

Green Extraction of Corn Silk Flavonoids Using NADES-UAE: Optimizing Conditions for Agricultural Waste Valorization

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Abstract

Corn silk (of *Zea mays* L.) is an agricultural waste that has not been optimally utilized, even though it is known to contain flavonoid compounds with various pharmacological activities, such as antioxidant, anti-inflammatory, and antidiabetic effects. The flavonoid compounds in corn silk have the potential to be developed as natural raw materials for applications in the pharmaceutical and cosmetic fields. However, the extraction process for these active compounds often still uses organic solvents like methanol, which are toxic, flammable, and have negative impacts on health and the environment. Therefore, this study aimed to optimize the Ultrasound Assisted Extraction (UAE) method using a more environmentally friendly alternative solvent, namely Natural Deep Eutectic Solvent (NADES). The study was conducted in two main stages: selection of the best NADES composition and molar ratio from combinations of choline chloride with urea, glycerol, lactic acid, and malic acid; and optimization of UAE parameters including ultrasonic power, time, and temperature using the Response Surface Methodology (RSM) with the Box-Behnken Design (BBD) model. The determination of total flavonoid content was performed using the colorimetric method with AlCl_3 reagent and quercetin standard, and the measurements were taken using a UV-Vis spectrophotometer. The results showed that the NADES combination of choline chloride:urea (1:2) with the addition of 30% aquades produced the highest flavonoid content compared to 80% methanol solvent. The optimal conditions for UAE extraction were obtained at 100 % ultrasonic power, 30 minutes of extraction time, and a temperature of 70°C, resulting in a total flavonoid content yield of 96.02 ± 0.48 mgQE/g of corn silk powder. This study concluded that the combination of NADES and UAE method can significantly enhance the extraction yield of total flavonoids while also providing a more sustainable and environmentally friendly solution for the utilization of agricultural waste, such as corn silk.

Keywords: Corn Silk; Flavonoid; Green Solvent; NADES; UAE.

Abbreviations: Box-Behnken Design (BBD); Natural Deep Eutectic Solvent (NADES); Quercetin Equivalent (QE); Response Surface Methodology (RSM); Ultrasound Assisted Extraction (UAE).

INTRODUCTION

Corn (*Zea mays* L.), a member of the Poaceae family, is one of the most widely utilized food crop commodities in Indonesia. Primarily, corn serves as a source of carbohydrates, but it also contains proteins, vitamins, and minerals (Nawaz et al., 2018). Beyond its role as a food commodity, corn is also used in livestock feed and the biofuel industry (Amin, 2022). Currently, the utilization of corn plants predominantly focuses on the kernels, while other parts, such as corn silk, are often regarded as waste. In countries like China and the United States, corn silk is traditionally consumed as herbal tea to promote health (Lapčík et al., 2023). Extracts from corn silk have demonstrated various pharmacological activities, including lowering blood pressure, regulating blood sugar levels, and exhibiting anti-inflammatory and antioxidant effects. Phytochemical analyses have

revealed that corn silk contains flavonoids, polyphenols, phenolic acids, fatty acids, and terpenoids, with flavonoids being the most abundant bioactive components (Wang et al., 2024). The total flavonoid content in methanol extracts of corn silk has been found to be significantly higher than that of corn husk extracts, indicating the potential of corn silk as a natural source of flavonoids with beneficial pharmacological activities (Dong et al., 2014).

Flavonoid compounds identified in corn silk include maysin, rutin, genistein, apigenin, quercetin, luteolin, and catechin (Wang et al., 2024). The extraction efficiency of flavonoids from corn silk is influenced by the type of solvent used. Methanol extracts have shown higher flavonoid content compared to ethanol extracts (Haslina and Eva, 2017; Haslina et al., 2019a; Haslina et al., 2019b). However, conventional solvents like methanol have several drawbacks, including flammability, non-

biodegradability, and potential long-term health risks (Jauregi et al., 2024). The toxic properties of methanol can be mitigated through evaporation, but this process is time-consuming for obtaining natural extracts. Therefore, there is a need for safer and more environmentally friendly solvents that allow for quicker and simpler extraction processes.

Natural Deep Eutectic Solvents (NADES) have gained attention in recent years as eco-friendly solvents derived from natural materials (Puspita et al., 2023). NADES are formed from the combination of a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) in a specific molar ratio., creating a stable eutectic mixture at room temperature. They offer advantages such as low cost, simple preparation, reusability, biodegradability, and adjustable viscosity. The viscosity of NADES can be modified by adding water, which facilitates the transfer of analytes during the extraction process (Shikov et al., 2022). Common materials used for extracting flavonoids include Choline Chloride (ChCl), urea (U), glycerol (Gly), lactic acid (LA), and malic acid (MA) (Liu et al., 2024).

The extraction of flavonoids from corn silk using NADES has shown promising results, indicating that this method could be more efficient than traditional extraction techniques. However, conventional extraction methods, such as maceration, require long durations and large volumes of solvent. Therefore, this study will employ the Ultrasound-Assisted Extraction (UAE) method, which utilizes ultrasonic waves for a shorter process, avoiding high-temperature exposure, and requiring less solvent (Medina-Torres et al., 2017). Factors affecting UAE extraction yield include ultrasonic power, time, and temperature (Kumar et al., 2021). These conditions can interact, influencing the total flavonoid yield, necessitating the optimization of UAE conditions through appropriate experimental design. The aim of this research is to optimize the extraction of flavonoids from corn silk using NADES in conjunction with the UAE method. By identifying the optimal composition and ratio of NADES and optimizing UAE conditions, this study seeks to enhance the efficiency of flavonoid extraction while promoting a more sustainable and environmentally friendly approach to utilizing agricultural waste.

MATERIALS AND METHODS

Study Area

This research was conducted at the Pharmacognosy and Phytochemistry Laboratory, Pharmacy Department, Faculty of Mathematics and Natural Sciences, Universitas Udayana, and the Forensic Science and Criminology Laboratory Unit, Universitas Udayana.

Chemical and Materials

The chemicals used in this study included methanol 80% (Smart-Lab), distilled water, choline chloride (Himedia),

urea chemical pure grade and glycerol pharmaceutical grade were purchased from Rofa Laboratory Centre, 90% food grade lactic acid (Subur Kimia Jaya), food grade malic acid (Subur Kimia Jaya), quercetin standard (Sigma), aluminum chloride (Merck), and sodium acetate (Merck).

Sample Preparation

Corn silk (*Zea mays L.*) were obtained from Badung Regency, Bali Province, Indonesia. Corn silk was collected from maize plants ready for harvest, specifically the silk emerging from the husks. Dried corn silk specimens were cut into small pieces, then ground into a fine powder using a grinder and sieved using 60-mesh.

NADES Synthesis and Characterization

NADES were synthesized by mixing defined molar ratios of hydrogen bond acceptor (HBA) to hydrogen bond donor (HBD) components (Table 1). NADES was prepared using the heating-stirring method with a magnetic stirrer (Cimarec⁺). The mixture was stirred at 400 rpm and heated at 80°C until a clear and homogeneous solution was formed (Ozkan, 2023). The formed NADES was then added with 30% (v/v) distilled water and stirred again until a homogeneous mixture was obtained. Viscosity of NADES was determined using an Ostwald viscometer (Iwaki Pyrex), pH was measured using a pH-meter (Kedida CT-6020A), and a pycnometer (Iwaki Pyrex) was used to determine the density of NADES. The measurements were performed at ambient room temperature ($\pm 32^\circ\text{C}$).

Table 1. Molar ratio combination of NADES.

NADES Code	Component		Molar Ratio
	HBA	HBD	
NADES-1	Choline chloride	Urea	1:2
NADES-2	Choline chloride	Glycerol	1:1
NADES-3	Choline chloride	Glycerol	1:2
NADES-4	Choline chloride	Glycerol	1:3
NADES-5	Choline chloride	Lactic acid	1:1
NADES-6	Choline chloride	Lactic acid	1:2
NADES-7	Choline chloride	Lactic acid	1:3
NADES-8	Choline chloride	Malic acid	1:1

Screening and Selection of NADES for Extraction

Screening and selection of the formulated NADES as extraction solvents was conducted using ultrasonic-assisted extraction (UAE) under the following conditions: a solvent-to-solid ratio of 20:1, ultrasonic power at 100% with a frequency of 40 KHz, temperature of 60°C, and extraction time of 20 minutes. Then, centrifugation was performed at 4000 rpm for 20 min, the supernatant was collected and then filtered using filter paper. Each NADES extract was stored in a dark container until further analysis.

In this study, 80% methanol, which are volatile organic solvents, were used as comparators. Separately, extraction of the dried powder of corn silk using the methanol was performed using the same procedure to obtain methanol extract. All extraction procedures were carried out in triplicate. The optimal solvent was selected based on its total flavonoid extraction capacity.

Determination of Total Flavonoid Content (TFC)

The determination of the total flavonoid content (TFC) was performed using the aluminum chloride colorimetric method previously described by Chandra et al. (2014) was employed with some minor modifications. A stock solution of quercetin (1000 µg/mL) was prepared by dissolving 10.0 mg of quercetin in 10 mL of methanol. Standard quercetin solutions were then prepared at concentrations of 40, 50, 60, 70, and 80 µg/mL. For sample preparation, 0.5 mL of the solution was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 1.8 mL of distilled water. The mixtures were thoroughly homogenized and incubated for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer (UV mini Shimadzu 1240) at 436 nm. The results were expressed as milligrams of quercetin equivalents per gram of the dried samples (mg QE/g).

NADES-UAE Optimization Using Response Surface Methodology

The optimal conditions for the extraction of flavonoid compounds from corn silk using NADES-UAE were determined through response surface methodology (RSM) with the aid of Design-Expert software. The optimization process involved three independent variables: ultrasonic power (X_1 , 40–100%), extraction time (X_2 , 10–30 minutes), and extraction temperature (X_3 , 50–70°C). A total of 17 experimental runs, including five replicates at the center point, were carried out based on the Box–Behnken Design (BBD). An experimental approach was employed to generate response data, with total flavonoid content, measured using the same analytical procedure, serving as the response variable.

Data Analysis

All experiments data obtained were statistically analyzed and presented as means $\pm$ standard deviation (SD). The best solvent to be used in the optimization stage was determined by performing statistical analysis using one-way ANOVA, followed by a post hoc Least Significant Difference (LSD) test using IBM SPSS Statistics version 30.0 software. Meanwhile, the optimization data for the UAE conditions were analyzed using the Design-Expert 13.1 software package through analysis of variance (ANOVA). Additionally, a one-sample t-test was performed using the same software to detect significant differences between the samples. Significant difference among groups was considered at $p < 0.05$.

RESULTS AND DISCUSSION

NADES Synthesis and Characterization

All eight NADES combinations prepared under identical processing conditions successfully formed clear and homogeneous solutions at room temperature, indicating successful NADES formation. The dilution of these NADES with 30% (v/v) distilled water was carefully controlled to ensure a distilled water content of less than 50%, in order to preserve their molecular structures and prevent any negative impact on their performance (Rocha et al., 2023). Based on visual observation, the diluted NADES remained stable as clear and homogeneous liquids during storage.

The characterization results of all NADES types are presented in Table 2. The pH is determined by the individual and combined acidity and basicity properties of the HBA and HBD components (Sazali et al., 2023). NADESs containing lactic acid and malic acid as HBD components tended to exhibit low pH values (<2), while those with glycerol as the HBD component showed neutral pH values. NADESs formulated with urea as the HBD exhibited high pH values (>8). The lowest density was recorded for NADES-5 at 1.12 ± 0.01 g/mL, while the highest density was observed in NADES-8 at 1.22 ± 0.01 g/mL. High viscosity can reduce the mobility or diffusion of compounds in solution, thereby hindering mass transfer and slowing down the extraction process. NADES-1 showed the lowest viscosity value at $2.6 \times 10^{-3} \pm 0$ kg/m.s, whereas NADES-8 exhibited the highest viscosity value at $2.34 \times 10^{-2} \pm 0$ kg/m.s. These results are attributed to the strong hydrogen bonding interactions between the components of the NADES, which lead to a tightly packed molecular network structure, thereby increasing both density and viscosity.

Table 2. Characterization Results of NADES: pH, Density, and Viscosity.

NADES	Characteristics		
	pH	Density (g/mL)	Viscosity (kg/m.s)
NADES-1	8.43 ± 0.02	1.14 ± 0.01	$2.6 \times 10^{-3} \pm 0$
NADES-2	5.09 ± 0.01	1.15 ± 0	$5.4 \times 10^{-3} \pm 0$
NADES-3	5.13 ± 0.02	1.16 ± 0.01	$6.2 \times 10^{-3} \pm 0$
NADES-4	5.37 ± 0.02	1.16 ± 0	$8.0 \times 10^{-3} \pm 0$
NADES-5	1.53 ± 0.01	1.12 ± 0.01	$3.5 \times 10^{-3} \pm 0$
NADES-6	1.12 ± 0.01	1.13 ± 0	$4.4 \times 10^{-3} \pm 0$
NADES-7	1.02 ± 0.01	1.14 ± 0	$6.9 \times 10^{-3} \pm 0$
NADES-8	0.67 ± 0.01	1.22 ± 0.01	$2.34 \times 10^{-2} \pm 0$

Note: The values presented are expressed as mean $\pm$ standard deviation; measured at 32°C.

Selection of The Optimal NADES for Extraction

In general, various factors affect the efficiency of natural deep eutectic solvents (NADESs) in extracting bioactive compounds from plant sources. The variation in component types and molar ratios within NADES formulations plays a crucial role in determining their extraction performance, particularly for flavonoids. This

is due to the fact that essential properties of NADES—such as polarity, viscosity, and their ability to form hydrogen bonds—are strongly influenced by the specific combination of constituents (Wu et al., 2022). This screening was conducted to evaluate the effectiveness of each NADES in extracting flavonoids, in comparison with 80% methanol as a conventional solvent (Figure 1).

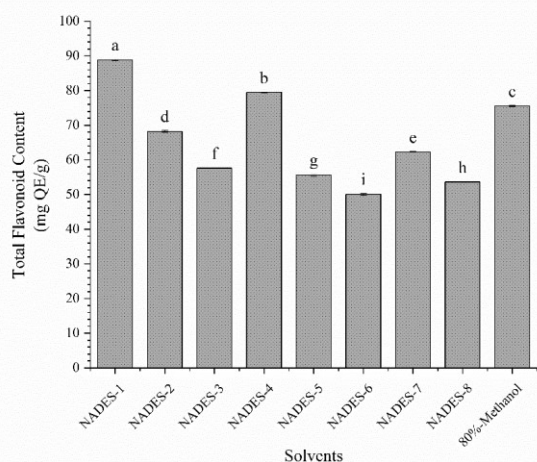


Figure 1. Total flavonoid content of each solvent. Extraction was performed at 60°C for 20 minutes with 100% ultrasonic power. Different letters indicate significant differences based on LSD test ($p < 0.05$).

Based on the results shown in Figure 1, each NADES exhibited different capabilities in extracting flavonoids from corn silk, as reflected by the total flavonoid content. Among them, NADES-1 (choline chloride:urea = 1:2, mol/mol) yielded the highest total flavonoid content at 88.85 ± 0.23 mgQE/g of powdered material, followed by NADES-4 (choline chloride:glycerol = 1:3, mol/mol) with 79.47 ± 0.11 mgQE/g. NADES-1 had the lowest viscosity among the tested solvents. Lower viscosity facilitates better mass transfer, thereby enhancing extraction efficiency. In terms of pH, NADES-2 exhibited a basic environment ($\text{pH } 8.43 \pm 0.02$) and also resulted in a high flavonoid content. This is consistent with the findings of Rismawati and Ismiyati (2017), who reported that an increase in pH tends to enhance flavonoid extraction, with a decline observed at pH 10.

The results demonstrated that NADES-1 achieved higher total flavonoid content than the conventional solvent, 80% methanol (75.55 ± 0.17 mgQE/g). NADES-1 exhibited the highest extraction efficiency among all tested NADESs and 80% methanol. This finding aligns with a study by Hikmawanti et al. (2023), which also reported that NADES composed of choline chloride:urea (1:2) significantly outperformed methanol in extracting flavonoids from *Pluchea indica* L. leaves. The superior performance of NADES in flavonoid extraction compared to conventional solvents is attributed to their ability to form a stable and complex hydrogen-bonding network between the hydrogen bond donor and acceptor components. These hydrogen bonds are believed to induce more pores and microcracks in plant materials

during extraction, thereby facilitating the release of intracellular compounds (Du et al., 2024; Gao et al., 2024). NADES exhibit strong penetration and permeability properties, allowing them to disrupt the lignin and cellulose structures in plant cell walls. This disruption facilitates the release of intracellular compounds into the extracellular environment (Du et al., 2024). According to Scanning Electron Microscope (SEM) observations by Gao et al. (2024), extraction with NADES results in surfaces that are rougher and more irregular in shape, resembling corrosion damage. This effect is likely due to the ability of NADES to dissolve or hydrolyze cellulose, leading to cell wall degradation. As a result, NADES can expose and release a greater quantity of target compounds compared to conventional solvents (Gao et al., 2024). Based on all the results obtained, NADES-1 (choline chloride:urea = 1:2) was selected as the solvent for use in the optimization stage of the UAE conditions.

Optimization of Ultrasound-Assisted Extraction

The optimization study of extraction conditions using Ultrasound-Assisted Extraction (UAE) was conducted using Response Surface Methodology (RSM) with the Box-Behnken Design (BBD) approach. The independent variables selected for the optimization were based on previous studies that identified the most effective extraction conditions. The response data obtained from the optimization process are presented in Table S1. The UAE conditions in runs 1, 2, 4, 7, and 13 were identical (70% ultrasonic power, 20 minutes, and 60°C), resulting in relatively consistent experimental total flavonoid contents ranging from 82.80 to 83.29 mgQE/g. This consistency demonstrates the good reproducibility of the extraction method. The highest experimental flavonoid content was recorded in run 16, with a value of 92.47 mgQE/g, obtained under conditions of 100% ultrasonic power, 20 minutes, and 70°C. This result closely matched the predicted value of 92.48 mgQE/g, indicating a high level of accuracy in the predictive model. In contrast, the lowest flavonoid content was observed in run 9 (71.48 mgQE/g), conducted under suboptimal conditions of 40% ultrasonic power, 10 minutes, and 50°C. The total flavonoid content results were modeled into a response function equation in relation to the selected variables. Model selection was evaluated using Analysis of Variance (ANOVA), based on key statistical parameters including the sequential p -value, lack of fit p -value, adjusted R^2 , and F -value. The results of the regression model selection based on statistical analysis indicate that the quadratic model was the most suitable, as shown in Table 3. The recommended model—a quadratic model—was statistically significant ($p < 0.0001$), with a non-significant lack of fit ($p = 0.0993$) (Table 4). This indicates that the model sufficiently fits the experimental data and can reliably explain the effect of the variables on the response.

Table 3. Regression model selection.

Source	Sequential <i>p</i> -value	Lack of fit <i>p</i> -value	Adjusted R ²	Predicted R ²	
Linear	<0,0001	0,2327	0,9974	0,9964	
2FI	0,3230	0,2358	0,9976	0,9956	
Quadratic	0,0140	0,9939	0,9992	0,9993	Suggested
Cubic	0,9939		0,9986		

Table 4. ANOVA model results.

Source	Sum of Square	df	Mean Square	F-value	<i>p</i> -value
Model	550.63	9	61,18	2149,34	<0,0001 (sig.)
X ₁ -Ultrasonic Power	189.48	1	189,48	6656,40	<0,0001
X ₂ -Time	105.89	1	105,89	3719,84	<0,0001
X ₃ -Temperature	254.30	1	254,30	8933,90	<0,0001
X ₁ X ₂	0.0015	1	0,0015	0,0510	0,8278
X ₁ X ₃	0,3265	1	0,3265	11,47	0,0116
X ₂ X ₃	0.0015	1	0,0015	0,0510	0,8278
X ₁ ²	0,5060	1	0,5060	17,78	0,0040
X ₂ ²	0,0010	1	0,0010	0,0343	0,8582
X ₃ ²	0,1027	1	0,1027	3,61	0,0993
Residual	0,1993	7	0,0285		
Lack of fit	0,0036	3	0,0012	0,0247	0,0993 (not sig.)
Pure error	0,1956	4	0,0489		
Cor total	550,83	14			

The selected quadratic model showed a high coefficient of determination (R²) of 0.9996, indicating a strong agreement between the experimental and predicted values. The predicted R-squared (0.9993) and adjusted R-squared (0.9992) values differed by less than 0.2, demonstrating good predictive ability of the model. The small difference between these values suggests that the model not only fits the data used to build it but also accurately predicts new data. Adequate precision, a parameter used to assess the model's ability to distinguish signal (model variation) from noise (error), was 162.3618—well above the threshold of 4—indicating a satisfactory signal-to-noise ratio and confirming the model's reliability in navigating the design space (Gao et al., 2024). The ANOVA results indicated that ultrasonic power (X₁), extraction time (X₂), and extraction temperature (X₃) had statistically significant effects on the total flavonoid content ($p \leq 0.05$). Among these variables, temperature (X₃) was identified as the most influential factor, followed by ultrasonic power (X₁) and extraction time (X₂). Ultrasonic power affects the size and intensity of cavitation bubbles formed during the ultrasonic extraction process. Higher power levels generate a greater number of cavitation bubbles—both large and small—which, upon collapsing, produce strong shock waves. These shock waves enhance mixing between the solvent and plant material, thereby improving the extraction yield of flavonoids. Bioactive compounds in plants are typically bound to the matrix through physical adsorption and chemical interactions. Increasing the extraction temperature can weaken these interactions, facilitating the release of secondary metabolites into the solvent. Moreover, elevated

temperatures reduce solvent viscosity, thereby enhancing mass transfer and diffusion rates, ultimately leading to more efficient extraction. Additionally, longer extraction times generally improve yield by allowing extended contact between the solvent and plant matrix, promoting optimal diffusion of target compounds into the solvent (Gao et al., 2024). The quadratic equation describing the effects of ultrasonic power (X₁), extraction time (X₂), and extraction temperature (X₃) on the total flavonoid content (TFC) of corn silk extract is expressed as follows:

$$Y \text{ (TFC)} = 82,77 + 4,87X_1 + 3,64X_2 + 5,64X_3 + 0,0190X_1X_2 - 0,2875X_1X_3 + 0,0190X_2X_3 - 0,3467X_1^2 + 0,0152X_2^2 - 0,1562X_3^2$$

The 3D response surface plots were used to visualize the interaction effects between two variables on the total flavonoid content. The plots demonstrated that increasing ultrasonic power and extraction time (Figure S1a), increasing temperature and ultrasonic power (Figure S1b), as well as increasing temperature and extraction time (Figure S1c), generally led to an increase in total flavonoid yield.

Based on the results of the RSM-BBD analysis, the optimal conditions for flavonoid extraction from corn silk using the UAE method were determined to be an ultrasonic power of 98.604%, extraction time of 29.527 minutes, and temperature of 69.919°C, with a predicted total flavonoid yield of 95.775 mgQE/g. To validate the reproducibility and reliability of the RSM design, experimental verification was conducted using the model-predicted optimal conditions. These optimized parameters were derived from regression analysis of the

variables and 3D response surface plots, while accounting for instrumental operational constraints. However, due to equipment limitations, the optimal conditions were adjusted to an ultrasonic power of 100%, extraction time of 30 minutes, and temperature of 70°C. These adjusted optimal conditions were then validated through actual experiments to verify the accuracy of the predictive model. The experimental results showed that the total flavonoid content obtained was 96.02 ± 0.48 mgQE/g. A one-sample t-test was conducted to statistically compare the experimental and predicted values, revealing no significant difference between them (Table 4). This indicates that the quadratic model used was able to accurately predict the flavonoid yield based on the combination of the three tested variables.

Table 4. Predicted and actual values of optimum conditions.

Parameters	Predicted values	Experimental conditions
Ultrasonic Power (%)	98,604	100
Time (menit)	29,527	30
Temperature (°C)	69,919	70
TFC (mgQE/g)	95,775 ^a	96,02 ^a

Note: The letter a indicates results that are not significantly different based on the one sample t-test ($p > 0.05$).

CONCLUSIONS

Based on the study, the combination of choline chloride:urea in a 1:2 molar ratio was identified as the most effective NADES formulation for extracting flavonoids from corn silk compared to other NADES types. This specific NADES not only outperformed other formulations but also demonstrated a significantly higher flavonoid extraction capacity than the conventional 80% methanol solvent when used to extract flavonoids from corn silk (*Zea mays* L.). Furthermore, the optimal extraction conditions for maximizing flavonoid yield using the UAE method were determined to be an ultrasonic power of 100%, an extraction time of 30 minutes, and a temperature of 70°C, with a total flavonoid content of 96,02 mgQE/g.

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