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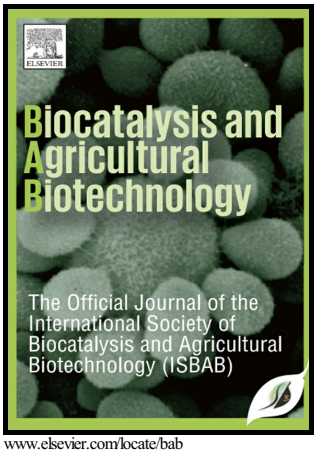

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Obtaining hesperetin 7-O-glucosyl 6''-O-laurate, a high lipophilic flavonoid ester, from *Citrus* waste

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ABSTRACT

A biotechnological process that combines the treatment of *Citrus* processing waste to extract a high-valuable biomolecule, neohesperidin (NEO), and its further modification by biocatalysis to enhance the lipophilicity of its flavonoic moiety is presented. NEO was extracted from immature fruits of *Citrus aurantium* and hydrolyzed by a commercial alpha-rhamnosidase to obtain hesperetin 7-glucoside (HG). NEO and HG were mono-acylated with vinyl laurate by using Novozym 435 in their primary OH-groups, but the esterification reaction was substantially faster for HG than NEO. Furthermore, the activation energy of the reaction lowers as solvents' log-P decreases linearly. Under best conditions, HGL was obtained at gram scale with a simple downstream process. The solubility in n-octanol increased with each biocatalytic step (NEO<HG<HGL), reaching a difference of 2 orders when comparing HGL with its parent molecules. This dramatic change in hydrophobic/hydrophilic balance opens

many possibilities to use the product in lipophilic media as an active agent in functional foods, therapeutic and cosmetic industries.

Keywords

Neohesperidin; hesperetin 7-O-glucoside; flavonoid ester; *Citrus* processing waste; enzymatic esterification.

1. Introduction

Neohesperidin (NEO) is a flavonoid unique to *Citrus* which is found in significant concentrations in some orange varieties from where it can be obtained (Macoritto et al., 2004). *Citrus* flavonoids have proven to be quite valuable due to their antioxidant, anti-allergy, vasotonic, anti-inflammatory properties (Benavente-García & Castillo, 2008) and antimicrobial activity (Tripoli et al., 2007). In addition, they are substances Generally Recognized as Safe (GRAS) by the FDA. It is therefore reasonable to assume their potential as a component in functional foods which, in turn, would enhance human health and prevent both rancidity and microbial contamination.

In the last few years, new characteristics have been determined about NEO. It prevents aflatoxin contamination from *Aspergillus flavus* (Salas et al., 2016), it has anti-osteoclastic effects in vitro and in vivo, and it possesses therapeutic potential as a natural anti-catabolic treatment in osteoporosis (Tan et al., 2017). Given these characteristics, this molecule has potential use *per se*. However, the transformation of NEO could lead to derivatives with new properties, which would increase its demand, making its extraction and derivatization by the *Citrus* processing industry more attractive. The production of neohesperidin dihydrochalcone, a GRAS molecule

accepted by the FDA as a food additive for its sweetening power is a successful example.

NEO can be enzymatically hydrolyzed with α-rhamnosidases to generate rhamnose, a valuable sugar used as a precursor to diverse organic compounds (Ribeiro, 2011) and as an anti-aging agent in cosmetic products (Asselineau et al., 2011), and hesperetin 7-O-glucoside (HG). HG can be obtained starting from hesperidin (HES) too, but the partial de-glycosylation is conducted with rhamnosidases in water at mild conditions (50-60 ºC), were NEO is 9 times more soluble than HES at 25 °C and this ratio rose up to 32 at 50 °C. So, NEO is technically more adequate to obtain HG at higher scale in smaller reactors (Céliz et al., 2015).

HG inhibits the growth of hazardous molds and the production of their mycotoxins (Salas et al., 2011; Salas et al., 2012), effectively preventing bone loss in adult rats (Habauzit et al., 2009), as well as possessing other similar pharmaceutical properties as flavonoids with the same aglycone (Garg et al., 2001; Parhiz et al., 2015). However, the use of these properties is limited due to its low solubility in lipidic media.

Previous studies have demonstrated that introducing lipophilic moieties into flavonoids, not only can the physico-chemical properties be modified, but also their biological activities (to review this claim, see Danihelová et al., 2012 and de Araújo et al., 2017). Because of this, it is interesting to synthesize a hydrophobic HG ester since lipophilicity, cell membrane penetration, antimicrobial activity and other biological properties could be enhanced, expanding the applications of this molecule in the food, therapeutic and cosmetic industries.

Lipases are industrial biocatalysts involved in several reactions, occurring in aqueous medium as well as non-aqueous medium. In addition, most of the synthetic reactions on industrial scale are carried out in organic solvents (Kumar & Kanwar, 2012). The advantages to employ lipases for synthetic reactions in organic solvents and the effects of them on lipase activity have been well reviewed recently. They are often immobilized for operational convenience and to enable biocatalyst reuse, thus reducing enzyme costs (Kumar et al., 2016). From the vast array of commercially available immobilized lipases, undoubtedly Novozym 435 (*Candida antarctica* lipase B, CALB, immobilized on Lewatit) is one of the most used for esterifying natural compounds and was successfully used in the acylation of flavonoid glycosides (de Araújo et al., 2017). Current literature reports the use of the commercial preparation Lipozyme RM IM (*Rhizomucor miehei* lipase, RML, immobilized on an anion exchange resin) for esterification of flavonoids in organic media as well. These two biocatalysts have high availability and are relatively low-cost, making them viable for industrial purposes (Franssen et al., 2013). Concerning the acyl-donor, the addition of a C-12 alkyl rest to HG would generate a compound that could interact with the cell membrane more effectively and would have more antibacterial activity than the original molecule, especially against Gram-positive (Céliz et al., 2010; Céliz et al., 2011). Vinyl esters generate hydroxyethylene as a leaving group (unlike fatty acids that generate water). It tautomerizes to acetaldehyde (boiling point 20.2 °C) that quickly evaporates, shifting the equilibrium towards products. Therefore, vinyl laurate is a promising acyl donor to esterify HG.

In this paper we carried out a biotechnological process that combines extraction from Citrus waste and biocatalysis to obtain the flavonoid ester hesperetin 7-O-glucosyl 6’’-

O-laurate (HGL). Additionally, compounds solubility and aspects of the enzymatic esterification were studied.

2. Materials and methods

2.1 Materials.

Neohesperidin (NEO) was obtained from naturally aborted immature fruits of *Citrus aurantium* as previously described (Macoritto et al., 2004). Firstly, these were ground to an average diameter of 2 mm and flavonoids were extracted in a fixed bed column with ethanol:water (25:50 v/v) at 25 ºC. The extract was then cooled at 4 ºC to achieve flavanones crystallization. The precipitate was filtered, washed and finally dried at 50 ºC. HG was obtained by enzymatic hydrolysis of NEO according a previous work (Céliz et al., 2015). Briefly, 500 ml of 20 mM NEO suspension was poured in an Erlenmeyer flask at 50 °C. The hydrolysis was initiated by adding 2.8 ηkat mL^{-1} (against p-NPR) of a α-rhamnosidase from *Aspergillus niger* (Tanabe Seiyaku Co, Japan). The reaction was carried out at 50 ºC for 24 h. Then, the reactor was kept at 4 ºC for 6 h. The reaction media was filtered using filter paper to recover the NEO-HG mixture. It was washed with cool distilled water. The solid was suspended in 300 mL of buffer and the reaction was carried out at 50 °C for another 24 h at the same enzyme concentration. Finally, the reactor was kept at 4 °C for another 6 h. The precipitate obtained was filtered, washed with distilled water and dried at 50 °C. The HG obtained had purity by HPLC close to 100 %. Vinyl laurate was from Fluka (USA). Novozym 435 and Lipozyme RM IM were gifts from Novozymes Latin America Limited (Brazil). Molecular sieves (sodium aluminosilicate) with 4 Å pore diameter was from SIGMA (USA). Solvents and all other reagents were of analytical grade.

2.2 Synthetic reaction.

Water was removed from the solvents and vinyl laurate by adding molecular sieves (30 mg mL^{-1}) and keeping them in the presence of the adsorbent. Substrates (NEO and HG) were weighed and placed on screw cap vials with Teflon seal to reach final concentrations of 5 or 20 mM (NEO Mw 610 g mol^{-1} and HG Mw 464 g mol^{-1}). Then 15 mg molecular sieves per milliliter of reaction media were added. Later, 6 mL of an appropriate mixture of vinyl laurate and solvent were added to reach the acyl-donor/flavonoid molar ratio desired (between 2 and 20). The reactors were incubated with gentle axial shaking (180 rpm) at temperatures ranging from 40 to 70 ºC. The reaction was started by adding 25 mg of immobilized enzyme. At regular time intervals, 50 μL aliquots were extracted and appropriate dilutions of these samples were analyzed by HPLC.

2.3 Obtaining hesperetin 7-O-glucosyl 6''-O-laurate.

HGL was obtained on gram scale using Novozym 435 and acetonitrile (AcN) as solvent. In a typical experiment 190 mL of AcN, 3 g of molecular sieves (15 mg mL^{-1}), 1.85 g of substrate (final HG concentration 20 mM) and 10 mL of vinyl laurate (200 mM) were poured into a screw cap 500 mL glass bottle, which was incubated with axial shaking (180 rpm). The temperature was set to 60 ºC prior to the addition of 1 g (5 mg mL^{-1}) of Novozym 435 to start the reaction. After 48 h, the conversion reached 100 %. Then, the immobilized enzyme and the molecular sieves were separated from the reaction medium by filtration and the AcN was recovered in a rotating evaporator at 50 ºC. The solid was then washed three times with hexane, centrifuging each time at 1075

xg (2000 rpm, r = 240 mm) for 5 min. A greenish white powder was obtained. Purity was checked by HPLC.

2.4 HGL structural identification.

^{1}H, ^{13}C and 2D NMR experiments were measured on a Bruker Avance DMX500 NMR spectrometer operating at 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C) using standard Bruker software.

^{1}H (500.13 MHz, DMSO-d_6): δ 5.35 (1H, dd, 2.7, 12.9 Hz, H-2), 3.09 (1H , dd, 12.9, 17.2 Hz, H-3-ax), 2.75 (1H , dd, 2.7, 17.2 Hz, H-3-eq), 6.17 (1H, d, 2.2 Hz, H-6), 6,23 (1H, d, 2.2 Hz, H-8), 6.96 (1H, sbr, H-2'), 6.93 (1H, d, 8.3 Hz, H-5'), 6.92 (1H, dbr, 8.3 Hz, H-6'), 3.87 (3H, s, OCH_3), 4.92 (1H, dbr, 7.5 Hz, H-1''), 3.46 (1H, dd, 7.3, 8.8 Hz, H-2''), 3.31 (2H, m, H-3'', H-4'', overlapped with the solvent signal), 3.67 (1H, ddd, 2.0, 7.2, 8.5 Hz, H-5''), 4.14 (1H, dd, 7.2, 11.7 Hz, H-6_a''), 4.44 (1H, dd, 2.0, 11.7 Hz, H-6_b''), 2.28 (2H, dd, 7.2, 7.3 Hz, H_α to C=O), 1.45 (2H, dd, 7.2, 7.3 Hz, H_β to C=O).

^{13}C (125.76 MHz): δ 80.1 (d, C-2), 44.4 (t, C-3), 197.0 (s, C-4), 163.4 (s, C-5*), 97.6 (d, C-6), 165.5 (s, C-7), 96.4 (d, C-8), 163.4 (s, C-9*), 103.6 (s, C-10), 131.0 (s, C-1'), 113.6 (d, C-2'), 146.4 (s, C-3'), 147.9 (s, C-4'), 111.9 (d, C-5'), 118.2 (d, C-6'), 100.5 (d, C-1''), 77.0 y 73.9 (d, C-2''+ impurities), 71.2 (d, C-3'', C-4''), 75.1 (d, C-5''), 63.9 (t, C-6''), 55.8 (s, OCH_3), 34.4 (t, C_α of C=O), 25.4 (t, C_β to C=O), 174.1 (C=O).

2.5 Solubility.

The NEO, HG and HGL solubility were determined in the solvents used for the synthesis at 50 ºC and in water and n-octanol at 20 ºC. Saturated solutions were kept overnight at the selected temperatures. Later, they were centrifugated. Supernatants

were filtered by Nylon membrane 0.45 μm (Microclar, Argentina), diluted appropriately and analyzed by HPLC.

2.6 HPLC.

The concentrations of the compounds were determined by HPLC using an autosampler Autoinjector 234, an isocratic peristaltic pump Pump 37, an UV-detector at 280 nm Detector 118, all by Gilson (France). The results were recorded on an integrator Chromatopac C-R8A by Shimadzu (Japan). A GraceSmart RP-18 reverse-phase column (25 cm long, 4 mm internal diameter and 5 μm particle size by Grace, USA) was used. Samples (20 μL) were eluted isocratically with 80:20 acetonitrile:water as mobile phase at 0.8 mL min^{-1} flow-rate. Calibration curves for the analyzed compounds were performed using purified samples; the areas obtained by HPLC were transformed into conversions according to the equation:

$$Conversion = \left(\frac{Area^{ester}}{Area^{ester} + Area^{substrate}} \right) * 100\%$$

This calculation is based on the fact that the molar absorptivity of the substrate and response factors were the same, regardless of the group linked to the 7-O-hesperetin position.

2.7 Data processing.

Data analysis and graphs were performed using GraphPad Prism Software for Windows versions 5.00 GraphPad Software (San Diego, California, USA, www.graphpad.com).

3. Results and Discussion

3.1 *Enzyme selection.*

Two biocatalysts viable for industrial purposes were assayed: Novozym 435 and Lipozyme RM IM.

Regardless the solvent tested, both enzymes were able to carry out the HGL synthesis, generating HPLC chromatograms with a single peak for the product. The HMBC experiment allowed to conclude that in all cases a monoacylated ester was obtained, being hesperetin 7-O-glucosyl 6''-O-laurate its structure. This was determined for a correlation between the carbonyl group at δ_C 174.1 with H-6''$_a$ and H-6''$_b$. This result is in agreement with other works. For example, Ardhaoui *et al.* esterified quercetin, hesperidin, rutin and esculin with fatty acids using CALB in 2-methyl-2-butanol. For all molecules, only flavonoid monoester was produced, being the primary OH on the sugar moiety the reactive group (Ardhaoui et al., 2004).

Novozym 435 proved to be more efficient than Lipozyme RM IM, in all comparisons. Using Lipozyme RM IM, conversion did not reach 40 % in any of the solvents, after one day reaction at 50 °C. On the other hand, Novozym 435 achieved full HG conversion, in three of the four solvents tested, after 24 hours at the same temperature (Figure 2).

The superiority of CALB over RML aligns with previous research on flavonoid ester synthesis. For example, Gao *et al.* (2000) in the esterification of catechin-7-O-glucoside with aromatic acids using TBOH/pyridine and acetonitrile/pyridine mixtures, and Céliz *et al.* in the synthesis of prunin 6''-O-laurate in different organic solvents (Céliz & Daz, 2011). In light of the outcomes, the following optimizations were conducted with only Novozym 435.

3.2 Temperature and solvent effect.

HGL concentrations were determined as a function of time at different temperatures in each solvent. The initial HG concentration was 5 mM because it was the highest, at which the substrate was completely dissolved at 50 ºC in all solvents (Table 1). The HGL concentrations (mM) versus time (h) profiles were transformed to initial rate (mM min^{-1}) versus solvent log-P graphs (Figure 3-A). Then, considering a pseudo 1^{st}-first order kinetic model with low enzyme saturation [$v_{initial}$(mM min^{-1})=K(min^{-1} mg-biocatalyst^{-1})**E* (mg-biocatalyst^{-1} *Substrate(mM)], K values were calculated. Subsequently, the simplest dependence of K on temperature (Arrhenius equation) was used to estimate the activation energy (E_{ac}) in each solvent (Figure 3-B) and then, these E_{ac} were plotted versus solvents' log-P (Figure 3-C).

When temperature ranged from 50 to 60 °C, a substantial improvement in the synthesis of HGL was observed for all solvents, while, from 40 to 50 °C or from 60 ºC to 70 °C, the reaction rate improvements were less significant. It was therefore decided to use 60 ºC to carry out the production of HGL at gram scale.

Regarding solvent effect, it is common to obtain simplified conclusions correlating different solvent properties (hydrophobicity, solubility in water, ability to donate or accept electron pairs, polarizability, dielectric constant, etc.) with the variable of interest, such as enantioselectivity (Hirose et al., 1992), initial rates (Hazarika et al., 2003) or conversion (Watanabe et al., 2009), among others.

In the esterification of HG with vinyl laurate using Novozym 435, it was found that, for the solvents used, conversion increases while solvents' log-P decreases linearly; AcN being the solvent where the reaction had the fastest initial rate. In addition, the joint

analysis of temperature, solvents' log-P and initial rate allowed to determine that increasing solvents' log-P, increased E_{ac} linearly. In the synthesis of other similar flavonoid ester, prunin 6''O-laurate, by Novozym 435 the same analysis was conducted and an identical dependence between solvents' log-P and E_{ac} was recorded (data presented in Supplementary Figure 1).

Xu *et al.* pose the question of whether the rate of an enzymatic reaction proceeding through a charged transition state, such as esterification with CALB (De Oliveira et al., 2009), it can be increased by increasing the active-site polarity in an organic solvent. They concluded, while working with subtilisins in THF, that increasing the polarity of the microenvironment within the active site increases the process rate substantially and they suggest that solvent polarity is an important factor in stabilizing the tetrahedral intermediate (Xu et al., 1994). Our result would be a further argument for this hypothesis, since as the solvents' log-P decrease, Eac decreases, which agree with a transition state of lower energy. However, it should not be forgotten that the solvent affects the biocatalytic process in many other ways, especially if the enzyme is immobilized (Kumar et al., 2016).

3.3 Molar ratio effect.

In order to obtain HGL at grams scale, HG concentration was increased to 20 mM and the effect of the vinyl laurate/HG molar ratio over the conversion was studied at 60 ºC. Figure 4 summarizes the conversions reached after 8 h of reaction in each solvent.

It was observed that the substrate was completely solubilized only in the media with THF. In the others, the reaction media became slightly lacteally cloudy with HG partially precipitated. However, conversions close to 100 % were obtained after 8 h with

10 vinyl laurate/HG molar ratio or more. Previous research demonstrated that the esterification of glycosyl flavanones can progress seamlessly with the substrate mostly non-dissolved (Céliz et al., 2012). Doubling molar ratio from 10 to 20 did not improve conversion significantly, so the first was assumed to be better conditions to obtain HG at gram scale.

3.4 Advantages of esterifying HG instead NEO.

The esterification of HG and NEO (both 20 mM) was compared at 60 ºC in the four solvents. The unique translucent reaction medium with flavonoids dissolved totally was which had THF as solvent. Regardless the solvent used or appearance of the reaction media, the ester formation rate was substantially faster for HG than NEO (Figure 5). Thus, eliminating rhamnose moiety from NEO had an advantage with respect to the aims of performing the efficient lipophilization of the aglycone moiety.

This could be explained by the differences of solubility between the substrates, which arise 1-fold order on pure solvents (see Table 1) and maybe by the fact that HG is smaller and more hydrophobic, therefore it should have higher compatibility with the hydrophobic CALB active-site. Supporting this result, Gao *et al.* also observed that the acylation rates of diglycosyl flavonoids were lower compared to monoglycosyl flavonoids (Gao et al., 2000). Unfortunately, they did not compare compounds with the same aglycone, and therefore other interactions could have taken place. Pedersen *et al.* (Pedersen et al., 2002) suggested that bigger substrates would have a greater steric hindrance on the active site, which would reduce the rate of ester formation.

3.5 Solubility.

Despite the attention that flavonoids attract, few studies have been done with regard to their solubility. There are several reasons to determine solubility. First, the use of a compound on a matrix is closely tied to its solubility in that medium. It is worth mentioning that in recent years there has been an increasing amount of research which includes the determination of the solubility of compounds, and further evaluates, as a main objective, the potential for food and drug industries (Petereit & Saal, 2011).

A central point in this paper is to determine whether the esterified derivative has higher solubility in lipophilic matrices than the parent flavonoid. It was also of interest to evaluate the solubility of HG in the solvents used in the synthesis, since the substrate was slightly soluble in 3 of the organic solvents used.

Eliminating rhamnose moiety from NEO to generate HG resulted in a derivative with higher solubility in the solvents employed for the synthesis (Table 1). A similar result was found by Chebil et al. (Chebil et al., 2007) when working with rutin, isoquercitrin and quercetin (di-glycoside, mono-glycoside and aglycone), in which 1-fold order of solubility in acetonitrile between the di-glycoside and the mono-glucoside (0.5 and 5.4 mM, respectively) was recorded.

Table 1 also presents the solubility of NEO, HG and HGL in water and n-octanol. As clearly shown, the lipophilization of HG with a 12 carbons alkyl chain was effective to enhance its solubility in n-octanol, making it over 80 times more soluble than HG and 160 times than NEO. The log-P determined for HG was -0.12 while HGL log-P cannot be measured since its solubility in water was not detected.

4. Conclusions

Neohesperidin can be obtained at large scale from *Citrus* processing waste easily. Hydrolyzing it by rhamnosidases generates rhamnose and HG, a more soluble substrate in the organic solvent assayed which is mono-esterified faster and in its primary OH-group by CALB. Furthermore, the activation energy of the reaction lowers as solvents' log-P decreases. HGL could be obtained at gram scale with a simple downstream process, being substantially more soluble in n-octanol than NEO or HG. This proves the efficiency of the biotransformation in order to solubilize the aglycone in lipophilic media. HGL is a promising high lipophilic semi-synthetic flavonoid ester with industrial potential. The next step is to assay its properties and toxicity to test its scope.

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Figure 1. Scheme of the synthesis of HGL from NEO by a two steps enzymatic process.

Figure 2. Conversion profiles for the esterification of HG with vinyl laurate using Novozym 435 (solid line) and Lipozyme RM IM (dashed line) in (■) AcN, (○) acetone, (●) THF and (□) TBOH at 50 ºC. Experimental conditions: HG 20 mM, vinyl laurate 100 mM, total volume 6 mL. Means and SD of two independent values are presented.

Figure 3. A) Initial rates for the synthesis of HGL vs. solvents' log-P in (■) AcN, (○) acetone, (●) THF and (□) TBOH at 40 ºC (•••), 50 ºC (••), 60 ºC () and 70 ºC (—). Means and SD of 2 independent values are presented. B) ln K vs. 1/T plot for activation energies determination according to Arrhenius law with their SD. C) Activation energies vs. solvents' log-P with their SD. Experimental conditions: HG 5 mM, vinyl laurate/HG molar ratio 10, molecular sieves 35 mg mL^{-1}, Novozym 435 25 mg, total volume 6 mL.

Figure 4. Effect of vinyl laurate/HG molar ratio on the HG conversion reached after 8 hours at 60 ºC. Experimental conditions: HG 20 mM, molecular sieves 30 mg mL^{-1}, Novozym 435 50 mg, total volume 3 mL. The results are the mean of 3 independent determinations with SD.

Figure 5. Conversion profiles in the esterification of **HG** (full line) and **NEO** (dashed line) with vinyl laurate in (■) AcN, (○) acetone, (●) THF and (□) TBOH at 60 ºC. Experimental conditions: substrate 20 mM, vinyl laurate 400 mM, molecular sieves 60 mg mL^{-1}, Novozym 435 17 mg mL^{-1}. The results are the mean of 3 independent determinations with SD.

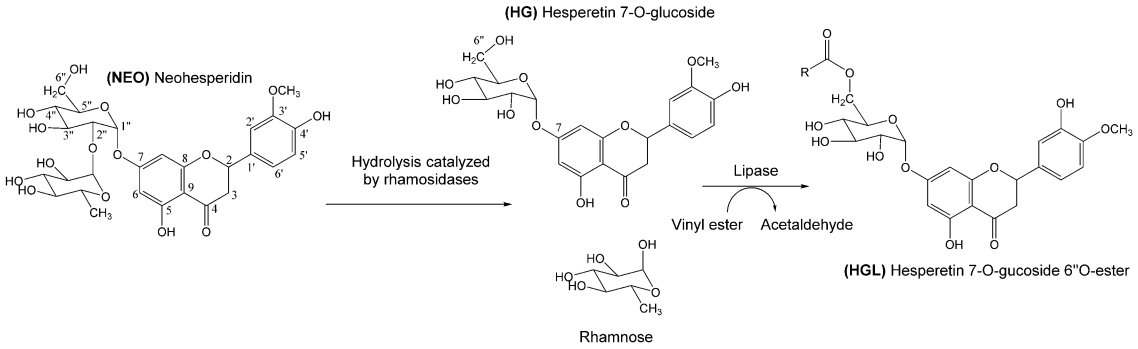
Table 1. Solubility of NEO, HG and HGL in different solvents.

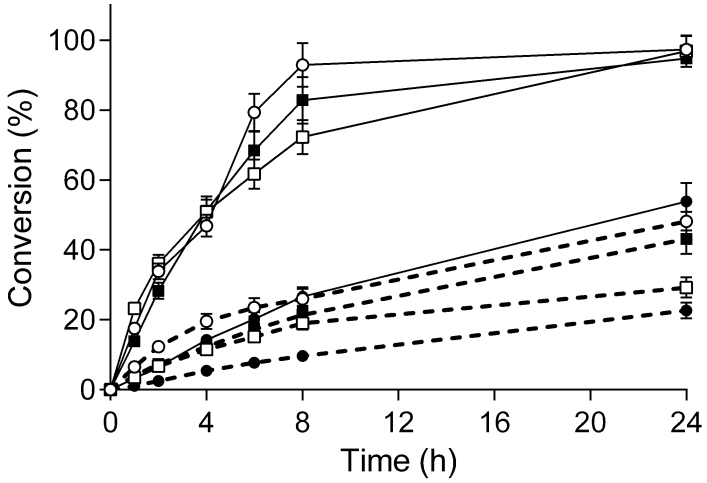
		Solubility (mM)		
	Solvent	Neo	HG	HGL
50 ºC	Acetone	2.3	11	128
	Ter-butanol	0.7	7.8	15
	THF	14	133	217
	Acetonitrile	0.8	5.0	73
20 ºC	Water	2.6	0.8	ND
	n-Octanol	0.4	0.8	66

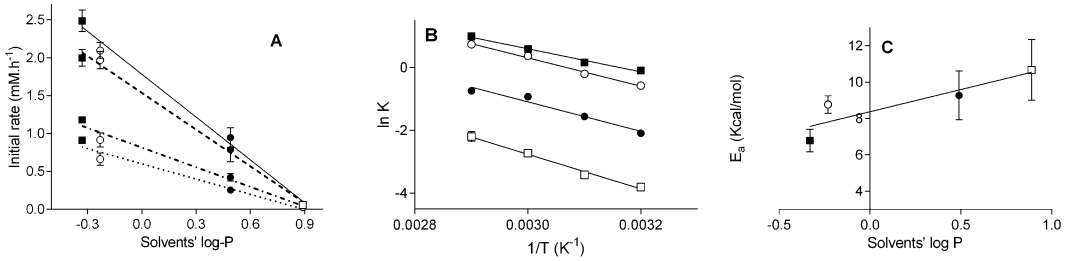
Means of 3 independent values are presented. The SD were below than 10 %.

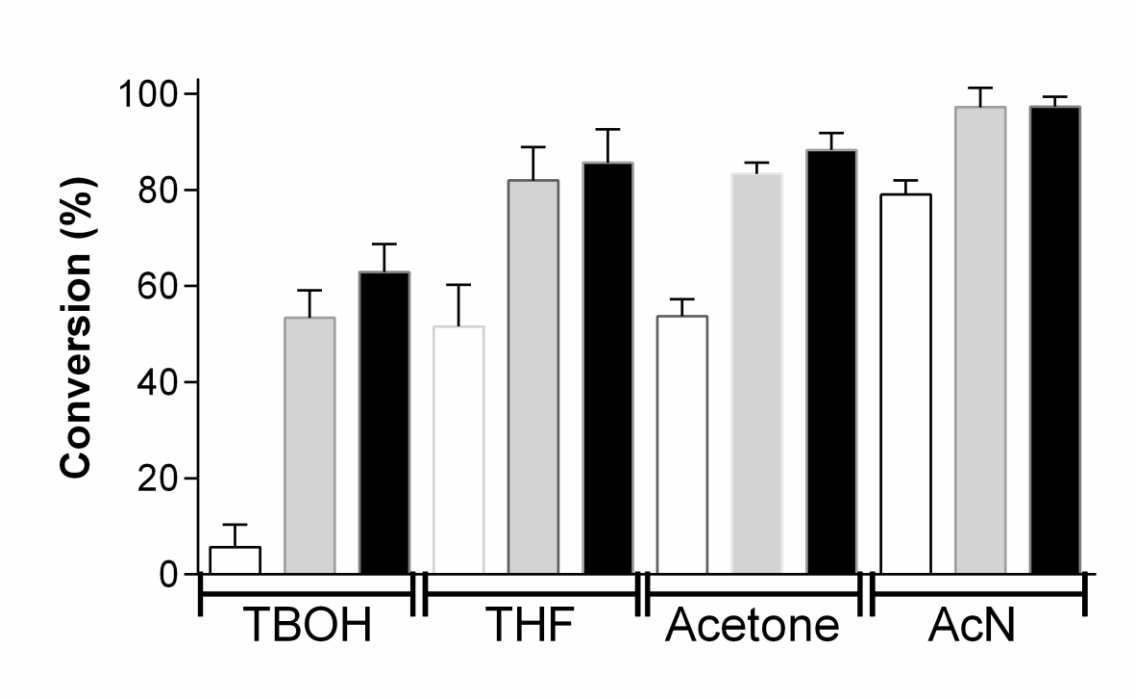
Highlights

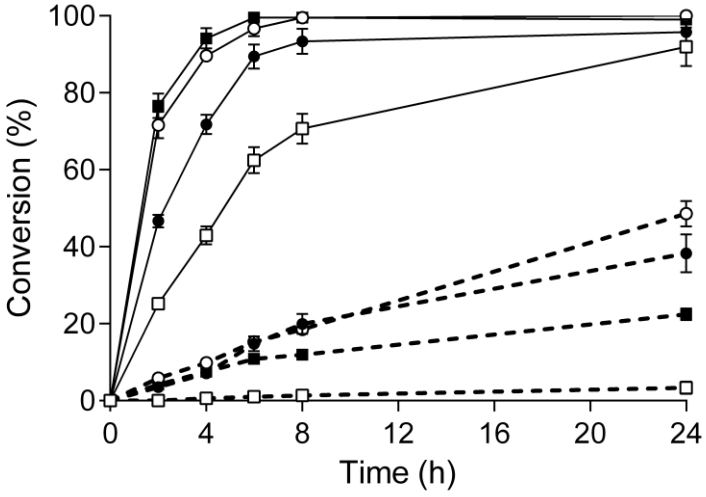
- A new high lipophilic flavonoid ester, HGL, was obtained from *Citrus* waste.
- HG was more suitable than NEO for esterification by CALB.
- In the lipophilization by CALB, Eac lowers as solvents' log-P decreases.
- HGL was around to 2 orders more soluble in n-octanol than NEO or HG.
- HGL could be obtained at gram scale with a simple downstream process.

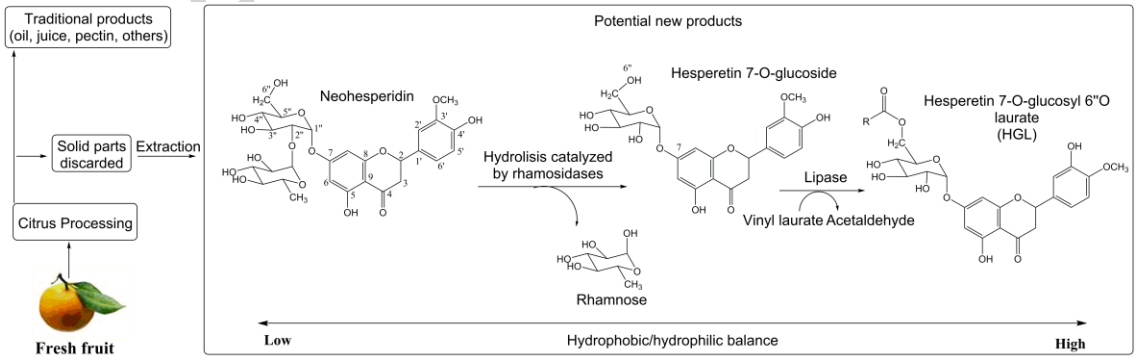

(NEO) Neohesperidin
(HG) Hesperetin 7-O-glucoside
Hydrolysis catalyzed by rhamosidases
Lipase
Vinyl ester
Acetaldehyde
(HGL) Hesperetin 7-O-gucoside 6''O-ester
Rhamnose


Conversion (%)
Time (h)


A
Initial rate (mM.h-1)
Solvents' log-P
B
ln K
1/T (K-1)
C
Ea (Kcal/mol)
Solvents' log P


Conversion (%)
100
80
60
40
20
0
TBOH
THF
Acetone
AcN


Conversion (%)
100
80
60
40
20
0
0
4
8
12
16
20
24
Time (h)


Traditional products (oil, juice, pectin, others)
Solid parts discarded
Extraction
Citrus Processing
Fresh fruit
Potential new products
Neohesperidin
Hydrolisis catalyzed by rhamosidases
Rhamnose
Hesperetin 7-O-glucoside
Lipase
Vinyl laurate
Acetaldehyde
Hesperetin 7-O-glucosyl 6"O laurate (HGL)
Low
Hydrophobic/hydrophilic balance
High