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Article

A green process for the extraction and purification of hesperidin from Mexican lime peel (*Citrus aurantifolia* Swingle) extendible to *Citrus* genus

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Abstract: The processing of Mexican limes generates great amounts of peel as a byproduct. Lime peel is mainly rich in the flavonoid hesperidin, whose bioactivity is oriented mainly to cardiovascular diseases and cancer. The purpose of this work was to develop a green process for the extraction and purification of hesperidin from Mexican lime peel. The extraction of hesperidin was investigated on a laboratory scale by varying the solvent composition and the solid-to-solvent ratio. The best conditions (solid-to-solvent ratio of 0.33 g/mL and 60% ethanol) were used for the extraction of hesperidin in a pilot scale (Volume = 20 L). The kinetics of the extraction was studied to find the maximum hesperidin concentration at 100 min. The concentrated extract had a hesperidin content of 0.303 mg/mL. Next, a purification process using adsorption resins was assessed. Through static tests, it was determined that higher adsorption efficiencies were achieved with the EXA-118 resin and diluted extract (4:6 ratio with 10% DMSO). Finally, the adsorption of hesperidin from the diluted extract (hesperidin concentration of 0.109 mg/mL) was carried out at 25°C in a column packed with 80 ml of EXA-118 resin. The mean recovery efficiency of hesperidin from the extract was almost 90%.

Keywords: *Citrus aurantifolia* Swingle; hesperidin; citrus byproducts; adsorption

1. Introduction

Citrus genus is the most important fruit tree crop in the world, with an annual production of approximately 135.8 million tons, which consists of oranges (71.4 million tons), tangerines and mandarins (28.7 million tons), lemons and limes (15.2 million tons) and grapefruit (8.4 million tons). These fruit have a high commercial value in both the fresh market and food industry [1,2].

The focus of the citrus processing industry has been the production of juices and essential oils. Approximately 33% of the citrus production in the world is used for the juice industry, and at least 50% of the whole fruit mass is residue. Therefore, considering these facts, the worldwide estimate of lime peel residue is 2.5 million tons per year. Clearly, this huge generated citrus waste, should be managed and industrialized properly, since it tends to constitute a severe environmental problem [2].

An attempt to reclaim some value from the residues generated by the citrus processing industry is to identify and extract the bioactive compounds within. *Citrus* peel has a high content of flavonoids,

and the flavanone glycoside hesperidin, is the most abundant [3] (Figure 1). The content of hesperidin in the tissues of Mexican lime is in the order of 197 mg/100 g of fresh tissue [4].

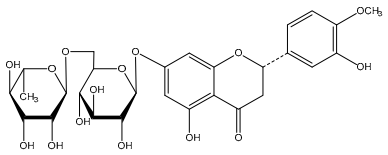

Figure 1. Chemical structure of hesperidin [5].

Hesperidin (3′,5,7-trihydroxy-4′-methoxy-flavanone-7-rhamnoglucoside) exhibits multiple biological properties: antioxidant, anti-inflammatory [6], antihypercholesterolemic [7], anti-hypertensive, anticarcinogenic, antimicrobial and antiallergenic. Hesperidin is neuroprotective and it has vasodilator and diuretic properties [8–10]. Hesperidin is also important in the pharmaceutical, cosmetic and food and beverages industries.

Traditionally, hesperidin has been obtained from the *citrus* peel using alkaline extraction. First of all, the peel is ground and washed to remove soluble solids, then it is blended with a water and NaOH solution (pH 11-11.5). After 1 h at room temperature, the insoluble solids are separated and the liquid phase is filtered. Mineral acids are then added to the filtered product to get the pH to 4-2-4.5, and the solution is heated at 40-45°C for 12 to 24 h. The hesperidin crystals formed that way are separated and dried. Generally, this procedure allows for the formation of a flavonoid complex, which has 60-70 percent hesperidin content. To obtain a higher hesperidin content (>95%), repeated crystallizations can be done [11]. As expected, this process is time consuming and requires a significant amount of acid and base. In addition, other compounds are simultaneously extracted, resulting in reduced efficiency and purity.

An alternative for the alkaline extraction method is the use of organic solvents. While methanol is an effective solvent for hesperidin extraction, toxicity limits its application. Ethanol is a substitute that is used as a solvent in the food industry. It has proven to be useful in the extraction of phenolic compounds in some *citrus* products [12,13]. In addition, ethanol is thought of as a bio-solvent because it can be produced from renewable resources [14]. The extraction process by itself presents low selectivity; so further purification of the required compound is necessary.

One of the most commonly used processes for flavonoid purification from extracts is resin adsorption. The alkaline treatment of peels and wastewater, coupled with the resin adsorption (styrene-divinylbenzene resins) step to obtain a more concentrated solution, which leads to more rapid crystallization, has been previously reported [15,16]. Additionally, the adsorption of hesperidin in 13 resins has been evaluated using model hesperidin solutions. Resin EXA-118, which has a high surface area, was most effective [17]. Resin FPX66 is a macroreticular, non-functionalized adsorbent resin designed for the juice processing market where local regulations allow for such use. Amberlite FPX66 can also be used for a wide variety of food processing applications to both recover high value materials and to purify and decolorize food and food additive streams.

Moreover, it does not exist many researches about extraction and purification of hesperidin from Mexican Lime, because all studies are focused mainly in the purification of hesperidin from orange (*Citrus sinensis*).

The objective of this present study was to evaluate a green, simple and economic alternative for the production of hesperidin from citrus peel. The process includes the hydroalcoholic extraction of hesperidin from Mexican lime peel and further purification using adsorption resins to increase recovery efficiency.

2. Materials and Methods

Mexican lime fruits, which originated from the region of Tecomán, Colima, México, were purchased at a local market (*Mercado de Abastos*, Jalisco, México) in February. Standard hesperidin was purchased from Sigma-Aldrich (>90%). The ethanol for extraction had a purity of 96%. DMSO (dimethylsulfoxide) had a purity of 90% (Karal). The solvents used for analysis were HPLC grade.

The resins used were Relite EXA118 (Mitsubishi Chemical, Italy) and Amberlite™ FPX66. Their main characteristics are shown in Table 1.

Table 1. Main Characteristics for Amberlite FPX66 and Relite EXA-118.

Resin	Amberlite FPX66	Relite EXA-118
Matrix	Non-functionalized macroreticular aromatic polymer	Styrene and divinylbenzene copolymer with high degree of hydrophobicity
Appearance	White spheres	Light brown translucent spheres
Superficial area (m^2/g)	≥700	1200 aprox.
Porosity (mL/g)	≥1.4	2.3
Particle size (mm)	0.6 - 0.75	0.3 - 0.71
Specific gravity (g/mL)	1.015 - 1.025	1.01

2.2. Hesperidin content

To quantify flavonoids in *Citrus* peels, the total hesperidin content in the Mexican lime peel was determined based on the procedure proposed by Nogata et al. (2006). Three limes were squeezed and 1 g of peel was taken from each. Each sample was dried at 70°C in a thermobalance (AND) and ground in a mortar. Next, 100 mg of each sample were placed in individual test tubes, where three consecutive 24-h extractions were performed. Each extraction was done with 1.5 mL of 1:1 v/v solution of methanol and dimethyl sulfoxide (DMSO). The three extracts were combined and the concentration of hesperidin was determined by HPLC.

2.3. Extraction

2.3.1. Lab-scale extraction

There are many factors that affect extraction yield. In this work, we chose to study the solid-to-solvent ratio and the solvent composition. The remaining operating conditions were selected based on previous studies of hesperidin extraction from Persian lime peel [18,19].

Extraction was performed in 250-mL Erlenmeyer flasks, at 50°C for 4 h, with 120 rpm agitation in an orbital shaking incubator (New Brunswick G25). Fresh peel was used as the extraction material, and the solvent volume was 50 mL. At this stage of the process, 2 kg of Mexican limes were washed to remove impurities. They were then squeezed and the seeds were manually removed. Finally, the peel was ground up in a Moulinex blender, until the particle size was less than 1 cm.

The solid-to-solvent ratio and the solvent composition were varied according to the 2^3 experimental design (with two central points) and were duplicated. The central points were added to observe the linearity of the response variable. The controlled variables were the percent ethanol in the solvent (0, 60), the percent DMSO in the solvent (0, 20) and the solid-to-solvent ratio in g/mL (0.1, 0.33). The response variables were the concentration of hesperidin in the extract and the extraction yield.

The use of DMSO was proposed because hesperidin has processing difficulties due to its low solubility in water (<20 mg/L) [20]. Its solubility is greatly increased in DMSO, at a concentration of 122 mg/mL [21]. DMSO is considered to be a green solvent and is one of the least toxic organic chemicals known [22]. Because it has low chronic and acute oral toxicity, it offers a delivery option for difficult-to-dissolve medications [23].

The extracts were separated from the exhausted solids with a strainer and stored at 8°C in amber containers. The concentration of hesperidin in the extracts was determined by HPLC, and the yields were calculated. Statistical analysis was conducted with STATGRAPHICS Centurion XVI®.

2.3.2. Pilot-scale extraction

The same conditions that were selected from the lab-scale extraction stage were used in the pilot scale. Waxes and impurities were eliminated by washing 50 kg of Mexican limes with water. Next, the fruit were fed through a screw press operated at a frequency of 30.0 Hz. to separate the juice and some of the seeds from the peel. The remaining seeds were removed manually. The peel was ground in an industrial blender with a 5 L capacity (International) until the particle size was smaller than 1 cm. The juice and the seeds were reserved in refrigeration for other research work. Three extractions were made on the peel. Each extraction was performed using 20 L of solvent for 3 h at 50°C. A pilot extraction tank with stirring and temperature control was used. The extracts from each run were combined and filtered through a polypropylene cartridge of 1 μm in a Serfilco Labmaster unit. Finally, the extract was concentrated in a Büchi R-220 rotavapor at 45°C and 500 mmHg. The filtered and concentrated extract was refrigerated at 8°C.

2.4. Purification

Resins FPX66 and EXA-118 were chosen to evaluate their capacity to adsorb hesperidin from the extract. The resins were activated by an overnight treatment with 2 bed volumes (BV) of 96% ethanol, and then were rinsed with 5 BV of deionized water before use. Once pretreated, the moisture content of the resins was determined in a thermobalance AND at 70°C (for dry weight calculations). To improve the solubility of hesperidin and ensure a homogeneous distribution of the solution, 10% DMSO was added to the extract.

2.4.1. Static tests

Adsorption kinetics

The concentration of hesperidin in batch tests was monitored to evaluate the time for adsorption equilibrium. Adsorption was performed by adding 1.7 g of resin (dry weight) to 50 mL of extract in a flask at 25°C. The mixture was stirred (150 rpm in an orbital New Brunswick G10 shaker) for 5 h. Samples of the extract (1 mL each) were taken at 0, 20, 40, 60, 90, 120, 180, 240 and 300 min and were analyzed for total flavonoids (see section 2.6). The analysis for total flavonoids was chosen because the test comprises of a simple colorimetric method and because hesperidin accounts for most of the flavonoids present in the extract.

Adsorption efficiency

A multifactorial experimental design (6x2x2) was used to evaluate adsorption efficiency. The experiments were performed in duplicate. The variables were the resin type (FPX66 and EXA-118), the temperature (25 and 40°C), and the initial concentration of hesperidin (six levels, where the extract was diluted with the 10% DMSO solution).

Adsorption was performed by adding 1 g of resin (dry weight) to 30 mL of extract in a flask. The temperature was 25°C and mixture was stirred (150 rpm in New Brunswick G25 shaker) for 3 h. The final concentration was measured by HPLC and the results were analyzed using the variance analysis with the Statgraphics Centurion XVI software®.

2.4.2. Dynamic tests

A fixed bed with 80 mL of resin was used to evaluate the dynamic adsorption and desorption of hesperidin from the diluted extract of Mexican lime peel. The temperature, initial hesperidin concentration (dilution) and resin were selected from the static tests. Ascending flow was used and this was done for both operations. The purification operation was performed in cycles comprising the following steps:

1. First wash: The column was washed with 1 L of deionized water (5 mL/min).
2. **Adsorption**: 1 L of diluted extract was passed through the column (5 mL/min). Samples at 25 mL, 50 mL and 100 mL were collected at the exit of the column, until the whole volume had been treated.

3. Second wash: The column was washed with 250 mL of deionized water (5 mL/min) to remove any remaining extract (water does not desorb the hesperidin from the column).
4. **Desorption**: 1 L of ethanol 96% was passed through the column (2.7 mL/min) to recover the adsorbed hesperidin. Samples at 50 mL and 100 mL were collected until there was no volume of ethanol left in the column.

The evaluation of the purification process consisted of three complete cycles. All of the collected samples were analyzed by HPLC.

2.5. HPLC analysis

High performance liquid chromatography (HPLC) was used to determine hesperidin content in all of the samples. Each sample was filtered with a 0.45 µm syringe filter and 10 µL were injected to the unit. The chromatograph was a Varian Prostar and the column used was the Phenomenex Geminic C6-phenyl 150x4.5 (5 µm). The mobile phase consisted of water (40% acidified with 0.1% acetic acid) and methanol (60%), and it had a flow rate of 1 mL/min. The equilibrium time was 3 min, and the analysis time was 7 min. Identification was done with a UV detector at 280 nm. All solvents were filtered through a 0.45 µm membrane and sonicated for 20 min.

2.6. Total flavonoids

To quantify the total flavonoids, a colorimetric method was used, which consisted of taking 250 µl of the extract sample and diluting it in 1.25 mL of distilled water. Next, 75 µl of 5% $NaNO_2$ solution was added and the sample was left to stand for 6 min. Subsequently, 150 µl of 1 M NaOH solution and 775 µl of distilled water were added obtain a final volume of 3 mL. The sample was stirred in a vortex mixer, and the absorbance was immediately measured at 510 nm in a Thermo Genesys 10 UV spectrophotometer [24].

3.Results and discussion

3.1. Hesperidin content

Three limes were randomly selected, and a fraction of their peels was dried. The total hesperidin content was determined by extraction using a mixture of 50% ethanol and 50% DMSO. The average concentration of the extracts, moisture content and dry and wet weight calculations are shown in Table 2 (quantified by HPLC).

Table 2. Total hesperidin content in Mexican lime peel.

Extract concentration (mg/mL)	0.078 ± 0.021
Dry peel concentration (mg/g)	3.528 ± 0.962
Moisture content (%)	81.57 ± 1.92
Fresh peel concentration (mg/g)	0.653 ± 0.208

The standard deviation of the hesperidin content in Table 2 shows that there is significant variability between the samples. Other authors have reported a content of 1.97 mg/g in a fresh peel of *Citrus aurantifolia* Swingle [4]. By comparison, we obtained a substantially lower concentration of 0.65 mg/g. These results indicate that important differences can be observed among fruit of the same species. Factors such as growing conditions (e.g., weather, soil type and irrigation), harvest time, storage conditions and size influence the flavonoid content, which subsequently hinders the appropriate contrast of the results obtained in each study [25,26]. On the other hand, moisture content had a low impact on the variation, and the average value was similar to previous reports [27].

3.2. Extraction

3.2.1. Lab-scale extraction

The main objective was to implement a green process to recover the hesperidin content in citrus peels. The first stage of the selected process was solvent extraction. This is because prior to the adsorption process, it is possible (and highly desired) to eliminate the solvent from the extract by evaporation, recover it and reuse it in the extraction step. The first step was to evaluate (at lab-scale) the influence of the solid-to-solvent ratio and the solvent composition over two response variables: the concentration of hesperidin in the extract and the extraction yield.

The behavior of the first response variable is depicted in Figure 2. The variance analysis proved that the three studied factors significantly affected ($p<0.05$) the content of hesperidin in the extracts.

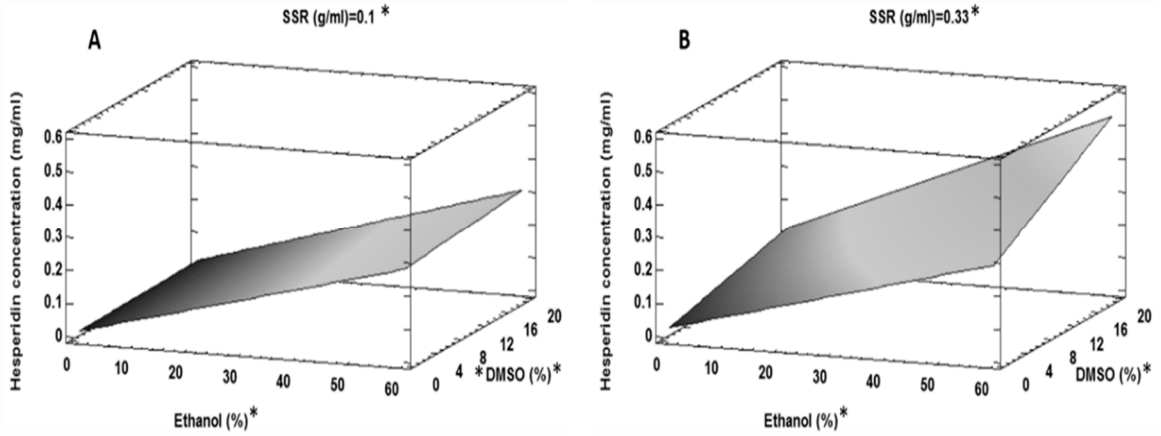

Figure 2. Concentration of hesperidin in the extract for a solid-to-solvent ratio (SSR) of 0.1 g/ml (A) and 0.33 g/ml (B). *Significant factors in the variance analysis ($p<0.05$).

The interaction between the solvent-to-solid ratio (SSR) and the DMSO percentage was also significant. It can be noted from Figure 2 that higher levels of the three factors (SSR=0.33 g/mL, 60% Ethanol, 20% DMSO) maximized the hesperidin concentration, with a value of approximately 0.5 mg/mL. A remarkable difference between Figures 2A (SSR=0.1 g/mL) and 2B (SSR=0.33 g/mL) is the effect of the DMSO concentration. At a DMSO content of 0%, the hesperidin concentration remains similar in both figures (approximately 0.3 mg/mL). However, at a 20% DMSO content in the solvent, the hesperidin concentration is greatly increased when the SSR is 0.33 g/mL, compared to the SSR of 0.1 g/mL, where the change is subtler. This indicates that when the SSR is lower (0.1 g/mL), an aqueous solvent with 60% ethanol is adequate to extract almost all of the hesperidin. Meanwhile, when the SSR is 0.33 g/mL, the same solution becomes saturated and the addition of 20% of DMSO increases the ability of the solvent to extract more hesperidin. Finally, the central points displayed a linear response of the hesperidin concentration of the extract.

The extraction yield was calculated as 'mg of hesperidin per gram of fresh peel', and a variance analysis was performed. The results from ANOVA showed that the percent Ethanol and SSR significantly affected the extraction yield ($p<0.05$). The behavior of this response variable is shown in Figure 3. It is noted that an SSR of 0.1 g/mL and 60% Ethanol, maximized the extraction yield, thereby obtaining a value of approximately 3 mg/g. The fact that the extraction yield is lower for higher SSR at 60% ethanol, confirms that the solvent was getting saturated. It is also evident that the DMSO percentage by itself did not have an important effect on this variable. Nonetheless, the results from ANOVA suggest that the interaction between the SSR and the %DMSO was significant. However, the effect of this interaction is much less evident for the extraction yield than the concentration of hesperidin in the extract. The response of this variable was also linear, as tested with the central points of the experimental design.

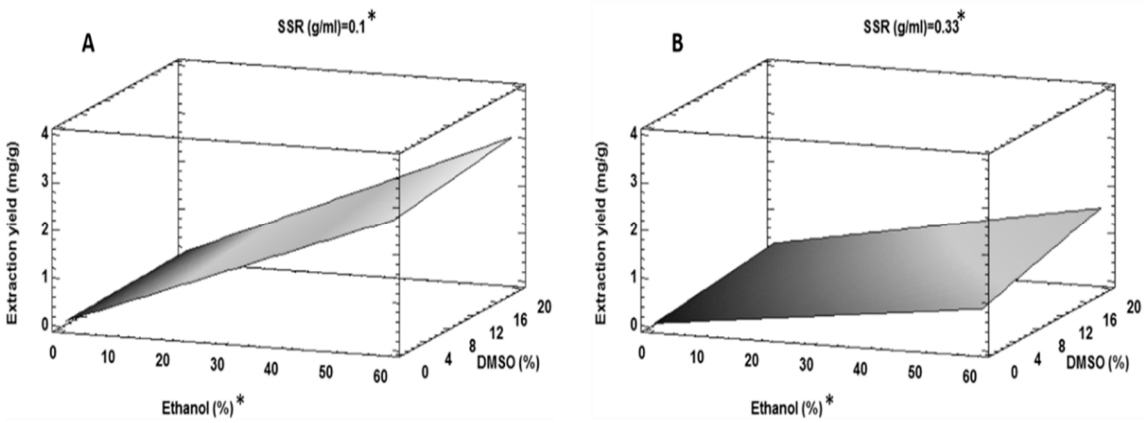

Figure 3. Extraction yield for a solid-to-solvent ratio (SSR) of 0.1 g/ml (A) and 0.33 g/ml (B). *Significant factors in the variance analysis ($p<0.05$).

These results obtained from the laboratory are of high relevance for the selection of operating conditions at an industrial level. This selection has to be carefully made, and it depends on the objectives and a cost-benefit analysis. For example, the highest extraction yield possible (if the amount of peel available is limited) could be obtained, despite requiring higher amounts of solvent, which would result in having a lower concentration of the extract. On the other hand, lower quantities of solvent can be used on an unlimited peel resource to obtain hesperidin, even though not all of the hesperidin can be collected.

In this particular study, even though the extraction yield was not optimal, we wanted to obtain a higher concentration of hesperidin in the extracts for the adsorption purification step. This step employed the use of an SSR of 0.33 g/mL, 60% ethanol and 20% DMSO. However, the use of DMSO implies its recovery through vacuum evaporation and it represents an increase in the cost and time of process. For that reason, we decided to do the extraction without DMSO and to use it only before the resin adsorption process to increase the solubility of hesperidin and facilitate its adsorption.

3.2.2. Pilot-scale extraction

The results obtained from the lab-scale extraction step were used in the 20 L pilot scale extraction, where 6.6 kg of peel, 12 L of 96% ethanol and 8 L of deionized water were used. Extract samples were taken every 20 min. Figure 4 shows the mean concentration from the three extractions. It is observed that from 0 to 100 min, the hesperidin concentration increased to a maximum concentration, followed by a slight decreasing trend. The average final concentration was 0.136 g/mL.

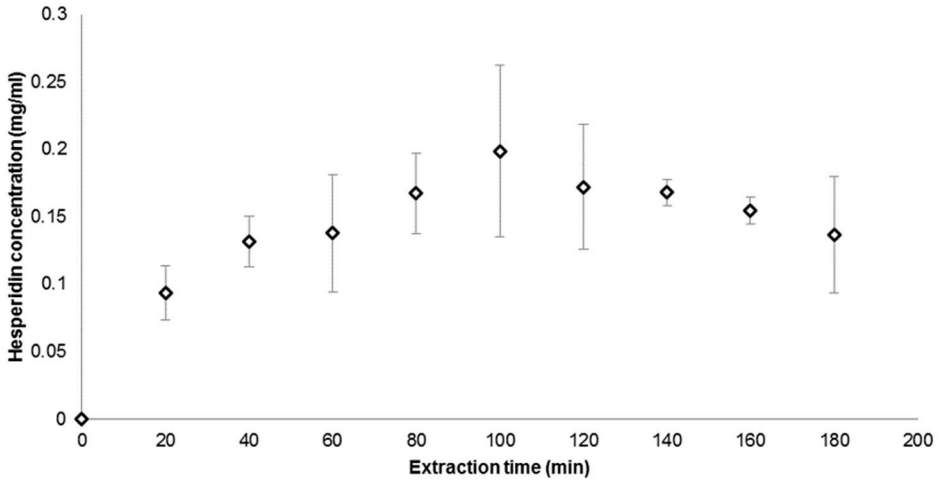

Figure 4. Pilot scale extraction kinetics.

The efficiency of the hydroalcoholic extraction was 2.25 mg/g of dry peel. Once the extract was filtered and concentrated, a hesperidin concentration of 0.303 mg/mL was achieved.

The proposed extraction conditions require a low concentration of alcohol (60%). To reduce energy consumption, this process was done at a reduced temperature of 50 °C for an extraction time of 1 h and 40 min.

It is very common to use milling and drying (hot air or freeze drying) as conditioning steps for polyphenol extraction processes [2]. However, freeze drying is an expensive process, which prevents from using it at an industrial scale, specially for high water content products such as citrus fruits [28,29]. Hot air drying is cheaper, but it has the disadvantage of exposing the sample to heat and oxygen for extended periods of time [2]. This is why simpler and more economic methods are preferred nowadays.

There are few studies approaching the extraction of bioactive compounds of fresh and untreated citrus wastes, either due to the difficulty of having an homogenous particle size or because the water promotes enzymatic reactions. However, if it is processed immediately this problem is avoided and costs are lowered by eliminating the drying operation and reducing the processing time [30]. Besides, the mass transfer in liquid-solid extractions involves the use of dry and grinded material, which shortens the extraction time. The particle size can be controlled when fresh material is used, as in the case of polyphenol extraction from oranges, which can controlled using calibrated steel cubes [30].

Therefore, it is relevant to state that prior drying of the peels was not required in the process proposed in this study, which translated into shorter operation times. This process makes use of fresh peels from the citrus juice industry that exit the pressing operation.

3.3. Purification

3.3.1. Static tests

The aqueous extract was diluted with 10% DMSO and reached a hesperidin concentration of 0.272 mg/mL. This extract was used in the static tests.

Adsorption kinetics

In order to perform fast adsorption experiments with both resins in further steps, we determined the equilibrium time using a quick method measuring absorbance of total flavonoids. The adsorption kinetics was investigated by allowing the resin to be in contact with the diluted extract for 5 h. In the case of both resins (FPX66 and EXA-118), the concentration decreased rapidly in the first 50 min. As seen in Figure 5, the adsorption kinetics for EXA-118 resin shows that almost all of the flavonoids were adsorbed in this time frame. Regardless, 180 min was established as an appropriate time to ensure that the adsorption efficiency test reached the equilibrium state. This equilibrium time is in accordance with several works of flavonoid adsorption using resins, were equilibrium times ranging from 60 to 200 minutes haven been reported [31–34].

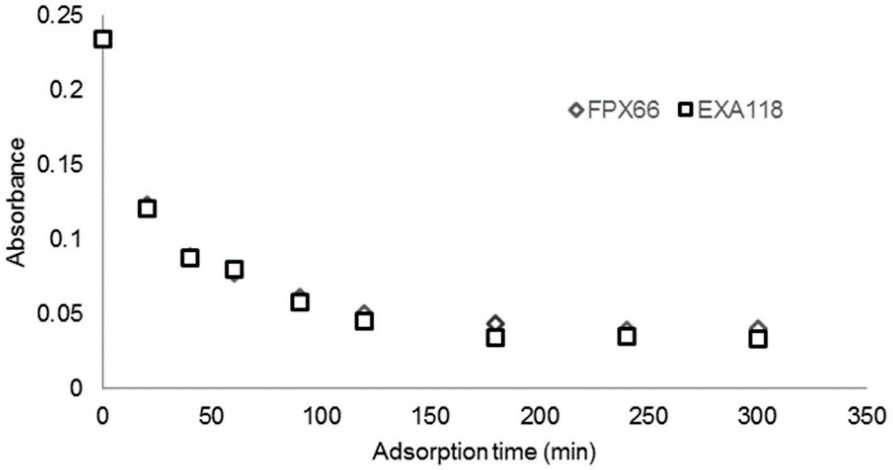

Figure 5. Adsorption kinetics of total flavonoids for EXA-118 and FPX66 resins.

Adsorption efficiency

To investigate the effects of the variables (resin, temperature and initial concentration) for adsorption efficiency, a variance analysis was performed. Six initial concentrations, shown in Table 3, were used. The dilutions were made with a 10% DMSO solution.

Table 3. Initial concentrations for the adsorption efficiency tests.

Dilution	Hesperidin concentration (mg/mL)
0	0.273
1	0.218
2	0.164
3	0.109
4	0.054
5	0.027

The hesperidin concentration was measured after equilibrium was reached (in approximately 3 h). The adsorption efficiency was calculated according to the following equation (1):

$$\varepsilon = \frac{C_0 - C_e}{C_0} \quad (1)$$

where C_e is the concentration of hesperidin at equilibrium (mg/ml), C_0 is the initial concentration of hesperidin, and ε is the efficiency.

The results showed that the type of resin and the initial concentration of the extract have a significant effect over the efficiency ($p<0.05$). Resin EXA-118 had a higher efficiency, which can be attributed to it having a higher superficial area. Based on the initial concentration, 4 homogeneous groups were found. On average, dilutions 4 and 5 exhibited higher average adsorption efficiencies.

3.3.2. Dynamic tests

Adsorption

From the results of the static tests, resin EXA-118 and 25°C temperature, were selected. Dilution 3, with an initial concentration of 0.109 mg/ml, was used in place of dilutions 4 and 5 despite having better efficiencies. This is because in an industrial operation with adsorption columns, the use of dilutions 4 and 5 may result in a higher operation time and higher DMSO requirements.

During the test, 1 L of diluted extract was passed through the column and all of the samples taken at the exit of the column did not show peaks in the chromatograms. This led to the conclusion that 100% of the hesperidin in the extract was adsorbed. This confirmed that the selected dilution was suitable for this operation. A 98% adsorption from model solutions of hesperidin at 40°C has been

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reported elsewhere [17]. In this work, adding DMSO to the extract helped achieve an even better adsorption capacity from a more complex mixture, and a 25°C temperature, which is an easier temperature to attain in an industrial operation, was proved to be equally useful.

Desorption

To recover the adsorbed hesperidin, 1 L of 96% ethanol was passed through the column. Only the four first collected fractions (50 mL each) at the exit of the column showed peaks during the chromatographic analysis. As a result, it took only 200 mL of ethanol to desorb and recover the hesperidin from a 1 L sample of extract, which had an initial concentration of 0.109 mg/mL. Figure 6 shows the recovery efficiency (mg of hesperidin recovered/mg of hesperidin adsorbed) achieved based on the volume eluted from the column.

The average recovery efficiency was almost 90%, and approximately 68% of the recovered hesperidin was in the first 100 mL that exited the column. The efficiency of purification in basis to the purity calculated from the chromatographic areas and concentrations of the extract and the fraction recuperated in the resins was of five.

It has been reported that the extraction of hesperidin is possible due to a conformation change of hesperidin to its anionic form (anion polyphenolate) at basic pH conditions [16]. The effect of the amount of $Ca(OH)_2$ over the extraction efficiency of hesperidin from orange peel has been previously studied, where the extracts were purified with the resin Kastell S-112, obtaining recoveries above 90%, using 0.5 N NaOH and 10% ethanol solutions, and recovering most of the hesperidin in the first 100 mL [16]. In this work it was possible to purify hesperidin with a similar high efficiency without the addition of $Ca(OH)_2$ or NaOH, showing that the separation of hesperidin is possible in acid conditions, with a process that reduces the economic and ecologic cost of the traditional alkaline treatment and that only uses solvents that can be recovered and reused. However, since all the hesperidin was absorbed, there is still opportunity to improve the 90% recovery efficiency attained in this work. This could be achieved by evaluating other desorption conditions, such as other solvents or mixtures of solvents, flow, temperature, etc.

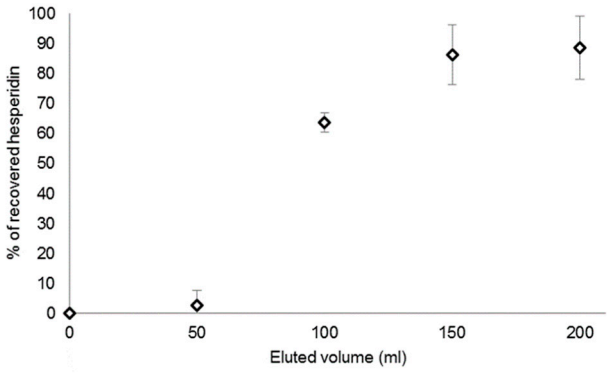

Figure 6. Hesperidin recovery efficiency during desorption.

Also, it is important to state that most of the literature about the extraction and purification of bioactive compounds are focused on orange (*C. Sinensis*), lemon (*C. Limon*), grapefruit (*C. Paradisi*), mandarin (*C. reticulata*) and there are very few studies focused on studying Mexican lime (*Citrus aurantifolia* Swingle) which is an important citrus fruit worldwide.

Finally, Figure 7 shows the general conditions of the method proposed in this work to extract and purify hesperidin from Mexican lime peel, and that could be extended to other citrus fruit.

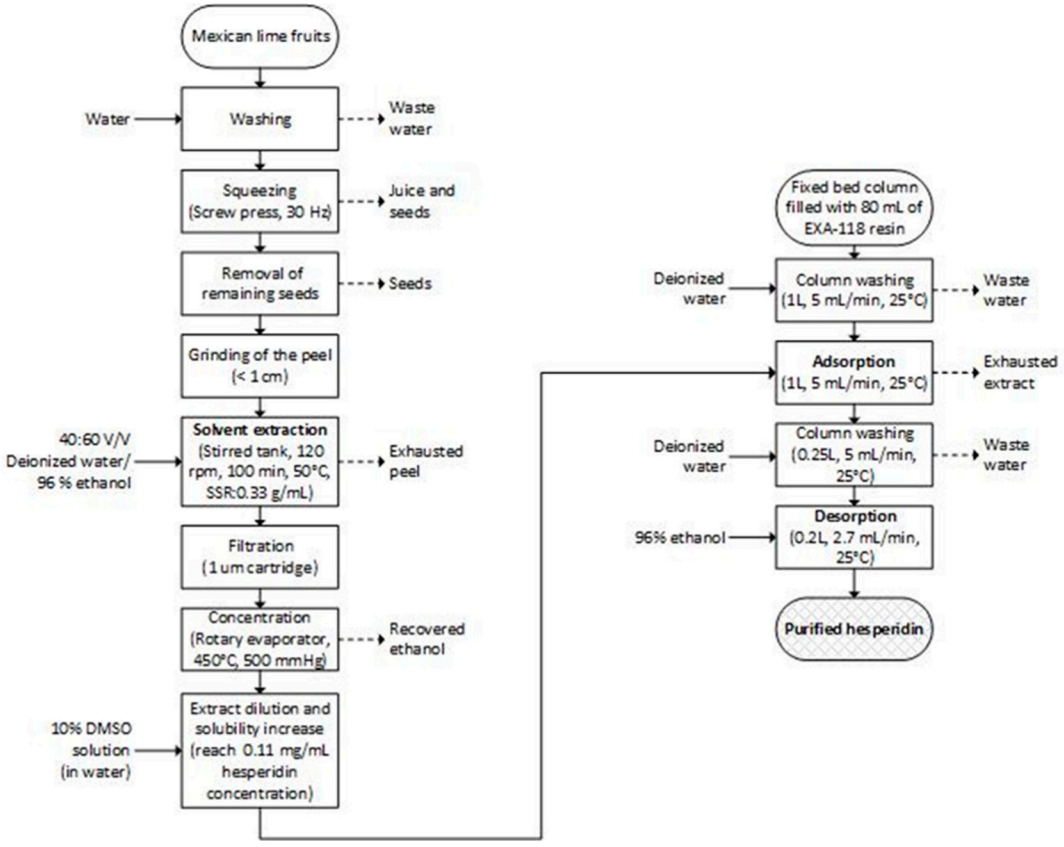

Figure 7. Proposed method by the authors, for the extraction and purification of hesperidin from Mexican limes peel.

4. Conclusions.

The purification process of hesperidin from Mexican Lime peel, comprising a hydroalcoholic extraction and a purification using a packed bed with resin EXA-118, presents advantages over the traditional alkaline process, since it does not require the use of acids and basis that represent an important environmental and economical cost. Therefore, this process offers an excellent alternative for its implementation on an industrial scale as a green technology. Based on the reduced number of unit operations (extraction, evaporation, filtration, adsorption and desorption), this process has been characterized for its simplicity and economic advantages.

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