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Highlights

- Rutin extraction from banana peels was assessed using organic acids.
- COSMO-RS estimated rutin solubility in organic acids (neat and hydrated).
- Hydrated acetic acid mixtures were selected as the best solvent.
- Solvent recovery and reuse had no significant impact on rutin extraction efficiency.
Valorizing banana peels by extracting rutin with hydrated organic acids

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Abstract

Bananas are a popular fruit worldwide and the fourth most important food crop. Banana peels, often discarded, contain valuable compounds known for their relevant biological activities, including rutin. In this study, organic acids, both in their pure and hydrated forms, were used as alternative solvents for rutin extraction. First, we assessed the ripeness of the bananas (green, semi-ripe and ripe), finding that ripe banana peels contained the highest rutin content. Then, COSMO-RS, a thermodynamic model based on quantum chemistry, was applied to determine the best organic acids for extraction, which were subsequently evaluated experimentally, with a 65 wt% acetic acid-water mixture yielding the best results (192 mg rutin/100 g DW). Next, extraction conditions were optimized using response surface methodology (RSM), resulting in 241 mg rutin/100 g DW at 27 ºC, 80 min, and a solid-to-liquid ratio of 1:15. Finally, it was observed that the solvent could be reused for two cycles under optimal operating conditions without a significant decrease in rutin extraction efficiency. This study demonstrates the potential of mixtures of hydrated organic acids for the extraction of value-added compounds from natural sources, offering a solution to reduce food waste while contributing to a circular economy.

Keywords: Solid-liquid extraction; food waste; alternative solvents; acid ethanoic; aqueous solutions; process optimization.
1. Introduction

Rutin, also known as vitamin P, is a flavonoid that possesses a range of health benefits attributed to its antioxidant, antimicrobial, antiallergic, and antifungal properties (Prasad & Prasad, 2019). While it can be found in more than 70 plant species (Gullón et al., 2017), its concentration varies considerably depending on the source of the biomass. In most cases, the rutin content in these sources is not very high, which makes it challenging to develop a large-scale process. Therefore, it is essential to identify rutin-rich sources that are readily available in substantial quantities and at a low cost in order to establish a viable industrial-scale process.

Bananas are widely consumed, and are the fourth most important food crop globally. Annually, over a staggering 105 million tons of bananas are produced, with Ecuador and the Philippines emerging as top banana-producing nations. Bananas with commercial interest belong to the Musaceae family and the Musa genus, with the Dwarf Cavendish species being particularly favored worldwide. Beyond their popularity, they offer a range of value-added compounds, such as dopamine, rutin, naringin, serotonin, tyramine, and histamine, their composition varying with factors such as species, location, production environment, and ripeness (Borges et al., 2019).

In addition to the fruit, the peels of bananas also contain valuable biocompounds. Zhang et al. (2019) demonstrated that it was possible to extract rutin from banana peels using a 95% ethanol solution, but before the extraction the authors boiled the biomass in water for 5 min to deactivate polyphenol oxidase. However, they only carry out the extraction and identification of compounds without quantifying the rutin content achieved in this process. Behiry et al. (2019) extracted 973 mg/100 g dry extract of rutin from banana peels by maceration and use of methanol as solvent for 3 days in a solid:liquid ratio of 1:4 rutin, showing them to be a promising source of rutin. Although the peels make up
to 40% of the total weight of fresh bananas, they are often overlooked and considered disposable - food waste. Thus, banana peels have the potential to become an example of transforming a disposable item into a value-added product, while contributing to the valorization of food waste within the framework of an integrated biorefinery and circular economy (Martínez-Inda et al., 2023).

Conventional methods such as Soxhlet extraction are preferred for the extraction of rutin (Gullón et al., 2017), but more innovative methods such as ultrasound- and microwave-assisted extraction and high-pressure extraction have also been used (Ameer et al., 2017; Kraujalis et al., 2015; Nam et al., 2015). These extraction methods have some disadvantages, including low efficiency, time-consuming and potential degradation of the extracted compounds, which are mainly associated with conventional methods such as Soxhlet extraction, and low selectivity and high energy costs, which are more related to the innovative methods (Gullón et al., 2017). Moreover, rutin extraction has been mainly performed with organic solvents such as methanol and ethanol (Gullón et al., 2017), from different types of banana (Behiry et al., 2019; Passo Tsamo et al., 2015; Yingyuen et al., 2020; Zhang et al., 2019). Therefore, more sustainable and efficient extraction solvents need to be identified.

Previous works by us and others have revealed the promising potential of organic acids and their water mixtures for the extraction of biocompounds (Benfica et al., 2020, 2021; Makris, 2017; Pappas et al., 2021; Tzima et al., 2015). Benfica et al. (Benfica et al., 2020, 2021) have shown that hydrated organic acids can provide yields similar to organic solvents in the extraction of L-dopa from Mucuna pruriens seeds, but with the advantage that hydrated organic acids are more selective in the extraction. This improved selectivity can be attributed to the tendency of water to enhance the selectivity of solvents (Ventura et al., 2017). Thus, the use of hydrated organic acids contributes to
the development of an extraction process that is both efficient and environmentally sustainable. However, it is important to note that further research is required in this area as it is still at an early stage. Various organic acids, such as lactic acid (E 270), acetic acid (E 260), citric acid (E 230), and tartaric acid (E 334), are approved as food additives (European Food Safety Authority, 2013), and are considered toxicologically safe. Moreover, they can function as highly efficient pH regulators for the preparation of mild acidic conditions due to their relatively low pK\textsubscript{a} values.

The COSMO-RS (Conductor-like Screening Model for Real Solvents) has been suggested as a fast alternative to the lengthy experimental screening traditionally employed for the selection of solvents (Jaapar et al., 2013; Jacotet-Navarro et al., 2018; Jeliński & Cysewski, 2018; Popović et al., 2022; Wojciechowski et al., 2020, 2021). Specifically, COSMO-RS can be used as a predictive tool for assessing the solubility of a target compound across various solvents. This approach has demonstrated its efficacy in numerous studies, as evidenced by the literature. COSMO-RS has demonstrated its efficacy in predicting the solubility of value-added compounds from rosemary in ethanol:water mixtures (Jacotet-Navarro et al., 2018) and deep eutectic solvents (Wojciechowski et al., 2020, 2021), ginger in hot water (Jaapar et al., 2013) and rutin in deep eutectic solvents (Jeliński & Cysewski, 2018; Popović et al., 2022). These studies highlight the applicability and reliability of COSMO-RS in predicting solubility behavior, offering valuable insights into solvent selection for extraction processes.

The aim of this study is to extract rutin from banana peels using organic acids, both in their pure and hydrated form. This study started with the determination of the most suitable ripening stage of banana peels to maximize the content of rutin, the desired compound. Then, COSMO-RS was applied as screening tool for the selection of the most promising solvents, also considering the influence of the water content of the
organic acids on the solubility of rutin. The best organic acids identified during the initial screening were then employed for the extraction of rutin from banana peels to establish the most efficient organic acid and its concentration. After, the extraction conditions for rutin, including temperature, extraction time, and solid:liquid ratio (biomass:solvent), were optimized using Response Surface Methodology (RSM). Finally, the solvent recovery and reuse were assessed under the optimal operating conditions for rutin extraction. This work is intended to contribute to the further development of sustainable and economically viable extraction processes for value-added compounds from food waste, in line with the goals of the United Nations 2030 Agenda to promote more sustainable practices.

2. Materials and Methods

2.1. Materials

The chemical compounds used in this work were: citric acid (CAS 77-92-9, purity: 99.5%) supplied by Panreac; acetic acid (CAS 64-19-7, purity: PA), methanol (CAS 67-56-1, purity: 99.8%), ethanol (CAS 64-17-5, purity: 99.0%) and acetonitrile (CAS 75-05-8, purity: 99.9%) acquired from Fisher Scientific; glycolic acid (CAS 79-14-1, purity: 99.0%) supplied by Sigma-Aldrich, lactic acid (CAS 79-33-4, purity: 90.0%) obtained from Riedel de Haen; rutin (CAS 250249-75-3, purity: 97.0%) acquired from Alfa Aesar; trifluoroacetic acid (CAS 76-05-1, purity: 99.5%) supplied by Acros organics. Ultra-pure water was employed for all experiments, subjected to double distillation, processed through a reverse osmosis system, and further purified using a Milli-Q plus 185 water purification device.
2.2. Biomass collection and selection

For the experimental study, green bananas (*Dwarf Cavendish* - *Musa acuminata Colla var cavendish*) from Madeira, Portugal, were acquired from a local market in Aveiro, Portugal. The bananas were divided into three equal parts for further processing. In the first portion, one-third of the bananas were peeled immediately, and peels were promptly frozen at -20°C. For the second portion, another one-third of the bananas were allowed to ripen until they reached a semi-ripe state, then peeled and the peels frozen. Finally, the remaining one-third of the bananas were left to fully ripen before peeling and subsequent freezing of the peels. The color of the banana peels determined the degree of ripeness, with green, green-yellow, and yellow banana peels being classified as semi-ripe and ripe, respectively. Before extraction, the banana peels at their respective desired stage of ripeness were immersed in liquid nitrogen. Following this, the frozen peels were ground into a fine “powder” utilizing a coffee grinder.

To select the optimal degree of ripeness for extracting rutin from banana peels, a solid-liquid extraction was performed for each type of banana peel (green, semi-ripe, and ripe). The extraction was performed using a Carousel apparatus from Radleys Tech, chosen for its ability to maintain a constant temperature and agitation. Ethanol was employed as solvent with a solid:liquid ratio of 1:10 (which means mass of banana peels per mass of solvent), at a temperature of 25 ± 1°C and a rotation speed of 300 rpm, during 60 min. The mixtures were accurately prepared on balance with a precision of ± 10^-4 g.

After extraction, the solvents were separated from the biomass via centrifugation (at 5600 rpm for 10 minutes using an Eppendorf 5804 centrifuge), and the resulting supernatant was filtered through 0.45-μm syringe filter. At least three independent
extractions were prepared and quantified, allowing for the determination of statistical parameters such as the mean and standard deviation of the extracted rutin content.

2.3. Quantification of rutin by HPLC-DAD

Rutin was quantified using high performance liquid chromatography (HPLC), utilizing an HPLC chromatography system (VWR Hitachi) equipped with a diode array detector (DAD). HPLC analyses were carried out using a reverse-phase analytical column C18 (250 × 4.60 mm), specifically the Kinetex 5 μm C18 100 Å column from Phenomenex. The mobile phase employed consisted of a gradient system, combining ultrapure water with 0.2% (v/v) trifluoroacetic acid (referred to as phase A) and acetonitrile with 0.2% (v/v) trifluoroacetic acid (referred to as phase B). Prior to use, both phases were degassed using ultrasound. The separation process followed this gradient profile: at 0 minutes, 90% of phase A; at 4 minutes, 0% of phase A; at 10 minutes, 90% of phase A; and maintained at 90% of phase A for the subsequent 20 minutes. The flow rate was set at 0.8 mL/min, with a 20 μL injection volume was employed. The column oven was maintained at 25°C, while the automatic injector operated at 10°C. The DAD was configured to a wavelength of 355 nm.

The calibration curve was established by dissolving the commercial standard, rutin, in a methanol-water mixture at a concentration of 50 wt%. The rutin calibration curve ranged from 0.008 to 0.165 mg/ml with an $R^2$ of 0.999. The retention time of rutin under the above conditions was 5.7 min.

The rutin content was calculated by dividing the weight of quantified rutin in the extract by the total weight of dry banana peels used. The results were expressed in milligrams of rutin per 100 grams of dry weight of biomass (mg/100 g DW). To determine the dry
weight of the banana peels, the water content of the biomass was assessed, which was 12 ± 2 wt.%.

**2.4. In silico solubility prediction of rutin using COSMO-RS**

COSMO-RS is a predictive model introduced by Klamt *et al.* (1998) that combines principles from quantum chemistry (Conductor-like Screening Model, COSMO) with statistical thermodynamics (Real Solvents, RS) (Catena *et al.*, 2020). In its first calculation stage, the solute under evaluation is embedded in a virtual conductor, performing the quantum chemistry simulation. The second one is the macroscopic step, resulting in the probability distribution with a specific charge density of a molecular area, *i.e.*, the σ-profile. In this study, we conducted geometry and charge density optimizations for all individual compounds using density functional theory (DFT) calculations, implemented via the TmoleX software (Steffen *et al.*, 2010), following the procedures outlined in a previous work (Wojeicchowski *et al.*, 2020).

After determining the geometry and σ-profile of each compound, we employed COSMOthermX (Eckert & Klamt, 2002) with the BP_TZVP_19.ctd parametrization to predict the relative solubilities of rutin in various solvents (both pure and hydrated) at a temperature of 25 °C. In this methodology, COSMOtherm calculates the logarithm of relative solubility between the solid compound (rutin, at infinite dilution) and the liquid solvent \( \log_{10}(x_S) \). The logarithm of the relative solubility \( \log_{10}(x_{RS}) \) in the solvent with the highest solubility value established as 0, and all other solvents were expressed relative to this reference solvent (Catena *et al.*, 2020; Yara-Varón *et al.*, 2016).

Additionally, the logarithm values of relative solubility were converted to the probability of solubility in percent \((\text{probability} \%)\) according to equation 1:
where, $x_{RS}$ is the relative solubility of rutin in the organic acid, $x_{RS \text{ maximum}}$ and $x_{RS \text{ minimum}}$ are the maximum and minimum values, respectively, of the relative solubility of rutin in all the acids studied. The probability of solubility values were categorized as follows: i) low probability of solubility (0-20%); ii) moderate probability of solubility (20-60%); and iii) high probability of solubility (60-100%).

2.5. Solid-liquid extraction: Influence of different organic acids in the rutin extraction

To extract rutin from banana peels, we employed various organic acids and their corresponding water mixtures. The solvents included acetic acid, citric acid, glycolic acid, and lactic acid, all used at concentrations of 100 wt%, 75 wt%, 50 wt% and 25 wt%. However, it's worth noting that citric acid and glycolic acid couldn't be used in their pure form due to their solid-state nature at room temperature. For comparison purposes, extractions were also performed with conventional solvents, namely methanol, ethanol, and water. Additionally, water acidified by adding 1 M hydrochloric acid at a pH of approximately 3 was included as another extraction solvent. Extractions were carried out using a Radleys Tech carousel according to the procedure described above. The subsequent treatment of the samples after extraction was also performed according to the protocol described above. For each solvent, at least three independent extractions were prepared and quantified. These data allowed us to calculate statistical parameters, including the mean and standard deviation of the extracted rutin content.
2.6. Optimization of the operational conditions

In order to optimize the extraction conditions for rutin, a response surface methodology (RSM) was performed to determine the most important parameters as well as their interactions. In a $2^k$ RSM, there are $k$ parameters that can influence the response. In this work, the effect of three parameters - factorial design $2^3$ - was evaluated: temperature ($T$), time ($t$), and the solid-to-liquid ratio ($S/L$ ratio). These parameters collectively influenced the ultimate response ($y$), which was the rutin content, as described by the following second-order polynomial equation:

$$y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$ (2)

where $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ represent the coefficients adjusted for the intercept, linear, quadratic, and interaction terms, respectively, while $X_i$ and $X_j$ denote the independent variables. A factorial planning with 20 points of extraction was performed (see Tables S1 and S2 in Supplementary Material). The results obtained underwent a thorough statistical analysis at the 95% confidence level. All statistical analyses and the creation of response surfaces were performed using Statsoft Statistica 11.0©.

2.7. Reuse of biomass and solvent, and recyclability of solvent

Three parameters were assessed for the development of a sustainable extraction process: i) reusing biomass, ii) reusing solvent, and iii) solvent’s recyclability. Biomass reuse was performed to determine whether the rutin present in the biomass had been fully extracted. For this purpose, three consecutive extractions were conducted using the same biomass under optimal operating conditions (80 min, 27 °C, solid:liquid ratio of 1:15, acetic acid-water mixture at 65 wt%). Following each extraction, the solid-liquid
mixture underwent filtration, and a fresh organic acid-water mixture was applied with the same biomass sample.

Then, the solvent reuse was tested until it was saturated with rutin. Specifically, the same solvent was reused for four consecutive extraction cycles, but new biomass was used in each cycle to achieve saturation. These experiments were conducted based on the optimal operating conditions that were identified.

Finally, the recyclability of the solvent was evaluated after its saturation with rutin (two consecutive solid-liquid extraction, with new biomass at each extraction, under optimal operating conditions). The solvent was recovered through evaporation under reduced pressure and reused for two consecutive extractions using the previously established optimal operating conditions. A total of three cycles were executed, including one initial cycle and two recycling cycles.

3. Results and discussion

3.1. Biomass selection

First, we investigated the impact of banana peel ripeness on the extracted rutin content. More specifically, three stages of ripeness were analyzed, namely green, semi-ripe and ripe banana peels, to determine the most suitable biomass for optimizing the rutin extraction process. For this study, methanol was used as extraction solvent since it is one of the most commonly used solvents in the literature for the extraction of rutin (Behiry et al., 2019; Gullón et al., 2017). The extracted rutin content for each ripening stage of the bananas is presented in Figure S1 and Table S3 in Supplementary Material. The results indicate a progressive increase in rutin content in the following order: green (10.7 ± 0.3 mg/100 g DW) < semi-ripe (22 ± 1 mg/100 g DW) < ripe (25.9 ± 0.4) mg/100 g DW. These results suggest that the rutin content increases as the bananas
ripen. Consequently, the subsequent rutin extraction investigations were focused on ripe banana peels, as they exhibited the highest rutin content among the ripening stages evaluated.

3.2. COSMO-RS prediction

After identifying ripe banana peels as the optimal biomass for rutin extraction, the next step was the selection of organic acids for this process using COSMO-RS. The σ-profiles serve as valuable indicators of a compound's chemical characteristics, including polarity and hydrogen bonding capabilities, which aid in understanding potential interactions between the target solute and solvents during extraction (Klamt et al., 2010). These histograms depict the polarized charge distribution (σ) of individual compounds. As illustrated in Figure 1, three distinct regions can be discerned: i) hydrogen bond donors, \( \sigma < -0.82 \, (e/\text{Å}^2) \cdot 10^{-2} \); ii) hydrogen bond acceptors, \( \sigma > +0.82 \, (e/\text{Å}^2) \cdot 10^{-2} \); and, iii) non-polar compounds in an intermediate region. Rutin presents a peak at around \(-1.8 \, (e/\text{Å}^2) \cdot 10^{-2}\) due to the acidic hydrogens of its hydroxyl groups (deep blue), indicating its hydrogen bond donor ability. A prominent positive peak originating from the lone pairs on the oxygen atoms of the hydroxyl groups (deep red) signifies a substantial hydrogen bond acceptor capability. The non-polar region is attributed to the hydrocarbon groups, characterized by a pronounced peak from the hydrogens, shifted towards the negative σ region, and a secondary peak from the carbons. Overall, the σ-profile suggests that given its large non-polar region rutin would be poorly soluble in water but its hydrogen bond acceptor character indicates that acidic compounds with a good hydrogen bond donor ability could be good solvents for rutin. Thus, the potential of various organic acids, and their mixture with water, was evaluated to solubilize rutin.
The evaluation of the relative solubility ($\log_{10}(x_{RS})$) allows to compare the solubility of a given solute in several different solvents, at the same conditions (COSMOlogic, 2018). For this, rutin was considered as a solid solute and calculations were conducted at 25 °C, taking into account both pure and hydrated solvents (75 wt% of organic acid and 25 wt% of water). The results of $\log_{10}(x_{RS})$ (where the highest solubility was designated as 0) obtained are in Table S4 in Supplementary Material. Then the solubility of rutin in different solvents was expressed as percentage of probability of solubility (see section 2.3 for more details), and the results obtained are presented in Figure 2.
Figure 2. Probability of solubility of rutin in different solvents pure and hydrated, at 25 °C, predicted by COSMO-RS.

Results from Figure 2 and Table S4 in Supplementary Material indicated a better overall probability of solubility of the hydrated solvents than the pure ones. The water added to the acid seems to favor its solvation ability, besides reducing the viscosity of the liquid solvents, which enhances the mass transfer in solid-liquid extraction (Vilková et al., 2020).

Among the organic acids investigated, it was found that citric acid, although identified as the most effective pure solvent, presents limitations since it is a solid at room temperature (melting point of 153 °C). When water was added to citric acid, it resulted in a decrease in the solubility of rutin, shifting from high to medium solubility. Despite the consistent mass of water added to all solvents, the molar fraction of water varied
based on the molar weights of the components. In the case of citric acid, a 25 wt% addition of water corresponds to approximately 78% molar fraction, which influences the affinity of the solvent for rutin.

Acetic acid, which demonstrated the second-highest likelihood of solubility among the pure solvents, emerged as the most promising solvent in its hydrated form. The addition of water to acetic acid enhances the overall polarity of the mixture and facilitates the formation of hydrogen bonds between the oxygens of rutin’s hydrogen bond acceptor zone and the acid hydrogens of water. This improvement in solvent performance contributes to the increased solubility of rutin. Additionally, employing hydrated acids improves the economics and the sustainability of a process.

The COSMO-RS screening identified hydrated acetic acid, mandelic acid, and lactic acid as the most suitable solvents for extracting rutin from banana peels (see Figure 2). Since acetic and lactic acids are approved as food additives (European Food Safety Authority, 2013) and rutin is a common dietary supplement (Gullón et al., 2017; Liu et al., 2020), these two organic acids were selected for experimental extraction. Citric acid was also chosen because not only because it is widely used as a food additive (European Food Safety Authority, 2013), but also, according to the results of COSMO-RS, it shows a very good solubility of rutin excellently as pure solvent (although, as mentioned above, this cannot be experimentally verified). Finally, glycolic acid was selected as part of the validation process for the results of COSMO-RS.

3.3. Solid-liquid extraction: Influence of different organic acids in the rutin extraction

The organic acids selected in the previous section were evaluated for rutin extraction in both pure and hydrated forms, with organic acid concentrations ranging from 100 to
25 wt%, to determine the most effective solvent for this process. The results obtained are shown in Figure 3A, with detailed information available in Table S5 in Supplementary Material. A comparison with the performance of the alcohols usually used in this process is presented in Figure 3B. It should be noted that citric and glycolic acids, as mentioned earlier, could not be used in pure form for the extraction process due to their solid nature.

For the acids studied, the addition of some water seems to have a positive effect on rutin extraction, since pure acids are not as efficient in the extraction of rutin as their water mixtures at a concentration of 75 wt%. Thus, the hydrated organic acids exhibit a higher extraction capacity for rutin, which can be due to a modulation of the solvent polarity due to co-solvency. Co-solvents can enhance the solubility of target compounds in biomass, thereby improving the extraction efficiency (Soares et al., 2019). This effect was particularly observed when acetic acid and water mixtures were used. Clearly, the dissociation constant ($pK_a$) of these acids (acetic acid ($pK_a = 4.64$); lactic acid ($pK_a = 3.78$), citric acid ($pK_a = 3.06$), glycolic acid ($pK_a = 3.53$)) is not relevant to understand the extraction performance of rutin ($pK_a = 7.62$) (“ChemAxon,” 2023). Overall, mixtures of organic acid-water exhibit better transport and solvation properties than their individual components, allowing an enhanced extraction performance of the hydrated acid.
Figure 3. Evaluation of the rutin content of extracts from ripe banana peels obtained using different solvents in the extraction. Extractions by applying (A) pure organic acids and their mixtures with water (wt%); or (B) pure alcohols and their mixtures with and water (wt%). Extraction conditions: $t = 60\ \text{min;}\ T = 25\ ^\circ\text{C}$ and $S/L\ \text{ratio} = 1:10$.

Regarding the structural differences between the organic acids, \textit{i.e.}, the hydroxyl group (-OH), the results showed that the addition of -OH to the extraction solvent leads to a lower rutin content. In particular, acetic acid clearly stands out from the other acids,
with acetic acid at 75 wt% leading to an extract with a rutin concentration of (182 ± 3) mg/100 g DW (Figure 3A) well above the performance of the alcohols conventionally used for this purpose with ethanol (at 50 wt%) being able to extract (42 ± 1) mg/100 g DW of rutin.

Moreover, the use of hydrated organic acids also proved to be much more effective than conventional solvents (alcohols and acidified water) in rutin extraction under the same conditions. The results showed that with the best solvent identified, namely the acetic acid-water mixture at 75 wt% (rutin content (182 ± 3) mg/100 g DW), it was possible to obtain a rutin content that was about four times higher than that obtained with the best conventional solvent, methanol at 50 wt% (rutin content (42 ± 1) mg/100 g DW).

Finally, before proceeding with the optimization, a detailed investigation was conducted to determine the impact of acetic concentration on rutin extraction. The obtained results are shown in Figure S2 (see Table S5 in the Supplementary Material for more details). Figure S2 confirms that the addition of water to the organic acid enhances rutin extraction, especially up to an acetic acid-water mixture of 65 wt%, resulting in a rutin content of (192 ± 1) mg/100 g DW. Consequently, the results indicate that the optimal solvent for achieving maximum rutin extraction from ripe banana peels is an acetic acid-water mixture at 65 wt%. Thus, this particular solvent concentration was selected for subsequent studies.

3.4. Optimization of extraction conditions

Once the optimal solvent and its concentration (acetic acid-water mixture at 65 wt%.,) was established, a RSM based on $2^3$ factorial design (three parameters and two stages) was performed to optimize the extraction conditions. This method enables the examination of the connection between the response variable (rutin content, mg/100 g
DW) and the independent parameters, while also allowing for the prediction of the optimal extraction conditions for maximizing rutin content. The independent parameters considered included temperature \( T, \, ^\circ C \), extraction time \( t, \, \text{min} \), and solid:liquid ratio \( S/L \, \text{ratio} \). The temperature varied from 25 \(^\circ\)C to 51 \(^\circ\)C, the extraction time from 10 min to 110 min, and the solid:liquid ratio ranged from 1:63 to 1:5.

Figure 4. Surface response plots for the rutin content using an acetic acid-water mixture at 65 wt% with the combined effects of: (A) time \( t, \, \text{min} \) and temperature \( T, \, ^\circ C \); (B) solid:liquid ratio \( S/L \, \text{ratio} \) and \( t, \, \text{min} \) and (C) \( S/L \, \text{ratio} \) and \( T, \, ^\circ C \).

The data obtained were subjected a 95% confidence interval analysis of variance (ANOVA) to assess the significance of the variables and their interactions on the rutin
content. The statistical analysis is shown in the Supplementary Material (Tables S6-S8 and Figures S3-S5), and the respective data are depicted in Figure 4. The $R^2$ of the polynomial equation for the extracted rutin content was 0.956 (explaining 95.6% of the observed variation), indicating no significant differences between the experimental and theoretical responses. In addition, the F-test (23.9) confirmed the global significance of the model (p-value, $1.3 \times 10^{-5}$, see Table S7 in Supplementary Material, please).

The Pareto chart provided in Figure S3 in the Supplementary Material shows that the two variables studied (temperature and solid:liquid ratio) were significant, while extraction time was not a significant variable by itself. However, the effect of extraction time combined with temperature, was one of the significant variables on rutin content. In summary, the following variables were statistically significant: $T$; $T*S/L$ Ratio; $S/L$ Ratio$^2$; $T^2$; $T*t$; $S/L$ Ratio. Temperature was the most significant variable but had a negative effect on the content of extracted rutin. It is well known that high temperatures can induce degradation of phenolic compounds. Rutin has been reported to be stable up to 100 ºC (Frutos et al., 2019), but this also depends on the time rutin is exposed to high temperatures. Chaaban et al. (2017) observed that rutin already shows some degradation at 70 ºC after 2 h ($< 10\%$). This aspect should be taken into account, especially if we want to perform successive extractions with the same solvent with fresh biomass samples, since this means that the extracted rutin will be exposed to high temperatures for a long time and thus can suffer some degradation even if subjected to moderate temperatures. Moreover, the quadratic variables have a negative effect (Figure 4 and Figure S3 in the Supplementary Material), which means that the maximum rutin content is reached in the interval of the studied variables. The optimal conditions to increase rutin content were as follows: an extraction temperature of 27 ºC for 110 min at a solid:liquid ratio of 1:15 (Figure S6 in Supplementary Material). These extraction conditions
gave an average value of rutin content of \((242 \pm 3 \text{ mg/100 g DW})\), which is very similar to the predicted value \((240 \text{ mg/100 g DW})\) according to the multiple regression obtained with the RSM analysis. Thus, the obtained regression value agrees perfectly with the experimental result.

Considering that time is not a significant variable, with only its effect combined with temperature being significant, the optimized extraction conditions were then evaluated using different extraction times, \textit{i.e.}, in addition to 110 min, extractions of 80 min and 50 min duration were also performed, as shown in Table S9 in the Supplementary Material. When the extraction time was reduced from 110 to 80 min, only a 0.6% decrease in rutin content was observed (from \((242 \pm 3)\) to \((241 \pm 2)\) mg/100 g DW). However, further reduction of the extraction time to 50 min already resulted in a 13% decrease in rutin content, as we could only obtain a rutin content of \((210 \pm 4)\) mg/100 g DW. Thus, although the optimal conditions for rutin extraction from ripe banana peels should be determined by an economic analysis between the recovered rutin and the extra cost required for a longer extraction time, here we will use the following optimized conditions: a acetic acid-water mixture at 65 wt\% and an extraction temperature of 27 °C for 80 min at a solid:liquid ratio of 1:15.

The rutin content obtained under these conditions \((241 \pm 2 \text{ mg/100 g DW})\) was somewhat higher even than the values obtained by Soxhlet extraction \((205 \pm 3)\) and \((230 \pm 4)\) mg/100 g DW of rutin with methanol and ethanol, respectively), that required longer extraction times (at least 360 min) and higher temperatures (60-80 °C) and thus where more costly.

In summary, the mild conditions and temperatures proposed here (acetic acid-water mixture at 65 wt\% and an extraction temperature of 27 °C for 80 min at a solid:liquid
ratio of 1:15), as well as the mixture of acetic acid and water, provide a competitive and more effective solvent for the extraction of rutin from banana peels.

3.5. Reuse of biomass and solvent

After determining the optimal operating conditions, biomass reuse was investigated to determine the maximum rutin content in the ripe banana peel. After a first extraction cycle was performed under the optimal conditions (cycle 1: 241 ± 2 mg/100 g DW), the biomass was recovered after extraction, and reused in another extraction cycle (cycle 2). It should be noted that new acetic acid-water mixtures at 65 wt% were used in each cycle while the same biomass sample was used. The results showed that rutin was essentially completely extracted in the first extraction cycle, since the rutin content obtained in the second extraction cycle was very low (0.94 ± 0.09 mg/100 g DW), as shown in Figure 5A (Tables S10 in Supplementary Material). Therefore, there is no need to reuse the biomass after the first extraction.

<table>
<thead>
<tr>
<th>(A) Biomass reuse</th>
<th>(B) Solvent reuse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total rutin content in biomass:</strong>*</td>
<td><strong>Total rutin content in solution after 3 cycles of extraction:</strong></td>
</tr>
<tr>
<td>241 ± 2 mg/100 g DW</td>
<td>508 ± 5 mg/100 g DW</td>
</tr>
<tr>
<td><strong>Rutin content (mg/100 g DW)</strong></td>
<td><strong>Rutin content (mg/100 g DW)</strong></td>
</tr>
<tr>
<td>Cycle 1</td>
<td>Cycle 2</td>
</tr>
<tr>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
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<td>100</td>
<td>0</td>
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</tbody>
</table>

*Each extraction was done with a fresh extraction solution and reusing the biomass.

*Each extraction was done with a fresh biomass and reusing the extraction solution. In cycle 4, the extracted amount of rutin is zero.

Figure 5. Rutin content from ripe banana peels with (A) biomass and (B) solvent reuse under optimal operating conditions: acetic acid-water mixture at 65 wt%; T = 27 °C; S/L ratio = 1:15; t = 80 min.
The reuse of the extraction solvent was then studied to determine the number of cycles required to saturate the solvent and maximize the cost, efficiency, and sustainability of the process. Successive extractions of rutin from fresh, ripe banana peels at optimal operating conditions for four consecutive cycles show that after the two first extractions the ability of the solvent to be further used decreases dramatically. The results obtained are shown in Figure 5B (Table S11 in the Supplementary Material). After three extraction cycles, the total rutin content in the acetic acid-water mixture increased from 241 to 508 mg/100 g DW corresponding to an increase in rutin concentration in the solvent from $0.13 \pm 0.01$ to $0.28 \pm 0.04$ g/L (Table S11 in the Supplementary Material). This value is in agreement with the rutin concentration observed in the extract obtained. This small difference could be attributed to the extraction of other low molecular weight compounds alongside rutin. Therefore, reuse of the solvent for two cycles appears to be feasible without significant loss of rutin.

Another necessary step to ensure the economic and sustainable viability of the extraction process under development is to confirm the recyclability of the solvent. After saturation of the extraction solvent (two solid-liquid extraction cycles), the solvent was recovered and recycled by evaporation under reduced pressure for a total of three cycles. A schematic representation of the proposed process is shown in Figure 6.
The results show that the rutin content decreases slightly with each cycle of the extraction process (1st cycle: \(480 \pm 10\) mg/100 g DW; 2nd cycle: \(467 \pm 12\) mg/100 g DW and 3rd cycle: \(446 \pm 10\) mg/100 g DW). It should be noted that the process requires an additional supply of fresh solvent, since we observed a loss of about 10% by weight of the mixture of organic acid and water during each complete extraction cycle. The stream recovered after evaporation of the extraction solvent in the vacuum dryer at low pressure was rich in rutin, with a purity of 80%, according to the HPLC-DAD method quantification used in this work. A further purification step may, or may not, be required depending on the desired application of rutin. Moreover, depending on the application, the liquid extract, or a concentrated liquid extract, can also be used directly as an end product, as acetic acid is a food additive approved by the Commission of the European Union (European Food Safety Authority, 2013).
4. Conclusions

The main aim of this study was to develop a process based on solid-liquid extraction to obtain rutin - a value-added biocompound with properties relevant to human health - from banana peels. The results showed that ripe banana peels are the best source of this compound. Then, COSMO-RS served as a screening tool for solvent selection, enabling a quick and qualitative in silico assessment of rutin's relative solubility in different organic acids. COSMO-RS calculations indicated that citric acid, lactic acid, and acetic acid are the most suitable organic acids for rutin extraction, whether in their pure form or when hydrated. Furthermore, it was observed that the presence of water has an impact on the efficiency of these organic acids in rutin extraction. The acetic acid-water mixture at 65 wt% was found to be the most efficient organic acid mixture studied, indicating that organic acids can be much more efficient than conventional solvents in rutin extraction. The extraction conditions were optimized by RSM, and (241 ± 2) mg/100 g DW of rutin was obtained at a temperature of 27 °C, an extraction time of 80 min, and a solid-liquid ratio of 1:15. Finally, three cycles of rutin extraction with recovery and reuse of the mixture of organic acids and water (one cycle + two recyclablility solvent cycles) were carried out to assess the economic viability and sustainability of the developed extraction process, and to demonstrate that solvent recyclablility is possible. Overall, it was shown that hydrated organic acids offer a viable alternative to alcohols for the extraction of rutin from natural sources, potentially advancing the development of more efficient and sustainable extraction processes.

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Graphical abstract

Rutin extraction

Solid-liquid extraction

Banana peels

COSMO-RS

Organic acids (neat & hydrated)

Optimization - RSM

ACETIC ACID-WATER mixture at 65 wt%

27 °C, 80 min, S/L ratio 1:15

Solvent recyclability

Dried extract rich in RUTIN

Vacuum dryer

Condenser

S1
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: