

Article

Valorization of Corn Silk as an Agricultural By-Product through the Optimization of Ultrasound-Assisted Extraction

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Abstract: Corn silk, derived from the stigmas of corn (*Zea mays*, Poaceae) flowers, possesses valuable bioactive compounds that can be harnessed for therapeutical, nutritional, and industrial purposes. This research aimed to optimize the extraction of bioactive compounds from corn silk, specifically flavonoids and total antioxidant capacity, by comparing it with classical extraction (infusion and maceration). The study identified significant factors influencing total flavonoid content (TFC) and antioxidant activity (assessed through an ABTS assay), including ethanol concentration, ultrasonic amplitude, and their quadratic interactions. This optimized combination of independent variables (37.89% ethanolic concentration, 48.58% ultrasound amplitude, and an extraction time of 38.72 min) yielded a balanced extraction, maximizing both TFC and antioxidant activity. Further analyses revealed significant total phenolic content, total flavonoid content (50.69 ± 0.36 mg GAE, 23.54 ± 0.01 mg QE, and 78.33 ± 0.94 mg TE/g extract, respectively), and potent in vitro antioxidant activity, affirming the efficacy of the optimized extraction. The study also explored the inhibitory effects on α -glucosidase ($IC_{50} = 411.67 \pm 25.39$ μ g/mL), acetylcholinesterase ($IC_{50} = 1166.4 \pm 399.38$ μ g/mL), and pancreatic lipase ($IC_{50} = 1519.67 \pm 209.04$ μ g/mL), showcasing potential antidiabetic and neuroprotective properties. While tyrosinase inhibition activity was not observed, the research emphasizes the nutraceutical potential of corn silk, offering valuable insights for its utilization and further exploration in the pharmaceutical and functional food industries.

Keywords: *Maydis stigma*; bioactivity; enzyme inhibition; Design of Experiment



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1. Introduction

Corn silk, also known as *Maydis stigma*, represents the stigmas of the corn plant (*Zea mays* L., Poaceae family), having a smooth, silky texture, hence the name. It is a very important part of the corn's reproductive system since it captures pollen particles from the atmosphere and ensures their safe passage to the plant's ovule, each clashing resulting in a corn kernel. Corn silk (CS) is considered a by-product of corn and is frequently discarded after cultivation. It constitutes approximately 3–5% of the overall weight of the corn plant, and as per projections from the United States Department of Agriculture, the anticipated worldwide corn yield for the period from 2023 to 2024 is estimated to reach 1214.45 million

metric tons [1]. On the other hand, according to Eurostat, the EU produced 270.9 million tons of cereals in 2022, of which 53 million tons were corn [2].

CS has long been known as a valuable herb, especially by the earliest human populations that started harvesting it, i.e., the Cherokee and Navajo native American tribes. These ancient tribes once used CS for infectious diseases, cardiovascular ailments, and urological problems [3]. Once corn began to spread worldwide through European colonialists, CS started to be used as a fair pain reliever for afflictions encountered in the renal pathological sphere, such as kidney stones, urinary tract infections (UTIs) or edema, and also in obesity [4].

The chemical composition of corn silk reveals a complex mixture of bioactive compounds that contribute to its medicinal properties. Various studies have aimed to identify and characterize these compounds, shedding light on the potential therapeutic effects of corn silk. Some of the key chemical constituents found in CS include flavonoids (CSF), such as quercetin, kaempferol, and rutin [5]. Corn silk is identified as an excellent source of flavones. Two novel flavone glycosides from corn silk are identified as 2''-O- α -L-rhamnosyl-6-C-3''-deoxyglucosyl-3'-methoxyluteolin and 6,4'-dihydroxy-3'-methoxyflavone-7-O-glucoside through spectral analyses [6]. Moreover, two flavone glycosides, isoorientin-2''-O- α -L-rhamnoside and 3'-methoxymaysin, were isolated from the *n*-butanol fraction [7]. The results suggest that corn silk extracts, especially the flavone glycosides, have the potential to serve as a valuable and readily accessible bioactive source of natural antioxidants.

CS has recently attracted worldwide attention from the scientific community, as there have been numerous articles published in the last ten to twenty years. These studies describe novel molecules found in the plant matrix that may be linked to the various types of CS. CS's diuretic properties are the most known and well documented in the literature. It is proven that CSF increases water excretion and has an impact on Na⁺ and K⁺ excretion and re-uptake [8–10]. Regarding the urinary tract, CSF has also been proven to exert protective effects on the epithelial renal cells by preventing the formation of oxalate crystals, thus manifesting an antilithiatic effect [11–14]. Moreover, the diuretic effect of CS tea presents ethnopharmacological relevance in traditional Romanian therapy [15].

Another well-documented effect is the antidiabetic one, through its constituent polysaccharides and flavonoids, molecules that exert inhibitory activity on α -glucosidase and through other unknown mechanisms [16,17]. Overweight, diabetic, and/or cardiovascular patients can benefit immensely from CS. Not only does it possess hypoglycemic effects, as mentioned before, but it also promotes weight loss [18] through inhibiting adipocyte formation [19,20]. It also helps to maintain a healthy lipid profile by decreasing levels of low-density lipoproteins, increasing blood high-density lipoproteins, and controlling cholesterol levels [21,22]. CS also helps to prevent inflammatory states and cellular deterioration by maintaining oxidative stress at regular levels in the organism through its potent antioxidant properties [23,24].

The retrieval of bioactive compounds involves a critical extraction step, accomplished through various methods like solvent extraction, mechanical expelling, supercritical extraction, and microwave extraction. Traditional extraction methods pose limitations concerning time, energy consumption, and solvent usage. Ultrasound-assisted extraction (UAE) emerges as a more efficient alternative, capable of extracting bioactive components in a significantly shorter time, at lower temperatures, and with reduced energy and solvent requirements. As a non-thermal extraction technique, UAE is better suited to preserve the functionality of bioactive compounds. However, understanding and optimizing the variables associated with UAE, including frequency, power, duty cycle, temperature, time, solvent type, and liquid/solid ratio, are essential for each specific by-product [25,26]. In the case of corn silk, despite numerous studies delving into its chemical composition and medicinal properties, there exists a noticeable gap in the literature regarding the optimization of extraction conditions. While several investigations have identified and characterized bioactive compounds in corn silk, such as flavonoids, polysaccharides, and antioxidants,

limited research has been conducted on the most effective parameters for extracting these compounds using UAE [26].

Considering this data, the aim of this study was to find the most efficient way to extract CS's bioactive compounds, namely, to obtain a maximum yield in extracted flavonoid and antioxidant phytochemical species, by changing three different extraction parameters: ultrasonic amplitude, time, and ethanol concentration.

2. Materials and Methods

2.1. Standards and Reagents

For the determination of total phenolic content (TPC), the subsequent reagents and standards were utilized: Folin–Ciocalteu reagent (F9252), sodium carbonate (Na_2CO_3) (223530), and gallic acid (G7384). In the evaluation of total flavonoid content (TFC), aluminum chloride (AlCl_3) and quercetin (Q4951) were employed. The measurement of antioxidant capacity necessitated the use of the following reagents and standards: ABTS (A1888), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), DPPH (D9132), sodium acetate (CH_3COONa), acetic acid (CH_3COOH), hydrochloric acid (HCl), ferric chloride hexahydrate ($\text{FeCl}_3 \times 6\text{H}_2\text{O}$), 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) (T1253), and Trolox (238813). Moreover, to assess the enzyme-inhibitory capacity of the samples, the following reagents and standards were employed: α -D-glucoside glucohydrolase from *Saccharomyces cerevisiae* (G5003), Electrophorus electricus cholinesterase (C3389), acarbose (A8980), galantamine (Y0001279), dimethyl sulfoxide (DMSO), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (D8130), 4-nitrophenyl- β -D-glucopyranoside (p-NPG) (N7006), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris base) (TRIS-RO), lipase from porcine pancreas (L3126), *p*-nitrophenyl butyrate (N9876), tyrosinase from mushroom (T3824), 5,5'-Dithiobis(2-nitrobenzoic acid) (D8130), and acetylthiocholine iodide (A5751). In instances where a product number is specified, all the reagents and standards were procured from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

2.2. Plant Materials

The corn used in this study was cultivated in Sighetu Marmăției, Maramureș County, Northern Romania (47°55' N, 23°53' E), utilizing a specific agro-technical approach and adhering to certain conditions. The corn was grown on Luvic clay-illuvial brown soil (podzolic), and the chosen cultivar was Marton Genetics MV 352, FAO 350 corn. The agro-technical approach involved the use of Equip[®] herbicide (Bayer Agrar Schweiz, Muttenz, Switzerland) in the V1 stage of development to ensure optimal growth conditions. The plant material was collected during the harvesting period of corn crops, and it was left to dry in a low-humidity ventilated room on wooden trays on a single layer, using the principles of classical drying, for several weeks until the weight was constant. Afterwards, it was stored in paper bags in a dry environment at the Pharmaceutical Botany Department at the "Tuliu Hatieganu" University of Medicine and Pharmacy in Cluj-Napoca. Before extraction, the plant material was ground into a fine powder for 25 s at 1000 rotations per minute using a laboratory mill (Grindomix[®] GM 200, Retsch GmbH, Haan, Germany). Then, it was passed through a 200 μM sieve to ensure there were no significant size differences between grounded plant particles. The final powdered material was kept in a dry atmosphere, in glass vials, until the extraction phase.

2.3. Extraction Procedures

To explore the effects of various solvents on the chemical composition of the extracts, CS was extracted using two traditional methods, namely infusion and hydroalcoholic solvent extraction (with 50% EtOH and 70% EtOH), in conjunction with a modern technique, ultrasonic-assisted extraction (UAE) using a Polsonic ultrasonic bath. These extraction methods were compared with an optimized UAE (using all the solvents mentioned beforehand and an ultrasonicator). For every type of extraction applied, the following protocols have been used (based on traditional methods and/or Pharmacopoeia regulations):

Infusion: One part of the plant material was mixed with ten parts of boiling water (*w/v*) and then left for 15 min in the ultrasound bath. The final sample was abbreviated as **MSI**.

Hydroalcoholic extraction: One part of plant material was mixed with ten parts of hydroalcoholic solution (*w/v*) in concentrations of 50 and 70%, respectively. The vials were kept in an ultrasound bath for 15 min at a constant temperature of 50 °C. The final samples were abbreviated as **MSE50** and **MSE70**.

Optimized UAE: A D-optimal experimental design was employed, taking into account three distinct process variables: ethanol concentration (0%, 50%, and 80%, where 0% corresponds to pure water), ultrasonic amplitude (30%, 40%, and 50%), and extraction duration (20, 30, and 40 min). During UAE, the microtip was precisely immersed 2 cm into the extract within a 50 mL falcon test tube, and the temperature was rigorously maintained below 25 °C. A magnetic stirrer, operating at 300 rpm, ensured the consistent homogenization of the sample. The final sample was abbreviated **MSO**.

Subsequently, all the samples were subjected to centrifugation and then filtered under pressure through paper filters. Afterwards, hydroethanolic extracts were subjected to a rotary evaporator to eliminate the ethanol, and the remaining aqueous solutions were lyophilized (−50 °C, 10 Pa, and 72 h) (Freeze Dryer BK-FD18S, Biobase, Jinan, China) to obtain dry powders. The final extracts have been kept in a dry place until further analysis in a desiccator. Before undergoing the biochemical assays, the dry samples were redissolved in either ethanol (for the antioxidant assays) or water (for the enzyme inhibition assays).

2.4. Total Phenolic Content (TPC)

Total phenolic content (TPC) was assessed as a preliminary phytochemical analysis using a previously described method based on the Folin–Ciocalteu method and adapted to 96-well plates [27]. Samples were diluted, and the determinations were realized in triplicate. They were mixed with Folin–Ciocalteu reagent and diluted beforehand (1:9, *v/v*). Following the incubation time (180 s), a solution of 1% sodium carbonate (80 µL) was added to the mix and then left to incubate once again for 30 min at room temperature. After this, the samples' absorbance was read at 760 nm using a SPECTROstar Nano Multi-Detection Microplate Reader (BMG Labtech, Ortenberg, Germany). The results were characterized as milligrams of gallic acid equivalents per gram of lyophilized extract (mg GAE/g extract).

2.5. Total Flavonoid Content (TFC)

The total flavonoid content of the CS samples was identified using a previously characterized method, the aluminum chloride method [28]. A quantity of 100 µL of CS sample was mixed with 100 µL of an AlCl₃ 2% solution. Following an incubation time of 15 min at room temperature, the samples' absorbances were read at 420 nm. Results were expressed as mg of Quercetin Equivalents (QE) per gram of lyophilized extract (mg QE/g extract). This method has also been determined as an output variable during DoE, where it has been expressed as mg QE/mL extract (to facilitate the quantification and comparison of experimental runs).

2.6. In Vitro Antioxidant Activity Assays

For the antioxidant activity assays, all dried CS extracts were re-dissolved in 50% ethanol (*v/v*) and diluted ten times to achieve an absorbance that was within linear limits. The assays described below were executed in compliance with methods that were already described by Babotă et al., adapted to microplate readers [29].

Radical scavenging activity of CS was examined using ABTS⁺ radicals and expressed as milligrams of Trolox Equivalents (TE) per gram of lyophilized extract (mg TE/g extract). Briefly, 200 µL of 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS) were mixed with 20 µL of CS sample, followed by an incubation time of 6 min. The absorbances

were measured at 760 nm against a solvent blank. This method has also been used as a response during DoE, where it has been expressed as mg TE/mL extract.

The ferric-reducing antioxidant power of CS was examined using a reagent that consists of ferric chloride (20 mM) mixed with hydrochloric acid (40 mM) and tri-2-pyridyl-s-triazine (TPTZ, 10 mM), using a ratio of 1:1:10 (*v/v/v*). The reaction mix was left to incubate for at least half an hour at room temperature, deprived of light. 175 µL of the obtained FRAP reagent were mixed with 25 µL of CS sample, followed by half an hour of incubation at room temperature in the dark. The samples' absorbance was measured at 593 nm. Results were reported like the aforementioned methods, as mg TE/g extract.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability of the CS samples was quantified as follows: 30 µL of CS samples were left to incubate in the dark for 30 min with a methanolic radical solution of DPPH reagent (0.002%). Afterwards, the absorbances were read at 517 nm against the solvent blank. Results were conveyed as mg TE/g extract.

2.7. In Vitro Enzyme Inhibition Assays

CS dried samples were re-dissolved in the various buffers used before doing the enzyme inhibition assays, and for each sample, 5 dilutions (concentrations ranging from 1 mg/mL to 10 mg/mL, and in some cases, from 0.1 mg/mL to 1 mg/mL) were prepared.

For the calculation of individual inhibition percentages (I%), the following formula has been applied, as per previous studies [30]:

$$I (\%) = \frac{100 * (Abs_{control} - Abs_{sample})}{Abs_{control}} \quad (1)$$

where $Abs_{control}$ represents the maximal absorbance of the reagents and Abs_{sample} represents the absorbance of the samples, after subtracting the reagent and sample blanks. Final results are expressed in terms of IC_{50} , as µg/mL, using an inhibitory logarithmic curve.

2.7.1. Inhibition of α -Glucosidase

α -glucosidase is a well-known enzyme with implications for the pathways of diabetic metabolism. The assay was executed on a fungal version of the enzyme, according to a previously described method [31]. All reagents and solutions used for this assay were prepared in phosphate buffer (pH 6.8, 0.1 M). A small quantity of enzyme solution with a concentration of 2 U/1 mL was combined with 50 µL of CS sample and left to incubate for at least 10 min in the dark at 37 °C. *p*-nitrophenyl- α -D-glucopyranoside (α -pNPG) is the substrate for this assay, as it gets hydrolyzed by α -glucosidase and helps measure the percentage of enzyme inhibition. A total of 50 µL of 2.5 mM α -pNPG was added to the mixture and left to incubate for another 10 min, hidden from light. The microplate was then read spectrophotometrically at a wavelength of 405 nm, using acarbose, and was used as a positive control.

2.7.2. Inhibition of Acetylcholinesterase

Acetylcholinesterase (AChE) is an endogenous enzyme that recycles acetylcholine, an endogenic neurotransmitter. Molecules and extracts that inhibit acetylcholinesterase are known to be valuable in neurodegenerative disorders, such as Alzheimer's disease, where there is a deficit of acetylcholine. Tris-HCl buffer (50 mM, pH 8) was prepared and used as a solvent for all reagents, as described before [32]. In a 96-well microplate, a mixture consisting of 25 µL of CS extract, 25 µL of AChE (0.078 U/mL), 125 µL of substrate (Ellman's reagent, DTNB, 0.9 mM), and 50 µL of buffer was added and left to incubate in a dark place at 37.5 °C for 15 min. Following this, 25 µL of acetylthiocholine iodide solution (ATCI, 4.5 mM) was added, and the mix was left to incubate for another 10 min. The microplate was then read at 405 nm using galantamine as a positive control.

2.7.3. Inhibition of Tyrosinase

Fungal tyrosinase was used to determine whether CS has beneficial effects on skin conditions. A 66 mM phosphate buffer was prepared and used as a solvent for all reagents, as described by an antecedent method [33]. Wells were filled with a mixture consisting of 40 μ L mushroom tyrosinase (46 U/mL), 40 μ L CS diluted sample, and 120 μ L phosphate buffer that was then left to incubate for 20 min at room temperature in the dark. After this, 40 μ L of L-DOPA (2.5 mM), the substrate, was added, and the mix was left again to incubate for 20 min under the same conditions. Following this, the absorbances were read at 475 nm, and the values of IC₅₀ were calculated using kojic acid as a positive control.

2.7.4. Inhibition of Lipase

Inhibition of porcine pancreatic lipase was tested for CS samples using a method described by Kim et al. [34]. A Tris buffer consisting of 100 mM Tris-HCl and 5 mM CaCl₂ with a pH of 7.0 was prepared and used as a solvent for the enzyme. A total of 20 μ L of this solution was mixed with 20 μ L of the sample and left to incubate for 15 min at 37.5 °C in the dark. Following this, 5 μ L of *p*-nitrophenyl butyrate (*p*-NPB), the substrate, was added, and the mixture was left to incubate for another 30 min under the same conditions. Lipase inhibition activity was then calculated through the absorbances read at 405 nm and expressed as IC₅₀ values (μ g/mL), using orlistat as a positive control.

2.8. Design of Experiments (DoE)

Design of Experiments (DoE) was accomplished using special software, namely MODDE 13.0 (Sartorius Stedim Data Analytics AB, Umeå, Sweden) [27]. Since the aim of the DoE was to maximize the recovery of bioactive compounds, a D-optimal type of design has been developed. As a result, the software allowed the automatic identification of the best combination of parameters from the experimental candidate set. Data analysis has been accomplished with the help of the same software, using regression and statistical analysis, and has been followed by the optimization function to search for candidate combinations of parameters that can induce potential maximal yields [35,36].

In addition to employing statistical methods in the Design of Experiments (DoE), the outcomes of *in vitro* assays were presented as the mean $\pm$ standard error based on experiments conducted in triplicate. Statistical analysis of the data was carried out using GraphPad Prism 9.3. software. To evaluate significant differences ($p < 0.05$) between species, a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test was utilized.

3. Results and Discussion

3.1. Experimental Design and Optimization

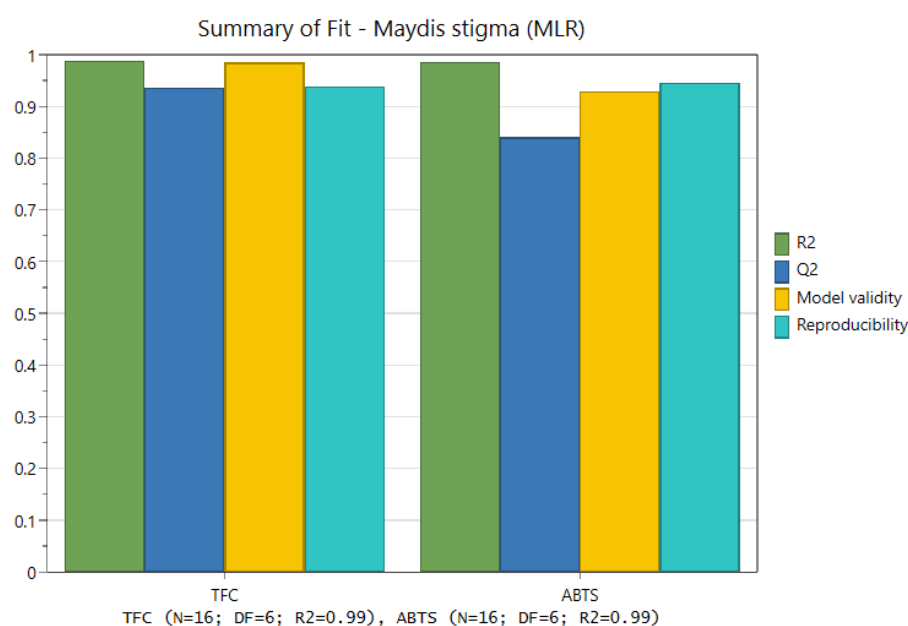
3.1.1. Experimental Design

The D-optimal DoE that has been generated for the current optimization process comprises three quantitative factors, namely EtOH concentration (0, 50, and 80%; in this case, 0% corresponds to pure water), ultrasound amplitude (30, 40, and 50%), and extraction time (20, 30, and 40 min). Considering the relevant composition of flavone and other flavonoid classes in CS, we have chosen TFC (determined through the AlCl₃ method) as the dependent variable. Furthermore, in order to assess and maximize the potential antioxidant effect of the final extract, we have included an additional dependent variable, namely the TEAC values determined through the ABTS radical assay (Table 1). This dual-dependent variable approach aimed to not only evaluate the flavonoid content but also maximize the potential antioxidant effect of the final extract. By utilizing this comprehensive experimental design, we sought to optimize the extraction process for CS, ensuring both a robust flavonoid profile and potent antioxidant properties in the resulting extract. Figure 1 contains a graphical representation of the statistical parameters for the experimental model that was developed.

Table 1. Experimental values for responses (TFC and ABTS) obtained during DoE for each of the chosen independent factors (EtOH concentration, amplitude, and time).

Exp. No.	EtOH (%)	Amplitude (%)	Time (min)	TFC (mg QE/g dw)	ABTS (mg TE/g dw)
1.	0	30	20	1.90	10.22
2.	50	30	20	3.82	16.00
3.	0	50	20	2.17	10.84
4.	80	50	20	2.58	10.13
5.	50	50	20	4.15	17.77
6.	80	40	20	2.33	8.75
7.	0	30	40	2.52	10.72
8.	0	50	40	3.25	10.79
9.	80	50	40	2.66	10.01
10.	50	40	40	4.04	16.96
11.	50	30	30	3.69	15.69
12.	80	50	30	2.57	9.44
13.	0	40	30	2.27	11.95
14.	50	40	30	4.28	16.74
15.	50	40	30	3.87	16.12
16.	50	40	30	4.01	17.67

Notes: EtOH represents the concentration of hydroethanolic mix (the value of 0 corresponds to water, *v/v*).

**Figure 1.** Graphical representation of parameters used for the statistical analysis of the DoE. All parameters are expressed for the two responses (TFC and ABTS).

The assessment of model fitting involved the examination of conventional and highly dependable statistical metrics. These metrics encompassed R^2 , signifying the goodness of fit by elucidating the proportion of response variation accounted for by the model. Additionally, Q^2 , a metric assessing the model's predictive capabilities, was considered to ascertain its performance in making accurate predictions. The evaluation further included the utilization of an ANOVA test and the determination of model reproducibility, a measure derived exclusively from the replicates explicitly defined in the design matrix. As can be seen from Figure 1, these parameters are all notably elevated, indicating a robust and reliable model fit. The substantial R^2 value signifies that a significant portion of the response variation is well-explained by the model, underlining its goodness of fit. Additionally, the elevated Q^2 value reflects the model's proficiency in making accurate predictions. The ANOVA test results further validate the model's statistical significance, while the

demonstrated model reproducibility underscores the reliability of the outcomes derived meticulously from the specified replicates in the design matrix. In summary, the depicted parameters in Figure 1 showcase the model's commendable performance and its capability to effectively capture and predict the observed phenomena.

3.1.2. The Influence of Experimental Factors on TFC and ABTS

The coefficient graph shown in Figure 2 can be used to identify the most relevant impact of individual and interaction factors on the assayed dependent parameters (TFC and ABTS). For this analysis, a confidence level of 0.90 has been chosen. Regarding TFC, the following factors have been statistically significant ($p < 0.05$): amplitude, extraction time, the quadratic interaction of EtOH concentration (sol $\times$ sol), and the interaction factor between EtOH concentration and time (sol $\times$ time). Regarding the antioxidant activity using the ABTS radical, the following factors have been statistically significant ($p < 0.05$): EtOH concentration, amplitude, and the quadratic interaction of EtOH concentration (sol $\times$ sol). There were no significant interaction factors identified, which suggests that in this case there was no synergic effect on the yield of antioxidant species.

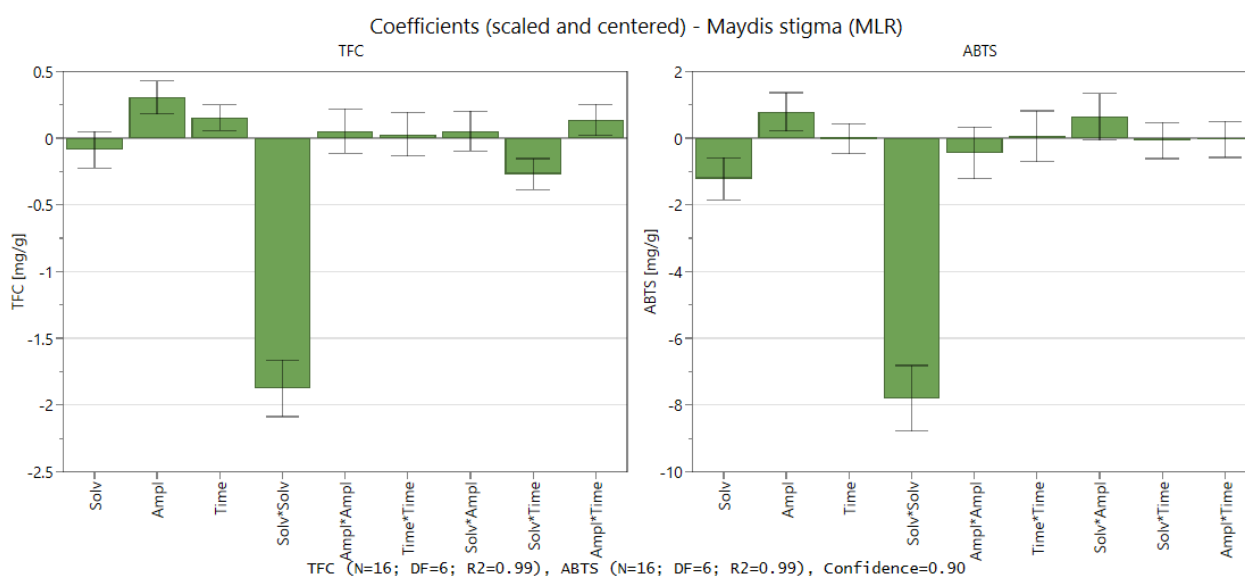


Figure 2. Coefficient plots representing the influence of individual and interaction factors on the outcome (quantified through TFC and ABTS methods) of the extraction process. Note: * represents the interaction between two factors.

Both the extraction of flavonoids and the antioxidant activity from corn silk were influenced by various factors. Ethanol concentration plays a crucial role in the extraction mix, as was previously suggested by various studies. Specifically, the utilization of water and ethanol mixtures induces a synergistic effect; therefore, an optimal concentration is necessary to assure the efficient recovery of flavonoids [37,38]. Different concentrations of ethanol can influence the solubility of various compounds, including antioxidants [39]; this fact was also noticed for ABTS as a dependent variable, where raising the concentration of ethanol in the extraction medium negatively influenced the *in vitro* antioxidant activity. To sum up, optimal ethanol concentrations may enhance the extraction of antioxidant and flavonoid species from corn silk, contributing to higher antioxidant activity in the final extract. Higher ultrasonic amplitude facilitates vigorous mixing and cavitation, improving the mass transfer of flavonoids [40], and this parameter showed a positive influence on both factors, TFC and ABTS. Extended extraction times enhance the thorough recovery of bioactive compounds; however, it may lead to the extraction of unwanted compounds or even to flavonoid degradation [41]. The extractive time was statistically significant only in the case of TFC. The response surface plot (RSP) in Figure 3 highlights the fact that ethanol

concentration shows a quadratic interaction in both cases, manifesting a negative influence; moreover, it induces non-linear effects, suggesting optimal ethanol concentrations can enhance solubility and extraction efficiency. The interaction factor between ethanol concentration and time considers their joint influence, necessitating a balanced approach for optimal extraction efficiency, and it presented a statistically significant negative influence only in the case of TFC. This result suggests an analogous theory as to the influence of ethanol concentration, indicating that the combination of higher concentration and time leads to a final decrease in flavonoid yield. Thus, it is highlighted once again that the identification of the optimal mix of parameters is crucial and dependent on the specific phytochemical class of interest. The extraction parameters that we have analyzed collectively impact physical and chemical processes, including solubility, mass transfer [42], and cell structure breakdown [43].

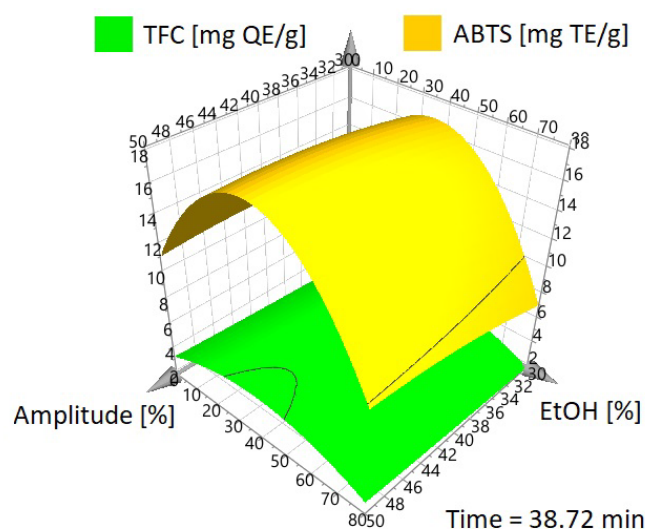


Figure 3. Three-dimensional response surface plot (RSP), showing the influence of model parameters on the two dependent variables (TFC and ABTS values) at the time period that was determined to be optimal (38.72 min).

3.1.3. Optimization of Extraction

There have been some previous studies regarding different optimization of extraction techniques applied to CS, aiming to optimize the extraction yield of flavonoids [44], polysaccharides using simple extraction [45], or pulsed electric field extraction [46]. Furthermore, we have chosen to develop a mathematical model using two dependent variables, specifically TFC and ABTS, aiming to maximize (a) the total yield of flavonoid species and (b) the total antioxidant activity of the phytochemicals that are successfully extracted.

To achieve this optimization, we employed the optimizer function within the MODDE 13.0 software, specifying the objective to maximize both TFC and ABTS responses. The predetermined maximal values were set at 4.71 mg/g for TFC (with a probability of failure of 8.1%) and 18.16 mg/g for ABTS (with a probability of failure of 3.4%). Through this optimization process, we identified a set of independent factors that could yield the highest outcomes. Specifically, the optimal combination involved a 37.89% ethanolic concentration, a 48.58% ultrasound amplitude, and an extraction time of 38.72 min. As it has been mentioned, this optimal time period was used for mapping the RSP in Figure 3.

Ethanol concentration, ultrasound amplitude, and extraction time are crucial factors in the extraction process. A specific ethanol concentration of 37.89% was deemed optimal, representing a balance between insufficient and excessive extraction. The chosen amplitude of 48.58% optimized mechanical forces for effective mass transfer of bioactive compounds, such as flavonoids. An extraction time of 38.72 min achieved a balance of extraction, allowing sufficient penetration of the solvent into plant material without risking unwanted extraction or degradation. This combination of parameters represented an optimal set of

conditions, systematically determined to maximize extraction efficiency for both flavonoid content using AlCl_3 (abbreviated TFC) and Trolox equivalent antioxidant capacity using the ABTS assay.

3.2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Different plant parts of *Zea mays* are known for containing secondary metabolites, especially polyphenolic species, which are typical for plant matrices [47]. In this study, we have used the Folin–Ciocalteu method for the in vitro approximate quantification of total phenolic species, and the results are presented in Table 2. As it can be noted, the highest content was determined for MSO (50.69 ± 0.36 mg GAE/g extract), followed by MSE70 and MSI (no significant difference), and lastly by MSE50, with the lowest content (39.73 ± 1.48 mg GAE/g extract). In accordance with our results, Dong et al. have identified the highest TPC in CS (399.4 ± 18.9 mg GAE/100 g extract), in comparison to corncob and cornhusk, using extraction with an 80% (v/v) hydroethanolic mix [47].

Table 2. Results for the TPC, TFC, and in vitro antioxidant activity (through ABTS-TEAC, DPPH, and FRAP assays).

Extract	TPC (mg GAE/g Extract)	TFC (mg QE/g Extract)	ABTS (mg TE/g Extract)	DPPH (mg TE/g Extract)	FRAP (mg TE/g Extract)
MSI	39.73 ± 1.48^b	8.69 ± 0.17^c	60.95 ± 1.56^c	24.80 ± 0.13^c	65.70 ± 5.11^c
MSE50	33.79 ± 1.51^c	8.68 ± 0.43^c	63.60 ± 1.37^b	22.62 ± 0.90^d	112.54 ± 2.86^a
MSE70	40.83 ± 1.89^b	12.43 ± 0.05^b	64.60 ± 1.36^b	33.747 ± 0.98^b	116.94 ± 2.01^a
MSO	50.69 ± 0.36^a	23.54 ± 0.01^a	78.33 ± 0.94^a	65.21 ± 0.09^a	101.02 ± 0.72^b

Notes: results have been expressed as average ($n = 3$) and standard deviation; individual superscript letters indicate significant statistical differences among every type of extract (every column).

CS is mentioned in the scientific literature for standing out due to the content of flavonoid species, such as flavone glycosides [47,48]. The aluminum chloride method is applied to the preliminary quantification of flavones and flavonols. It is worth mentioning that for the complexation of the compounds with Al^{3+} to occur, certain free hydroxyl groups are necessary [49]. Nonetheless, the difference between MSI and MSE50 has been insignificant, but MSE70 and MSO showed significantly higher values. The identification of the highest value of TFC for MSO (23.54 ± 0.01 mg QE/g extract) underscores the fact that the optimization process has been successful. Similarly, Nawaz, Aslam, and Muntaha have obtained a 0.43 ± 0.08 g CE/100 g dw value for a consecutive extraction of crude methanolic CS extract [50]. Furthermore, Dong et al. have identified a high TFC value for CS extract (956.8 ± 37.8 mg RE/100 g dw) using extraction with an 80% (v/v) hydroethanolic mix [47].

3.3. Total Antioxidant Capacity

Antioxidants are helpful molecules that guard the body against radical species, entities that wreak havoc in certain biochemical pathways if left unattended. CS's antioxidant activity is hugely documented in the literature, with many authors acknowledging its potency [51–54].

The results of in vitro antioxidant activity are shown in Table 2. As it can be noted, MSO activity was significantly higher in comparison to the other extracts, with the only exception of FRAP, where MSE70 showed the highest value (116.94 ± 2.01 mg TE/g extract). Finally, a similar trend as in the case of TFC has been observed with ABTS for the MSO sample in comparison to MSI, MSE50, and MSE70. This achievement highlights the efficacy of the optimized model that we have developed, indicating the applicability of the optimal parameters.

3.4. Enzyme Inhibition Potential of Corn Silk

Most pathology mechanisms usually imply one irregular-working enzyme, something that either works too much or something that does not work at all, or even something that

works just fine but inhibiting or inducing proves helpful in that disease, so in vitro assays are a helpful tool to explore whether one vegetal matrix has any potential in a specific disease or if it is fit to undergo further modulation, albeit at the extraction level or at a molecular level. This approach is fit for CS, too.

For example, previous studies have shown that CS is a fair inhibitor of α -glucosidase, an enzyme found in the intestines meant to break down bigger polysaccharide molecules into glucose, inducing higher blood sugar levels. Patients with diabetes mellitus benefit from α -glucosidase inhibitors as they help them maintain normal blood sugar levels. Some authors concluded that the molecules present in CS responsible for the antidiabetic effects are polysaccharides and flavonoids, but, to the best of our knowledge, none of them explored the individual impact of each of these two.

Polysaccharides are extensive molecules that are usually best extracted while using aqueous solutions, solutions where the pH is either acidic or alkaline, or while using ultrasounds or microwaves, but this is hugely dependent on the type of polysaccharide and on its source [55]. Polysaccharides usually precipitate when put in contact with alcohol, and, considering the solvents of our choice were water and ethanolic solutions, this leaves room to examine and correlate the inhibitory effect on α -glucosidase with the principal constituent—flavonoids in alcoholic extracts, polysaccharides in aqueous solutions.

As it can be observed in Table 3, the most relevant inhibitory activity that we have identified is the anti-glucosidase activity, and the samples MSE70 and MSO showed the best activity. According to the statistical analysis, there was no significant difference between these two samples ($p > 0.05$). The weakest activity has been identified for MSI, where the IC_{50} was more than double in comparison to MSE70 and MSO. Comparing these results with the therapeutic standard (Acarbose, with an IC_{50} of $52.04 \pm 2.30 \mu\text{g/mL}$), we can conclude that the hydroethanolic and optimized extraction yielded the most potent mix of compounds with in vitro antidiabetic activity. Probably, this yield could be explained based on the selective extraction of flavonoids in the presence of ethanol, which is a less polar solvent in comparison to water [56].

Table 3. IC_{50} values (expressed as μg of lyophilized powder/mL of solution) for the enzyme inhibitory activity for the four types of extracts.

Enzyme	Control	MSI	MSE50	MSE70	MSO
α -glucosidase	52.04 ± 2.30	1101.33 ± 36.46^c	451.6 ± 7.86^b	401.67 ± 20.84^a	411.67 ± 25.39^a
Lipase	68.17 ± 3.74	$10,079.33 \pm 581.97^b$	1508 ± 145.08^a	1535.67 ± 72.43^a	1519.67 ± 209.04^a
AChE	2.31 ± 0.14	ND	1166.4 ± 399.38^a	1222.67 ± 285.32^a	ND
Tyrosinase	56.27 ± 2.03	ND	ND	ND	ND

Notes: Different lowercase letters signify the presence of significant statistical difference among corn silk extracts. ND = no data/no activity; control standards: acarbose (α -glu), orlistat (Lip), galantamine (AChE), and kojic acid (Tyrosinase).

Another analysis done on CS extracts was the AChE inhibition assay. There was previous research done on CS where *ent*-kaurane diterpenoids were found, molecules such as kauralexin B1-B3 or kauralexin A1-A3, chemical entities with beneficial effects in Alzheimer's, as they were proved to manifest neuroprotective effects [57,58], so testing the inhibition of acetylcholinesterase was justified. As these compounds are non-polar, we did not expect to have these molecules extracted, but we did get results regarding AChE inhibition, so this proves a plus in effect when talking about CS benefits in neurological disorders. In our study, we have been able to identify only weak activity against AChE, with IC_{50} values of 1166.4 ± 399.38 and $1222.67 \pm 285.32 \mu\text{g/mL}$ for MSE50 and MSE70, respectively. There was no significant statistical difference between these two samples ($p > 0.05$).

Pancreatic lipase (PL) is an enzyme released into the duodenum that is crucial for the breakdown and assimilation of fats. In fact, PL is believed to be responsible for hydrolyzing 50–70% of dietary fats. Considering the pivotal role of PL in lipid digestion, it

emerges as a promising target for preventing obesity. The assessment of lipase inhibition potential provided valuable insights into the enzyme-inhibitory activity of the four types of extracts. Orlistat, a known lipase inhibitor, exhibited an IC_{50} value of $68.17 \pm 3.74 \mu\text{g/mL}$, establishing a baseline for comparison. In contrast, the natural extracts displayed varying degrees of inhibitory potential. The MSI extract demonstrated an IC_{50} value of $10,079.33 \pm 581.97$, while MSE50, MSE70, and MSO extracts exhibited values of 1508 ± 145.08 , 1535.67 ± 72.43 , and 1519.67 ± 209.04 , respectively. These results underscore the potential of MSE50, MSE70, and MSO extracts as inhibitors of pancreatic lipase. However, the results are modest in comparison with orlistat. In an animal study that focused on the effects and mechanisms of action of high maysin corn silk extract on body weight and fat deposition in mice, the high-fat corn silk (HFCS) group, receiving a high-fat diet along with high maysin corn silk extract, demonstrated significant reductions in body weight compared to the high-fat (HF) group that did not receive the corn silk extract [59]. Furthermore, kidney fat and epididymal fat pad weights were also significantly lower in the HFCS group. These findings suggest a promising role for corn silk extracts in modulating lipid metabolism and combating obesity.

Tyrosinase, a multi-copper enzyme with a widespread distribution across various organisms, holds significance in both melanogenesis and enzymatic browning processes. As a result, tyrosinase inhibitors are of interest for use in the cosmetics and medicinal fields as agents for depigmentation. Additionally, they hold potential in the food and agriculture industries as compounds to prevent browning [60]. Various screening methods have been employed to develop a range of natural, semi-synthetic, and synthetic inhibitors for this purpose. Research into its chemical composition led to the discovery of a novel flavone (Silkone A) and three known flavonoids, demonstrating antioxidant capacity and moderate inhibitory activities against the enzyme tyrosinase [61]. In addition, corn silk extract displayed significant inhibitory effects on melanin production in Melan-A cells without suppressing tyrosinase activity [62]. Corn silk extract exhibited tyrosinase inhibition activity with an IC_{50} value of $12.45 \mu\text{g/mL}$ [63]. Despite the presented results from the literature, the evaluation of four types of extracts did not show any tyrosinase inhibition activity. The variability in tyrosinase inhibitory activity observed may be attributed to factors such as variations in extraction methods, plant sources, and the inherent variability of natural products. To gain a more comprehensive understanding of the tyrosinase inhibitory potential of corn silk, further research is warranted to address these inconsistencies and identify optimal conditions for reliable and reproducible results.

4. Conclusions

Corn silk, or *Maydis stigma*, plays a vital role in corn plant reproduction by aiding pollen transfer. This research endeavors to provide valuable insights into the systematic optimization of corn silk processing, laying the foundation for its broader utilization in industries such as pharmaceuticals, cosmetics, and functional foods. MSO, representing an optimized corn silk ultrasound-assisted hydroethanolic extract, performs best in antioxidant assays, while MSE70 and MSO show potent anti-glucosidase activity, crucial for diabetes management. AChE inhibition hints at neurological benefits, and pancreatic lipase inhibition suggests potential in obesity prevention. Despite the literature support, tyrosinase inhibition is inconsistent, necessitating further research. In conclusion, corn silk's diverse benefits, from renal health to diabetes management, are underscored. Optimization enhances bioactive compound yields, indicating broader applications in health and industry. Future research studies should study the specific phytochemical composition of the developed extract, using chromatography and mass spectrometry, to potentially identify the flavonoid fractions that are recovered and to confirm the correlation between the polarity of the solvent mixture and the selectivity of flavonoid species that are recovered.

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