and maintained after acylation with a minimum decrease (Table 5).

Table 4
Log*P* value of naringin vs. acylated naringin.

Naringin	Naringin acetate	Naringin propionate	Naringin laurate
3.79 ± 0.89	3.98 ± 0.92	4.51 ± 0.92	9.30 ± 0.92

Table 5
Free radical scavenging decreased the antioxidant activity of naringin derivatives relative to naringin.

Naringin acetate Decrease rate	Naringin propionate Decrease rate	Naringin laurate Decrease rate
0.015% ± 3.8	3.63% ± 2.71	4.97% ± 2.8

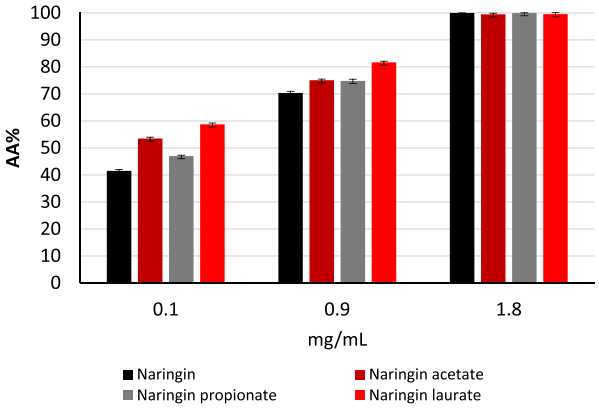


Fig. 6. Antioxidant activity for the β–Carotene-linoleic acid method.

3.5.2. β–Carotene-linoleic acid method

The results for the β–carotene method (Fig. 6) showed an increased antioxidant for acylated naringin, being laurate the highest. The degradation rate was calculated with [Equation 1], and the antioxidant activity rate was calculated with [Equation 2].

$$DR = \frac{In\,(A_0 - A_f)}{t} \times 100 \quad (1)$$

where A0: initial absorbance; Af: Final absorbance; and t: time.

$$\%\boldsymbol{AA} = \frac{\boldsymbol{DRControl} - \boldsymbol{DRSample}}{\boldsymbol{DRControl}} \times 100 \quad (2)$$

3.6. Oxidative stability

As shown in Table 6, the addition of flavonoids to the olive oil provided a protective action against its oxidation, increasing the time at the induction point from 2.6 min to 2.9 min in the naringin laurate sample.

4. Discussion

Lipases play a crucial role in various industries because they exhibit remarkable stability when exposed to organic solvents. They possess a diverse range of substrates on which they can act, demonstrating selectivity in their enzymatic reactions. Additionally, lipases possess the advantageous ability to catalyze reactions without requiring costly cofactors. Furthermore, they can be readily produced and remain highly active even under gentle reaction conditions [30]. One notable application is their crucial role in the enzymatic acylation of flavonoids. Lipases, with their ability to efficiently catalyze the acylation process, contribute signifi-

Table 6
Oxidative stability rate of olive oil (control) and olive oil plus flavonoids measured by the Rancimat method.

Control induction point in min	Naringin increase rate	Naringin acetate increase rate	Naringin propionate increase rate	Naringin laurate increase rate
2.65 ± 0.03	5% ± 0.05	5% ± 0.03	5% ± 0.05	10% ± 0.08

cantly to the production of acylated flavonoids with improved properties; the reaction yield is influenced by various factors, including the enzyme, substrates, molar ratio, solvent, and reaction conditions. In this study, we utilized *Candida antarctic*a lipase B, specifically in its commercially available immobilized form, from the brand Novozym® (N435), which is renowned for its exceptional selectivity and catalytic efficiency. N435 exhibits a remarkable ability to selectively recognize and interact with specific substrates, allowing for precise enzymatic modifications. This selectivity is attributed to the unique architecture of N435's catalytic site, which enables it to accommodate a wide range of substrates with varying structures. The catalytic site of N435 plays a crucial role in facilitating the enzymatic reactions, providing a favorable environment for the substrate to undergo acylation or other modifications [37]. By harnessing N435's selectivity efficacy, we successfully achieved the desired modifications of nutraceutical substrates in this study, showcasing the remarkable capabilities of N435 as a versatile biocatalyst for phenolic acylation [30,38]. Using a solvent offers numerous advantages due to its ability to buffer changes in the initial substrate composition, and maintain consistent physical properties of the reaction medium, particularly by ensuring a nearly constant low viscosity, however, the solvent impacts thermodynamically in reaction performances [39]. Acetonitrile was chosen as the reaction solvent based on our previous expertise in enzymatic modification of flavonoids [40] demonstrating its suitability by achieving conversions of above 90%. The selection of an appropriate solvent is pivotal for the success of chemical reactions, especially when working with enzymes. Acetonitrile has been widely employed in enzymatic modification of natural compounds due to its favorable properties and our prior experience in flavonoid modification using enzymes enabled us to assess the suitability of acetonitrile as the reaction solvent in this study [40]. One key advantage of acetonitrile is its capability to maintain a consistently low viscosity throughout the reaction process. This is particularly important for enzymatic reactions, as high viscosity can adversely affect enzymatic activity and, consequently, the efficiency of the modification. By ensuring a constant low viscosity, acetonitrile provides a favorable environment for enzymatic activity, potentially resulting in higher conversions of the target substrates (Fig. 3, Fig. 4, Fig. 5, Table 7). While not classified as a typical buffer solution, acetonitrile exhibits a buffering capacity analogous to that of conventional buffers, preserving the stability of a reaction mixture. This 'robust buffering capacity' of acetonitrile significantly contributes to the stability and efficiency of enzymatic reactions. By maintaining a consistent pH and composition within the reaction medium, acetonitrile establishes an optimal environment for enzymatic activities. Consequently, this stability not only enhances the overall effectiveness of enzymatic processes but also ensures the reproducibility of results, a fundamental requirement in scientific experimentation. Another advantage of acetonitrile is its ability to facilitate water partitioning. Water can either be a byproduct or a critical component in many enzymatic reactions. Acetonitrile aids in improving the separation of the aqueous and organic phases, which can be beneficial for the recovery and purification of reaction products. Our previous results in enzymatic modification of flavonoids using acetonitrile as the solvent support its suitability in this study. We achieved conversions of up to 90% using this solvent (Table 7), demonstrating its effectiveness and compatibility with the enzymes employed in the modification process. These positive outcomes validate our choice of acetonitrile as the reaction solvent in this study.

The molar ratio of substrates plays a crucial role and is influenced by the equilibrium constant and the reaction medium, as observed in previous lipase-catalyzed reactions [31,39]. Hence, we investigated molar ratios of 1:3, 1:5, and 1:10 for the three acyl donors, and the results were found to be dependent on both the molar ratio and the acyl donor's chain length, as illustrated in Fig 3, Fig. 4, and Fig. 5.

When comparing our reaction conditions to those of other studies (Table 7), where enzyme concentrations ranging from 3 to 50 g/L were used, we achieved higher conversions with a significantly lower enzyme concentration of only 1.6 g/L. Moreover, our reactions exhibited faster kinetics, with maximum conversion reached within 48 h, while other studies required significantly longer reaction times of 96 to 120 h. The exceptional efficiency of our enzymatic acylation can be attributed to the unique characteristics of N435, specifically its selective transesterification capability with specific fatty acid vinyl esters [41]. N435's ability to selectively target and modify the flavonoid structure enhances its efficiency in the acylation process. Additionally, the robust support of N435 ensures its stability in organic solvents, enabling the enzyme to be reused without a significant loss of activity, consistently achieving conversions above 90% (Fig 3, Fig. 4, Fig. 5) demonstrates the remarkable efficiency of this biocatalyst in our acylation reactions. The formation of monoacyl products in all three acylation reactions supports previous reports that N435 acylated position 6"-*O*- of the sugar moiety of flavonoids [42,43]. This finding aligns with our study, where the confirmation was obtained through the analysis of NMR and mass spectra, clearly exhibiting the signals of H-6a and H-6b, just as mentioned in the existing literature (Fig. S7, Fig. S8, Fig. S9, Fig. S10, Fig. S11, Fig. S12).

The chain length and structure of the acyl donor directly impact enzymatic synthesis [37]. The acylation of naringin with acetate and propionate (both short-chain donors) achieved complete conversion within 48 h, whereas similar conditions in other studies required 120 h to achieve substrate conversions ranging from 40% to 60%. Interestingly, when comparing our results of naringin laurate acylation with previous studies [44,45], we obtained higher conversions at similar reaction times while using a lower enzyme concentration. However, the acylation of naringin with laurate exhibited lower conversion and longer reaction time, suggesting that mass transfer limitations may arise when working with acyl donors of increased chain length. Furthermore, the slightly lower conversion observed with laurate indicates that a more hydrophobic solvent may be more suitable for longer-chain acyl donors, as in the case of acetone where Sun et al. [46] achieved 93% of conversion.

Several authors have synthesized naringin esters with various fatty acids of different chain lengths. However, to the best knowledge of the authors, acylation of naringin with vinyl propionate has not been conducted. In this study, the acylation reactions with vinyl propionate were performed, achieving conversions of 99% and obtaining a monoacylated product.

The impact of acyl chain length on the solubility of naringin was evident in our study. As the length of the acyl donor chain increased, the solubility of naringin showed improvement across

Table 7
Summary of similar reaction conditions used in various studies to synthesize naringin esters using N435 as biocatalyst.

Acil donor	Molar ratio	[Enzyme]	Solvent	Time	T	Conversion	Reference
Vinyl laurate	1:10	17 g/L	Acetonitrile	8 h	50°C	58%	[44]
Lauric acid	1:10	10 g/L	Acetone	96 h	50°C	71%	[45]
Lauric acid	1:5	12 g/L	Acetone	72 h	45°C	93%	[46]
Vinyl acetate	1:3	1.6 g/L	Acetonitrile	48 h	50°C	100%	This study
Vinyl propionate	1:3	1.6 g/L	Acetonitrile	48 h	50°C	99%	This study
Vinyl laurate	1:3	1.6 g/L	Acetonitrile	48 h	50°C	90%	This study

different solvents. For instance, naringin acetate significantly enhanced its solubility in water, exhibiting a 4-fold increase compared to the original solubility of naringin. On the other hand, naringin laurate demonstrated an impressive 82-fold increase in solubility in acetone, a solvent known for its ability to dissolve fats. Naringin propionate displayed intermediate solubility characteristics, showing increased solubility in organic solvents while remaining relatively unchanged in water when compared to the original solubility of naringin.

Previous studies involving enzymatic acylation with long-chain fatty acids have also reported similar influences on the solubility of naringin, where the modified molecule with long-chain fatty acids exhibited increased lipophilicity [47].

In addition, the altered partitioning behavior (Log*P*) observed between the organic and aqueous phases indicates a change in the affinity of the compounds for different environments. This characteristic has implications for their distribution and localization within biological systems. In pharmaceutical applications, the ability of these acylated compounds to selectively partition into specific cellular compartments or target tissues can significantly influence their therapeutic action. This altered partitioning behavior may also influence their interactions with other components, such as proteins or lipids, within the biological environment, further modulating their biological activities.

Regarding antioxidant activity, some authors have reported that the antioxidant activity decreases after acylation [44,48], this was also confirmed in our study, as we observed a reduction in antioxidant capacity by 1% to 5% during DPPH measurements. However, it is important to note that the antioxidant activity can be recovered *in vivo* through hydrolysis in the digestive system. This process releases the hydroxyl group at the site of acylation, thus restoring the antioxidant activity. This recovery allows for the potential utilization of this flavonoid in both the cosmetic and food industries, such as for the preservation of fatty foods. A drop in the antioxidant capacity in aqueous media for the laurate derivative was also expected since it is less soluble in polar media (Table 5).

The results for the β–carotene method (Fig. 6) showed an increased antioxidant for acylated naringin, presenting laurate with the highest activity. This was expected because it is a more hydrophobic derivative and therefore more soluble in lipids. Jasińska et al. [49] also reported for anthocyanins that in aqueous media, the %AA decreases with acylation, while in hydrophobic media, the %AA increases with the chain length of the acyl donor. This was also corroborated in the oxidative stability test, in which both naringin and its esters showed lipid protective activity in the olive oil that was used as a control (Table 6), with naringin laurate (with the longest chain used in this study) being the naringin ester with greater lipid protection. This result makes these compounds attractive to the pharmaceutical, food and cosmetic industries, having better solubility and improving their lipid antioxidant activity through enzymatic acylation [43,50].

The potential modulatory effect of short- and medium-chain fatty acids has been pointed out [51]. As these fatty acids are present in our naringin acylated derivatives, they could also contribute to this modulation. Indeed, recent studies have provided evidence of the modulatory effect of phenolic compounds, such as naringin, on the microbiota, although the mechanisms involved are not yet fully understood. Two main modes of action have been identified: firstly, certain microorganisms within the microbiota possess the ability to metabolize these phenolic compounds, and secondly, phenolic compounds can exhibit antimicrobial activity [9].

Although further investigation is warranted to fully elucidate the mechanisms underlying the modulation by phenolic compounds, the antimicrobial properties observed in some studies suggest that these compounds may help in maintaining a healthy gut environment by controlling the growth of potentially harmful microorganisms [52]. Moreover, the ability of certain gut microorganisms to metabolize phenolic compounds implies a mutual relationship between the microbiota and dietary compounds, potentially leading to a dynamic interplay that influences gut microbial diversity and function [53].

As our understanding of the gut microbiota's significance continues to evolve, harnessing the modulatory potential of dietary components like short- and medium-chain fatty acids and phenolic compounds may emerge as a valuable strategy for maintaining gut health and overall well-being.

5. Conclusions

Based on the results obtained in this study, it can be concluded that the reaction conditions for acylating naringin with acetate, propionate, and laurate resulted in a highly efficient conversion, surpassing 90% and demonstrating improved speed compared to previous studies under similar conditions. Notably, this enhanced efficiency is accompanied by a significant reduction in enzyme usage, which is particularly advantageous considering the high cost of the enzyme involved (N435). Achieving a lower enzyme consumption is a highly favorable outcome of this research.

Furthermore, the acylation of naringin led to enhanced solubility, thereby addressing a common limitation associated with its non-acylated form. Importantly, the acylated compounds exhibited preserved antioxidant activity in polar media and even showed increased activity in lipidic media. These enhancements hold considerable potential for expanding the application of naringin in the food and cosmetic industries.

In future studies, we aim to explore the impact of acylated naringin compounds on the modulation of the digestive microbiota.

Author contributions

- Study conception and design: JD Padilla de la Rosa; G Sandoval.
- Data collection: E Gutiérrez-Navarro; A Macías.
- Analysis and interpretation of results: E Gutiérrez-Navarro; J Solís; JD Padilla de la Rosa; G Sandoval.
- Draft manuscript preparation: E Gutiérrez-Navarro; J Solís.
- Revision of the results and approval of the final version of the manuscript: E Gutiérrez-Navarro; J Solís; A Macías; JD Padilla de la Rosa; G Sandoval.

Financial support

This research was funded by SEP-CONACYT 2018, grant number A1-S-34273.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

We thank CONACYT for financing the study (SEP-CONACYT 2018, No. A1-S-34273). We thank M.C. Carlos González for his support in analysis and logistics. We thank Dr. Sara A. Cortés-Llamas for her contribution to data analysis.

Supplementary material

https://doi.org/10.1016/j.ejbt.2023.12.003

Data availability

Several files of the experimental assays carried out are provided in the supplementary material.

References

[1] Barreca D, Mandalari G, Calderaro A, et al. Citrus flavones: An update on sources, biological functions, and health promoting properties. Plants 2020;9 (3):288. https://doi.org/10.3390/plants9030288. PMid: 32110931.
[2] Coello F, Peraza-Marrero M, Pinto-Catari I. Flavonoides: Micronutrientes con amplia actividad biológica. Revista de la Facultad de Medicina, Universidad Nacional de Colombia 2021;44:108–26.
[3] Tripoli E, Guardia ML, Giammanco S, et al. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. Food Chem 2007;104(2):466–79. https://doi.org/10.1016/j.foodchem.2006.11.054.
[4] Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem 2019;299:125124. https://doi.org/10.1016/j.foodchem.2019.125124. PMid: 31288163.
[5] Wojnar W, Zych M, Kaczmarczyk-Sedlak I. Antioxidative effect of flavonoid naringenin in the lenses of type 1 diabetic rats. Biomed Pharmacother 2018;108:974–84. https://doi.org/10.1016/j.biopha.2018.09.092. PMid:30372909.
[6] Ali AM, Gabbar MA, Abdel-Twab SM, et al. Antidiabetic potency, antioxidant effects, and mode of actions of *Citrus reticulata* fruit peel hydroethanolic extract, hesperidin, and quercetin in nicotinamide/streptozotocin-induced Wistar diabetic rats. Oxid Med Cell Longev 2020;2020:1730492. https://doi.org/10.1155/2020/1730492. PMid: 7327566.
[7] Jucá MM, Cysne Filho FMS, de Almeida JC, et al. Flavonoids: Biological activities and therapeutic potential. Nat Prod Res 2020;34(5):692–705. https://doi.org/10.1080/14786419.2018.1493588. PMid: 30445839.
[8] Padilla de la Rosa JD, Ruiz-Palomino P, Arriola-Guevara E, et al. A green process for the extraction and purification of hesperidin from Mexican lime peel (*Citrus aurantifolia* Swingle) that is extendible to the Citrus genus. Processes 2018;6 (12):266. https://doi.org/10.3390/pr6120266.
[9] Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, et al. Polyphenol-mediated gut microbiota modulation: Toward prebiotics and further. Front Nutr 2021;8:689456. https://doi.org/10.3389/fnut.2021.689456. PMid: 34268328.
[10] Sost MM, Ahles S, Verhoeven J, et al. A citrus fruit extract high in polyphenols beneficially modulates the gut microbiota of healthy human volunteers in a validated *in vitro* model of the colon. Nutrients 2021;13(11):3915. https://doi.org/10.3390/nu13113915. PMid: 34836169.
[11] Sakao K, Hou D-X. Effects and mechanisms of acylated flavonoid on cancer chemopreventive activity. Curr Pharmacol Rep 2020;6(5):286–305. https://doi.org/10.1007/s40495-020-00233-6.
[12] Choi G-Y, Kim H-B, Hwang E-S, et al. Naringin enhances long-term potentiation and recovers learning and memory deficits of amyloid-beta induced Alzheimer's disease-like behavioral rat model. Neurotoxicology 2023;95:35–45. https://doi.org/10.1016/j.neuro.2022.12.007. PMid: 36549596.
[13] Long J-Y, Chen J-M, Liao Y-J, et al. Naringin provides neuroprotection in CCL2-induced cognition impairment by attenuating neuronal apoptosis in the hippocampus. Behav Brain Funct 2020;16(1):4. https://doi.org/10.1186/s12993-020-00166-6. PMid: 32103758.
[14] Ahmed S, Khan H, Aschner M, et al. Therapeutic potential of naringin in neurological disorders. Food Chem Toxicol 2019;132:. https://doi.org/10.1016/j.fct.2019.110646. PMid: 20811644110646.
[15] Goldwasser J, Cohen PY, Yang E, et al. Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: Role of PPARα, PPARγ and LXRα. PLOS ONE 2010;5(8):e12399. https://doi.org/10.1371/journal.pone.0012399. PMid: 20811644.
[16] Shi Y, Tan YAN, Mao S, et al. Naringenin inhibits allergen-induced airway remodeling in a murine model of asthma. Mol Med Rep 2014;9(4):1204–8. https://doi.org/10.3892/mmr.2014.1940. PMid: 24534822.
[17] Wang W, Mao J, Chen Y, et al. Naringin promotes osteogenesis and ameliorates osteoporosis development by targeting JAK2/STAT3 signaling. Clin Exp Pharmacol Physiol 2021;49(1):113–21. https://doi.org/10.1111/1440-1681.13591. PMid: 34525226.
[18] Cai L, Wu H, Tu C, et al. Naringin inhibits ovarian tumor growth by promoting apoptosis: An *in vivo* study. Oncol Lett 2018;16(1):59–64. https://doi.org/10.3892/ol.2018.8611. PMid: 8910296.
[19] Passamonti S, Terdoslavich M, Franca R, et al. Bioavailability of flavonoids: A review of their membrane transport and the function of bilitranslocase in animal and plant organisms. Curr Drug Metab 2009;10(4):369–94. https://doi.org/10.2174/138920009788498950. PMid: 19519345.
[20] You HJ, Ahn HJ, Ji GE. Transformation of rutin to antiproliferative quercetin-3-glucoside by *Aspergillus niger*. J Agric Food Chem 2010;58(20):10886–92. https://doi.org/10.1021/jf102871g. PMid: 20886886.
[21] Pereira-Caro G, Polyviou T, Ludwig IA, et al. Bioavailability of orange juice (poly)phenols: The impact of short-term cessation of training by male endurance athletes. Am J Clin Nutr 2017;106(3):791–800. https://doi.org/10.3945/ajcn.116.149898. PMid: 28747329.
[22] Cao R, Wu X, Guo H, et al. Naringin exhibited therapeutic effects against DSS-induced mice ulcerative colitis in intestinal barrier–dependent manner. Molecules 2021;26:6604. https://doi.org/10.3390/molecules26216604. PMid: 34771012.
[23] Fidélix M, Milenkovic D, Sivieri K, et al. Microbiota modulation and effects on metabolic biomarkers by orange juice: A controlled clinical trial. Food & Funct 2020;11(2):1599–610. https://doi.org/10.1039/C9FO02623A. PMid: 32016250.
[24] Negatu DA, Gengenbacher M, Dartois V, et al. Indole propionic acid, an unusual antibiotic produced by the gut microbiota, with anti-inflammatory and antioxidant properties. Front Microbiol 2020;11:. https://doi.org/10.3389/fmicb.2020.575586. PMid:33193190575586.
[25] Gao X, Lin S-H, Ren F, et al. Acetate functions as an epigenetic metabolite to promote lipid synthesis under hypoxia. Nat Commun 2016;7(1):11960. https://doi.org/10.1038/ncomms11960. PMid: 27357947.
[26] Hernandez MAG, Canfora EE, Jocken JWE, et al. The short-chain fatty acid acetate in body weight control and insulin sensitivity. Nutrients 2019;11 (8):1943. https://doi.org/10.3390/nu11081943. PMid: 31426593.
[27] He J, Zhang P, Shen L, et al. Short-chain fatty acids and their association with signalling pathways in inflammation, glucose and lipid metabolism. Int J Mol Sci 2020;21(17):6356. https://doi.org/10.3390/ijms21176356. PMid: 32887215.
[28] Sheela DL, Narayanankutty A, Nazeem PA, et al. Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An *in silico* and *in vitro* study. Hum Exp Toxicol 2019;38 (7):753–61. https://doi.org/10.1177/0960327119839185. PMid: 30942101.
[29] Nitbani FO, Tjitda PJP, Nitti F, et al. Antimicrobial properties of lauric acid and monolaurin *in virgin* coconut oil: A review. Chem Bio Eng Rev 2022;9 (5):442–61. https://doi.org/10.1002/cben.202100050.
[30] Reyes-Reyes AL, Valero Barranco F, Sandoval G. Recent advances in lipases and their applications in the food and nutraceutical industry. Catalysts 2022;12 (9):960. https://doi.org/10.3390/catal12090960.
[31] Chebil L, Humeau C, Falcimaigne A, et al. Enzymatic acylation of flavonoids. Process Biochem 2006;41(11):2237–51. https://doi.org/10.1016/j.procbio.2006.05.027.
[32] Reyes-Reyes AL, Valero F, Sandoval G. Cloning, protein expression and biochemical characterization of *Carica papaya* esterase. Electron J Biotechnol 2023(61):61–8. https://doi.org/10.1016/j.ejbt.2022.11.004.
[33] Milisavljević A, Stojanović M, Carević M, et al. Lipase-catalyzed esterification of phloridzin: Acyl donor effect on enzymatic affinity and antioxidant properties of esters. Ind Eng Chem Res 2014;53(43):16644–51. https://doi.org/10.1021/ie5027259.
[34] Loucif K, Benabdallah H, Benchikh F, et al. Total phenolic contents, DPPH radical scavenging and β-carotene bleaching activities of aqueous extract from *Ammoides atlantica*. J Drug Delivery & Ther 2020;10(3-S):196–8. https://doi.org/10.22270/jddt.v10i3-s.4151.
[35] Nogales-Delgado S, Encinar JM, González JF. Safflower biodiesel: Improvement of its oxidative stability by using BHA and TBHQ. Energies 2019;12(10):1940. https://doi.org/10.3390/en12101940.
[36] Lu P, Chen Y, Tan M, et al. Chemical profiling by LC–MS/MS and HPLC fingerprint combined with chemometrics and simultaneous determination of 16 characteristic ingredients for the quality consistency evaluation of Shaoyao-Gancao Decoction. Biomed Chromatogr 2019;33(2):e4401. https://doi.org/10.1002/bmc.4401. PMid: 30277266.
[37] Doerr M, Romero A, Daza MC. Effect of the acyl-group length on the chemoselectivity of the lipase-catalyzed acylation of propranolol-a computational study. J Mol Model 2021;27(7):198. https://doi.org/10.1007/s00894-021-04808-y. PMid: 34115202.

[38] Sandoval G, Quintana PG, Baldessari A, et al. Lipase-catalyzed preparation of mono- and diesters of ferulic acid. Biocatal Biotransform 2015;33(2):89–97. https://doi.org/10.3109/10242422.2015.1060228.
[39] Sandoval G, Condoret JS, Monsan P, et al. Esterification by immobilized lipase in solvent-free media: Kinetic and thermodynamic arguments. Biotechnol Bioeng 2002;78(3):313–20. https://doi.org/10.1002/bit.10224. PMid: 11920447.
[40] González-Alfonso JL, Míguez N, Padilla JD, et al. Optimization of regioselective α-glucosylation of hesperetin catalyzed by cyclodextrin glucanotransferase. Molecules 2018;23(11):2885. https://doi.org/10.3390/molecules23112885. PMid: 30400664.
[41] Silvestrini L, Cianci M. Principles of lipid-enzyme interactions in the limbus region of the catalytic site of *Candida antarctica* Lipase B. Int J Biol Macromol 2020;158:358–63. https://doi.org/10.1016/j.ijbiomac.2020.04.061. PMid: 32380114.
[42] Milivojević A, Ćorović M, Carević M, et al. Highly efficient enzymatic acetylation of flavonoids: Development of solvent-free process and kinetic evaluation. Biochem Eng J 2017;128:106–15. https://doi.org/10.1016/j.bej.2017.09.018.
[43] Yang W, Kortesniemi M, Ma X, et al. Enzymatic acylation of blackcurrant (*Ribes nigrum*) anthocyanins and evaluation of lipophilic properties and antioxidant capacity of derivatives. Food Chem 2019;281:189–96. https://doi.org/10.1016/j.foodchem.2018.12.111. PMid: 30658747.
[44] Céliz G, Daz M. Biocatalytic preparation of alkyl esters of citrus flavanone glucoside prunin in organic media. Process Biochem 2011;46(1):94–100. https://doi.org/10.1016/j.procbio.2010.07.022.
[45] Mellou F, Lazari D, Skaltsa H, et al. Biocatalytic preparation of acylated derivatives of flavonoid glycosides enhances their antioxidant and antimicrobial activity. J Biotechnol 2005;116(3):295–304. https://doi.org/10.1016/j.jbiotec.2004.12.002. PMid: 15707690.
[46] Sun CQ, Johnson KD, Wong H, et al. Biotransformation of flavonoid conjugates with fatty acids and evaluations of their functionalities. Front Pharmacol 2017;2017(8):759. https://doi.org/10.3389/fphar.2017.00759. PMid: 29163154.
[47] Guo H, Yu J, Lei B, et al. T enzymatic esterification of naringin and the properties of naringin esterified derivatization. Ind Crops Prod 2022;178:. https://doi.org/10.1016/j.indcrop.2021.114372114372.
[48] Qian J, Gou L, Chen Y, et al. Enzymatic acylation of flavone isolated from extractive of bamboo leaves with oleic acid and antioxidant activity of acylated product. Eng Life Sci 2019;19(1):66–72. https://doi.org/10.1002/elsc.201800096. PMid: 32624957.
[49] Jasińska K, Fabiszewska A, Białecka-Florjańczyk E, et al. Mini-review on the enzymatic lipophilization of phenolics present in plant extracts with the special emphasis on anthocyanins. Antioxidants 2022;11(8):1528. https://doi.org/10.3390/antiox11081528. PMid: 36009246.
[50] Li HM, Xu TT, Peng QX, et al. Enzymatic acylation of rutin with benzoic acid ester and lipophilic, antiradical, and antiproliferative properties of the acylated derivatives. J Food Sci 2021;86(5):1714–25. https://doi.org/10.1111/1750-3841.15703. PMid: 33844282.
[51] Padilla-Camberos E, Arrizon J, Sandoval G. Effect of agave fructan bioconjugates on metabolic syndrome parameters in a murine model. Pharmaceuticals 2023;16(3):412. https://doi.org/10.3390/ph16030412. PMid: 36986511.
[52] Lippolis T, Cofano M, Caponio GR, et al. Bioaccessibility and bioavailability of diet polyphenols and their modulation of gut microbiota. Int J Mol Sci 2023;24(4):3813. https://doi.org/10.3390/ijms24043813. PMid: 36835225.
[53] Pei R, Liu X, Bolling B. Flavonoids and gut health. Curr Opin Biotechnol 2020;61:153–9. https://doi.org/10.1016/j.copbio.2019.12.018. PMid: 31954357.