

Process-driven Valorization of *Sonchus oleraceus* Leaves: Optimization of Microwave-assisted Aqueous Extraction of Antioxidant Phytochemicals

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Received December 13, 2025; Revised January 15, 2026; Accepted January 22, 2026

Abstract This study developed a green strategy to valorize *Sonchus oleraceus* leaves by optimizing microwave-assisted aqueous extraction of antioxidant phytochemicals. A three-factor Box–Behnken design quantified the individual and interactive effects of microwave power, extraction time, and particle size on total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and antioxidant capacity assessed by DPPH and ABTS. Extraction was performed in water at a solid-to-liquid ratio of 1:20 (w/v) using a pulsed microwave regime (5 s on, 15 s off) to limit boiling, followed by centrifugation and filtration. Quadratic models were retained for all responses, showing improvement over simpler structures and acceptable predictability across the design space. Experimental responses covered wide ranges, confirming strong process sensitivity. Numerical desirability optimization predicted an operating optimum at 378.39 W, 18.81 min, and 174 μm , with high predicted phytochemical yields and antioxidant activities. Validation at practical setpoints of 360 W, 20 min, and 180 μm produced TPC 99.82 ± 0.06 mg GAE/g ds, TFC 59.78 ± 0.09 mg RE/g ds, TTC 18.73 ± 0.04 mg TAE/g ds, DPPH 4.40 ± 0.04 mM TE/g ds, and ABTS 4.70 ± 0.05 mM TE/g ds. Taken together, these conditions represent a balanced operating window that supports efficient mass transfer while preserving redox-active phytochemicals under food-grade constraints.

Keywords: Process optimization, Phenolic compounds, Antiradical activity, Leaf powder processing

Cite This Article: William Tchabo, Emmanuel Akdowa Panyoo, Spéro Ulrich Koba Edikou, Ibrahima Kaba, Mohamed Lamine Dabo, Durand Dah-Nouvlessounon, and Joseph Dossou, “Process-driven Valorization of *Sonchus oleraceus* Leaves: Optimization of Microwave-assisted Aqueous Extraction of Antioxidant Phytochemicals.” *American Journal of Food Science and Technology*, vol. 14, no. 1 (2026): 1-12. doi: 10.12691/ajfst-14-1-1.

1. Introduction

Medicinal plants represent a major source of bioactive compounds with documented nutritional and health-promoting functions and remain central to traditional healthcare systems [1]. Among these, *Sonchus oleraceus*, widely utilized in African ethnomedicine, has been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities, with all parts of the plant considered pharmacologically active [2,3]. These biological effects are largely attributed to the plant’s richness in phytochemicals, which are increasingly recognized not only for their therapeutic relevance but also for their value as functional nutritional components [4].

Phytochemical investigations of *Sonchus oleraceus*

have revealed a diverse profile of secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, steroids, saponins, and glycosides [3,5]. Advanced metabolomic studies have identified more than fifty compounds in its fruits [6]. Among these, molecules such as rutin and synephrine have demonstrated strong inhibitory activity against α -amylase and α -glycosidase [2]. Together, these compounds underpin the reported antioxidant, antimicrobial, anticancer, and antidiabetic properties of *Sonchus oleraceus* [3]. Despite this growing biochemical and pharmacological evidence, systematic investigations of the extraction biochemistry of this species remain limited. In particular, few studies have examined how extraction conditions influence phytochemical recovery, antioxidant capacity, and process efficiency within a scalable, food-oriented framework.

From a food engineering and nutrition perspective, the

increasing demand for functional foods has intensified interest in plant-derived extracts enriched in antioxidant phytochemicals [7]. Functional foods are designed to provide physiological benefits beyond basic nutrition, notably through the regulation of oxidative stress, glucose metabolism, and inflammatory responses. Antioxidant-rich extracts contribute to these effects while also improving food stability by inhibiting oxidative deterioration [8]. Consequently, there is a clear need to develop extraction strategies that ensure high phytochemical yield, preserved bioactivity, and compatibility with food-grade processing conditions.

The extraction process is a critical determinant of phytochemical yield, antioxidant functionality, and bioaccessibility of plant-derived ingredients [9]. However, studies on *Sonchus oleraceus* have largely relied on solvent-based or qualitative approaches, with limited emphasis on process optimization or extraction kinetics. In contrast, microwave-assisted aqueous extraction (MAAE) has emerged as an efficient, process-intensified technique. It enhances mass transfer through rapid volumetric heating, improved solvent penetration, and disruption of plant cellular structures [10,11]. Microwave energy interacts directly with polar molecules, accelerating the release of intracellular phytochemicals while significantly reducing extraction time and solvent usage compared to conventional thermal methods [12].

In line with sustainable food processing principles, water represents a green, safe, and industry-relevant extraction medium [13]. It is non-toxic, inexpensive, and fully compatible with nutritional and functional food applications. Nevertheless, the efficiency of MAAE is highly dependent on operating conditions. Microwave power level (MP) controls the rate of heating and the extent of matrix disruption, but excessive power may lead to thermal degradation of sensitive phytochemicals [14]. Extraction time (ET) governs mass transfer and equilibrium attainment, while prolonged microwave exposure may promote oxidative or structural degradation [15,16]. Particle size ratio (PS) affects solvent accessibility, effective surface area, and microwave absorption uniformity, thereby influencing extraction kinetics and yield [17]. Despite their importance, the combined and interactive effects of these parameters on the extraction performance of *Sonchus oleraceus* remain poorly understood.

In this context, the present study adopts a process-driven valorization approach to optimize MAAE of antioxidant phytochemicals from *Sonchus oleraceus*. Response Surface Methodology (RSM) was employed to evaluate the effects of MP, ET, and PS as key process variables. Extraction performance was assessed through total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and antioxidant activity

determined by 1,1 diphenyl 2 picrylhydrazyl (DPPH) and 2,2' azino bis (3 ethylbenzothiazoline 6-sulfonic acid) (ABTS) assays. By explicitly linking processing conditions to phytochemical recovery and antioxidant functionality, this work aims to establish optimized, sustainable extraction conditions and support the valorization *Sonchus oleraceus* of as a functional ingredient for nutrition-oriented food applications.

2. Materials and Methods

2.1. Materials

Fresh leaves of *Sonchus oleraceus* were harvested in Ngaoundere, Adamawa Region, Cameroon. The plant material was authenticated by a botanical taxonomist at the Botanical Survey, Department of Science, University of Ngaoundere. The leaves were dried in a forced convection oven (UF110, Memmert GmbH, Schwabach, Germany) at 40 °C for 24 h. The dried leaves were milled using a hammer mill (FitzMill L1A, Fitzpatrick, Waterloo, ON, Canada). The powder was sieved using stainless steel meshes of 100, 250, and 400 µm in a laboratory sieve apparatus (BK-TS200, Biobase, Jinan, China). The size fractions were then packed in airtight polyethylene bags and stored at room temperature until further use. Reagents and chemicals, all analytical grade, were obtained from Sinopharm Chemical Reagent Co., Ltd. (China).

2.2. Microwave-assisted Aqueous Extraction

A household microwave oven (H30MOMS9HG, Hisense, Qingdao, China) was used for MAAE. Particle size fractions selected according to the experimental design, were dispersed in distilled water at a solid to liquid ratio of 1:20 (w/v) in an Erlenmeyer flask. The suspensions were irradiated at the power levels and extraction times specified by the design, using a pulsed regime of 5 s on followed by 15 s off to limit boiling [18]. After treatment, samples were centrifuged (BKC-TH21, Biobase, Jinan, China) at 8000 rpm for 15 min, then filtered. The filtered extracts were stored at -29 °C prior to analysis.

2.3. Experimental Design

RSM was applied to model and optimize aqueous extraction of phytochemicals from *Sonchus oleraceus* leaf powder. A three factor Box Behnken design (BBD) at three levels was used. MP (X_1), ET (X_2), and PS (X_3) were selected as the independent variables. Factor levels were defined from preliminary trials and are tabulated in Table 1.

Table 1. Coded and actual values of the independent variables in Box-Behnken Design

Independent variables	Symbols	Coded levels		
	X	-1	0	1
MP	X_1	360	630	900
ET	X_2	8	14	20
PS	X_3	100	300	500

MP -Microwave power (W), ET-Extraction time (min), and PS-Particle size (µm).

The experimental factors were coded according to the following equation:

$$x_i = \frac{X_i - X_{cp}}{\Delta X_i}; i = 1, 2, 3 \quad (\text{Eq.1})$$

Where x_i is the coded level of factor i , X_i is the real value, X_{cp} is the centre point value, and ΔX_i is the step change between coded levels 0 and 1.

The responses comprised TPC, TFC, and TTC alongside antioxidant activity determined by DPPH and ABTS assays. The dataset was fitted by multiple regression to a second order polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (\text{Eq.2})$$

Where Y denotes the predicted response, β_0 is the constant term, β_i , β_{ii} and β_{ij} correspond to the linear, quadratic, and interaction coefficients, respectively, and X_i and X_j are the independent variables.

2.4. Analytical Assays

2.4.1. Total Phenolic Content Assay

The TPC was quantified according to a slight modified Folin-Ciocalteu method, as described by Tchabo, Ma [19]. Concisely, the assay was performed by combining 0.5 ml of Folin-Ciocalteu reagent with 0.1 ml of filtered supernatant and 7.9 ml of deionized water. Following an incubation period of 1-8 min, 1.5 ml of sodium carbonate solution (20% w/v) was introduced. The reaction mixture was then incubated at $40 \pm 2^\circ\text{C}$ for 30 min in a water bath. Absorbance was then measured at 765 nm. Results were expressed as mg GAE/g ds.

2.4.2. Total Flavonoid Content Assay

The TFC was quantified using a modified aluminum chloride colorimetric assay, adapted from the method described by Tchabo, Ma [19]. Briefly, 1 mL of the filtered sample supernatant was combined with 4 ml of deionized water. Subsequently, 0.3 ml of a 5% (w/v) NaNO_2 solution was added to a 10 ml volumetric flask. After a 5 min reaction period, 0.3 ml of a 10% (w/v) AlCl_3 solution was introduced. One min later, 2 ml of a 1 M NaOH solution was added. The final volume was adjusted to 10 ml with 2.4 ml of deionized water, and the mixture was thoroughly vortexed. Absorbance was then measured at 510 nm. Results were expressed as mg RE/g ds.

2.4.3. Total tannin Content Assay

The TTC was determined using a Folin-Ciocalteu colorimetric method adapted from Haile and Kang [20]. In brief, 0.1 ml of the extract was combined with 7.5 ml of distilled water, followed by the addition of 0.5 ml of Folin-Ciocalteu reagent. The mixture was then reacted with 1.0 mL of sodium carbonate solution (35% w/v), and the final volume was brought to 10 ml using distilled water. After incubation at room temperature for 30 min, absorbance was measured at 700 nm. Results were

expressed as mg TAE/g ds.

2.4.4. DPPH Assay

The DPPH radical cation scavenging activity was determined according to Tchabo, Ma [21]. Concisely, 1 ml of extract was reacted with 6 ml of a freshly prepared DPPH solution (60 μM in methanol). Following incubation in darkness at room temperature for 30 min, the absorbance was recorded at 517 nm. Results were expressed as mM TE/g ds.

2.4.5. ABTS Assay

The ABTS radical cation scavenging activity was determined according to Tchabo, Ma [21]. Briefly, 125 μL of extract was reacted with 5 ml of a freshly prepared ABTS solution (2.45 mM ABTS in 140 mM ammonium persulfate). The mixture was incubated at room temperature for 15 min, and the absorbance was then measured at 734 nm. Results were expressed as mM TE/g ds.

2.5. Statistical Analysis

All extractions and analytical measurements were performed in triplicate, and data were reported as mean values. Response surface modelling and model fitting were carried out using Design Expert version 13 (Stat Ease Inc., Minneapolis, MN, USA). Treatments effects were assessed by analysis of variance, and mean differences were evaluated using Tukey HSD at $p < 0.05$ in OriginPro 2025 (OriginLab Corp., Northampton, MA, USA).

3. Results and Discussion

3.1. Experimental Results and Model Validation

The experimental dataset was generated using a three factor BBD comprising 15 runs, including three replicated center points estimate pure error and assess experimental repeatability (Table 2).

The responses spanned wide ranges across the factor space, confirming strong process sensitivity. TPC varied from 48.58 to 98.06 mg GAE per g ds, TFC from 31.70 to 60.26 mg RE per g ds, and TTC from 1.44 to 19.42 mg TAE per g ds. Antioxidant activities ranged from 2.53 to 4.39 mM TE per g ds for DPPH and from 2.50 to 4.71 mM TE per g ds for ABTS. The highest phytochemical contents were obtained in run 2 at 360 W, 14 min, and 100 μm , which produced the maximum TPC, TFC, and TTC. In contrast, antioxidant maxima occurred at different operating conditions, with the highest DPPH in run 1 at 630 W, 20 min, and 100 μm and the highest ABTS in run 11 at 360 W, 20 min, and 300 μm . The lowest overall MAAE performance was observed in run 13 at 900 W, 8 min, and 300 μm , which yielded the minimum TTC together with the lowest DPPH and ABTS values. These patterns indicate that MAAE severity did not increase responses monotonically and that response specific optima existed within the design space. Accordingly, the data were fitted to hierarchical polynomial models and evaluated using sequential p -values, lack of fit, and

predictive metrics (Table 3).

Table 2. Experimental design matrix and measured phytochemical contents and antioxidant activities of *Sonchus oleraceus* leaf extract

Runs	Independent variables			Responses				
	MP (W)	ET (min)	PS (μ m)	Phytochemicals			Antioxidant activities	
	X_1	X_2	X_3	TPC (mg GAE/g ds)	TFC (mg RE/g ds)	TTC (mg TAE/g ds)	DPPH (mM TE/g ds)	ABTS (mM TE/g ds)
1	630	20	100	89.31	57.86	18.97	4.39	4.61
2	360	14	100	98.06	60.26	19.42	4.25	4.56
3	360	14	500	66.54	40.85	13.94	3.29	4.05
4	630	20	500	83.61	52.84	12.62	3.70	4.63
5 ^a	630	14	300	82.12	51.31	14.90	4.02	4.62
6	900	14	100	67.37	45.01	14.44	3.44	3.56
7	900	14	500	57.78	41.76	8.27	3.04	3.48
8 ^a	630	14	300	80.90	52.44	17.15	3.94	4.39
9	360	8	300	74.95	46.34	11.77	3.25	3.62
10 ^a	630	14	300	81.52	52.40	15.64	3.97	4.56
11	360	20	300	94.84	57.11	17.91	4.10	4.71
12	900	20	300	80.67	53.07	13.89	3.75	4.19
13	900	8	300	51.85	31.70	1.44	2.53	2.50
14	630	8	100	72.99	47.65	9.67	3.03	3.48
15	630	8	500	48.58	33.92	3.90	2.57	2.76

^a Central points (used to determine the experimental error).

MP-Microwave power, ET-Extraction time, PS-Particle size, TPC-total phenolic content, TFC-total flavonoid content, TTC-total tannin content, DPPH-1,1 diphenyl 2 picrylhydrazyl, and ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid).

Table 3. Summary of model adequacy

Source	Sequential p-value	Lack of fit p-value	Adjusted R ²	Predicted R ²	Remarks
TPC					
Linear	< 0.0001	0.0082	0.8297	0.7504	
2FI	0.0728	0.0125	0.8975	0.8435	
Quadratic	0.0059	0.0637	0.984	0.9117	Recommended
Cubic	0.0637		0.9983		Aliased
TFC					
Linear	0.0002	0.0211	0.7769	0.6676	
2FI	0.0331	0.0393	0.891	0.849	
Quadratic	0.0063	0.1916	0.9825	0.9113	Recommended
Cubic	0.1916		0.9942		Aliased
TTC					
Linear	0.0002	0.1633	0.7756	0.6975	
2FI	0.7111	0.1309	0.7377	0.5179	
Quadratic	0.0032	0.7436	0.9678	0.9104	Recommended
Cubic	0.7436		0.9521		Aliased
DPPH					
Linear	0.0002	0.017	0.7732	0.7312	
2FI	0.7185	0.0133	0.734	0.6646	
Quadratic	0.0007	0.1546	0.9821	0.9068	Recommended
Cubic	0.1546		0.9952		Aliased
ABTS					
Linear	0.0004	0.0845	0.7388	0.6872	
2FI	0.6264	0.07	0.7079	0.6435	
Quadratic	0.0003	0.8946	0.9856	0.9727	Recommended
Cubic	0.8946		0.9721		Aliased

TPC-total phenolic content, TFC-total flavonoid content, TTC-total tannin content, DPPH-1,1 diphenyl 2 picrylhydrazyl, and ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid).

For all responses, the quadratic model was significant, with sequential *p*-values between 0.0003 and 0.0063, indicating that inclusion of quadratic terms significantly

improved model fit beyond the linear and two factor interaction structures. This supports selection of the quadratic model even when simpler models appear

significant, since significance does not necessarily imply adequacy when curvature exists in the response surface [22]. The quadratic models also provided the best overall fit and prediction, with adjusted R^2 values from 0.968 to 0.986 and predicted R^2 values from 0.907 to 0.973, showing that most of the response variability was explained within the studied domain [19]. The differences between adjusted and predicted R^2 were limited at 0.013 to 0.075, supporting reliable prediction across the design space [23]. Lack of fit was non-significant for all quadratic models, with p values between 0.064 and 0.895, indicating no systematic deviation between predicted and experimental values [19]. By comparison, the linear models showed weaker fit and significant lack of fit for TPC, TFC, and DPPH, while the two factor interaction models were generally non-significant and did not resolve the lack of fit. Although cubic models increased adjusted R^2 in some cases, they were aliased in this BBD. This means that some higher order terms were confounded and could not be estimated independently because the experimental points were insufficient to uniquely separate cubic effects [24]. As a result, the effects of each variable

can generate signals that become indistinguishable, and the cubic model is therefore not interpretable. On this basis, the quadratic model was retained for response surface analysis and optimization of microwave assisted aqueous extraction of *Sonchus oleraceus*.

3.2. Effects of Microwave Assisted Aqueous Extraction Parameters on Phytochemical Content

3.2.1. Effect on Total Phenol Content

The quadratic model for total phenolic content (TPC) was highly significant ($p < 0.0001$) and the lack of fit was not significant ($p > 0.05$), indicating that the selected polynomial adequately describes the data within the studied region (Table 4). Model precision was supported by low residual dispersion (SD = 1.86; CV = 2.47%), high explanatory power (adjusted $R^2 = 0.98$), good predictability (predicted $R^2 = 0.91$), and a strong signal-to-noise ratio (adequate precision = 32.15) (Table 4).

Table 4. Analysis of variance and regression coefficients of the quadratic response for phytochemical and antioxidant responses of *Sonchus oleraceus* leaf extract

Source	DF	TPC (mg GAE/g ds)		TFC (mg RE/g ds)		TTC (mg TAE/g ds)		DPPH (mM TE/g ds)		ABTS (mM TE/g ds)	
		CE	p -Value	CE	p -Value	CE	p -Value	CE	p -Value	CE	p -Value
Model	9	81.51	< 0.0001***	52.05	< 0.0001***	15.90	0.0003**	3.98	< 0.0001***	4.52	< 0.0001***
<i>Linear</i>											
X_1 (MP)	1	-9.59	< 0.0001***	-4.13	0.0001**	-3.13	0.0002**	-0.27	0.0002**	-0.40	< 0.0001***
X_2 (ET)	1	12.51	< 0.0001***	7.66	< 0.0001***	4.58	< 0.0001***	0.57	< 0.0001***	0.72	< 0.0001***
X_3 (PS)	1	-8.90	< 0.0001***	-5.18	< 0.0001***	-2.97	0.0003**	-0.31	< 0.0001***	-0.16	0.0031*
<i>Interaction</i>											
X_1X_2	1	2.23	0.0618	2.65	0.0051*	1.58	0.0201*	0.09	0.0651	0.15	0.0172*
X_1X_3	1	5.48	0.0020*	4.04	0.0008**	-0.17	0.7284	0.14	0.0161*	0.11	0.0537
X_2X_3	1	4.68	0.0040*	2.18	0.0114*	-0.15	0.7699	-0.06	0.2030	0.19	0.0076*
<i>Quadratic</i>											
X_1^2	1	-3.56	0.0144*	-3.05	0.0033*	-0.96	0.1072	-0.24	0.0019*	-0.36	0.0005**
X_2^2	1	-2.38	0.0578	-1.95	0.0202*	-3.69	0.0006**	-0.33	0.0005**	-0.41	0.0003**
X_3^2	1	-5.52	0.0023*	-2.03	0.0172*	-0.92	0.1182	-0.23	0.0025*	-0.25	0.0026*
Residual	5										
Lack of fit	3		0.0637		0.1916		0.7436		0.1546		0.8946
SD		1.86		1.12		0.94		0.08		0.09	
CV %		2.47		2.31		7.26		2.21		2.15	
Adj- R^2		0.98		0.98		0.97		0.98		0.99	
Pred- R^2		0.91		0.91		0.91		0.91		0.97	
AP		32.15		30.41		23.43		29.40		32.15	

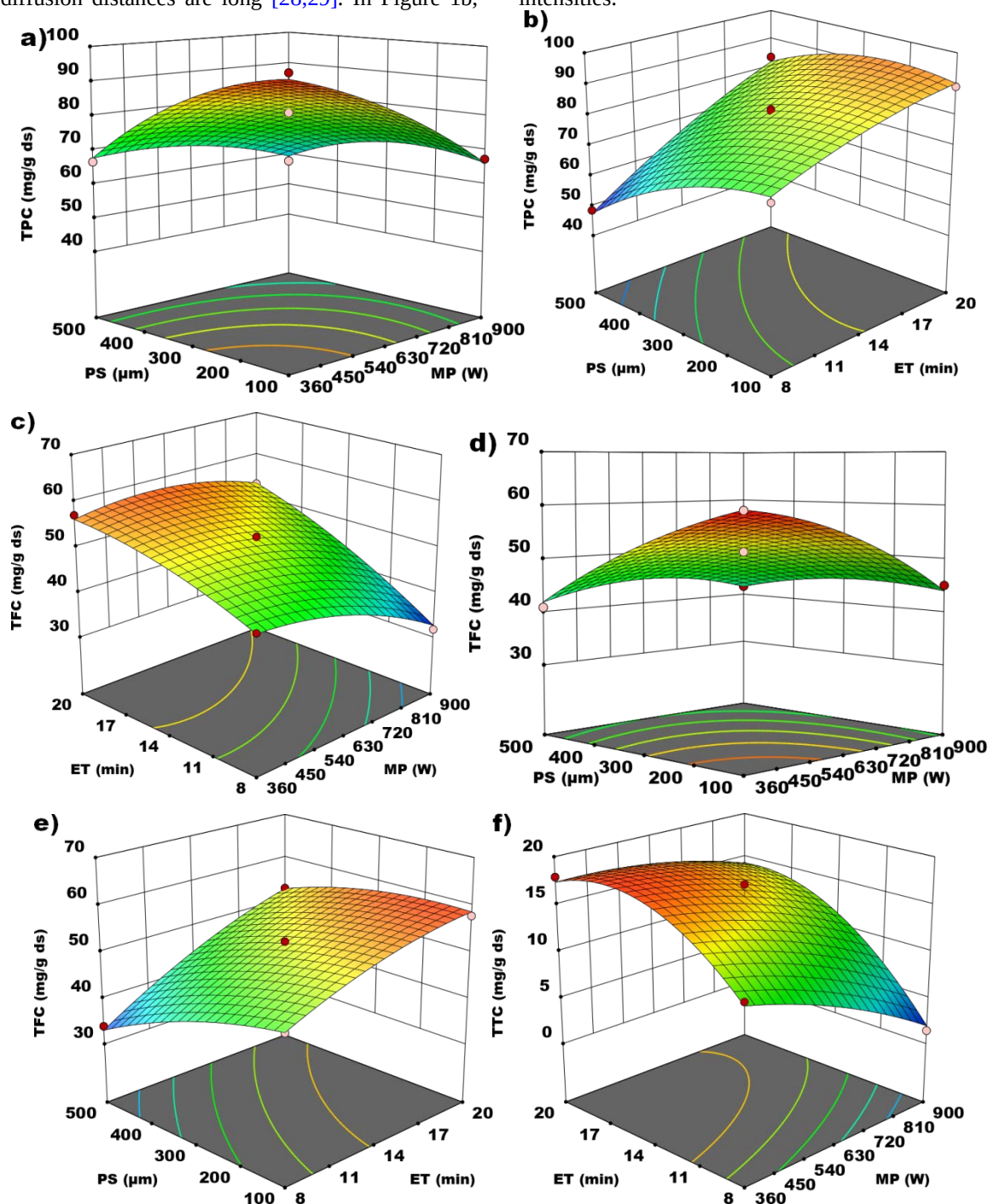
Total phenolic content (TPC), Total flavonoid content (TFC), Total tannin content (TTC), 1,1 diphenyl 2 picrylhydrazyl (DPPH), 2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) (ABTS), CE (coefficient estimate), DF (degree of freedom), Standard deviation (SD), Coefficient of variation (CV), Adjusted R-squared (Adj- R^2), Predicted R-squared (Pred- R^2), Adequate precision (AP), Microwave power (MP), Extraction time (ET), and Particle size (PS). Asterisks (*, ** and ***) indicate 5%, 0.1% and 0.01% significant levels, respectively.

At the linear level, MP reduced TPC (CE = -9.59, $p < 0.0001$), ET increased TPC (CE = 12.51, $p < 0.0001$), and PS reduced TPC (CE = -8.90, $p < 0.0001$) (Table 4). The positive time effect reflects progressive hydration of the solid matrix and sustained diffusion of soluble and weakly bound phenolics into the aqueous phase [25]. The negative PS effect indicates diffusion control, since comminution increases interfacial area and shortens intraparticle diffusion paths, thereby lowering mass transfer resistance [17, 26]. In contrast, the negative power effect suggests that, under the present aqueous conditions, the increase in

energy density promotes competitive reactions that reduce the measurable phenolic pool, including oxidation, condensation, and enhanced association with macromolecules [27], which can offset the benefit of faster cell disruption [10]. Nonlinear behavior was captured by significant negative quadratic terms for MP^2 (CE = -3.56, $p < 0.05$) and PS^2 (CE = -5.52, $p < 0.05$), whereas ET^2 was not significant ($p > 0.05$) (Table 4). This pattern indicates diminishing returns at high power and at the finest particle sizes, while the time effect remained close to linear across the tested range. Interaction analysis

showed that $MP \times PS$ ($CE = 5.48$, $p < 0.05$) and $ET \times PS$ ($CE = 4.68$, $p < 0.05$) were significant and positive, whereas $MP \times ET$ was not significant ($p > 0.05$) (Table 4). In Figure 1a, the positive $MP \times PS$ interaction indicates that increasing power partially compensates for the extraction penalty of coarser particles, because higher power improves internal heating and local permeability when diffusion distances are long [28,29]. In Figure 1b,

the positive $ET \times PS$ interaction shows that longer residence time similarly reduces the disadvantage of larger particles by allowing diffusion to proceed further toward equilibrium. Overall, TPC behaves as a composite response governed by mass transfer enhancement at longer times and smaller particles, but constrained by power driven chemical losses at the highest microwave intensities.



(a) TPC-Total phenolic content as function of MP-microwave power and PS-particle size, (b) TPC-Total phenolic content as function of ET-extraction time and PS-particle size, (c) TFC-Total flavonoid content as function of MP-microwave power and ET-extraction time, (d) TFC-Total flavonoid content as function of MP-microwave power and PS-particle size, (e) TFC-Total flavonoid content as function of ET-extraction time and PS-particle size, and (f) TTC-Total tannin content as function of extraction MP-microwave power and ET-extraction time.

Figure 1. 3D surface plots for the interactive effect of microwave power, extraction time and particle size on the phytochemicals of *Sonchus oleraceus* leaf extract

3.2.2. Effect on Total Flavonoid Content

The quadratic model for total flavonoid content (TFC) was highly significant ($p < 0.0001$) with a non-significant lack of fit ($p > 0.05$), and the diagnostic statistics indicated satisfactory model adequacy (SD = 1.12; CV = 2.31%; adjusted $R^2 = 0.98$; predicted $R^2 = 0.91$; adequate precision = 30.41) (Table 4). Linear effects followed the same directional pattern as TPC, but with different sensitivity. MP decreased TFC (CE = -4.13, $p < 0.001$), ET increased TFC (CE = 7.66, $p < 0.0001$), and PS decreased TFC (CE = -5.18, $p < 0.0001$) (Table 4). The positive time effect reflects gradual release of flavonoids as hydration and solvent access improve, whereas the negative PS effect again supports diffusion limitation. The negative power effect indicates that many flavonoid structures are chemically reactive under intense heating, so cleavage, oxidation, and rearrangement reactions can reduce the recoverable fraction despite greater physical disruption [12,30]. Unlike TPC, curvature was significant for all three factors, with negative quadratic terms for MP^2 (CE = -3.05, $p < 0.05$), ET^2 (CE = -1.95, $p < 0.05$), and PS^2 (CE = -2.03, $p < 0.05$) (Table 4). These effects indicate an interior optimum, where initial increases in severity improve release, but further increases progressively shift the balance toward chemical loss or extraction saturation [31]. All two factor interactions were significant and positive, including $MP \times ET$ (CE = 2.65, $p < 0.05$), $MP \times PS$ (CE = 4.04, $p < 0.001$), and $ET \times PS$ (CE = 2.18, $p < 0.05$) (Table 4). The response surfaces in Figure 1c and Figure 1e show that the effect of any single factor becomes stronger when paired with conditions that reduce the main bottleneck. Longer time amplifies the benefit of moderate microwave action by allowing disrupted tissues to equilibrate with the solvent [32,33], while smaller particles enhance the same synergy by shortening diffusion paths and improving heating uniformity [34]. Consequently, TFC is governed by a tighter processing window than TPC, with combined increases in time and comminution enabling efficient recovery at moderate power, but with clear curvature that reflects greater chemical sensitivity of flavonoids.

3.2.3. Effect on Total Tannin Content

The quadratic model for total tannin content (TTC) was significant ($p < 0.001$) and exhibited excellent lack of fit performance ($p > 0.05$), supporting reliable interpretation of factor effects (Table 4). Residual dispersion was low (SD = 0.94) and the model showed acceptable precision (CV = 7.26%, adjusted $R^2 = 0.97$, predicted $R^2 = 0.91$, adequate precision = 23.43) (Table 4). At the linear level, TTC decreased with MP (CE = -3.13, $p < 0.001$) and PS (CE = -2.97, $p < 0.001$), and increased with ET (CE = 4.58, $p < 0.0001$) (Table 4). The stronger time dependence reflects the physicochemical nature of tannins. These compounds are generally larger and more strongly associated with structural polysaccharides and proteins, and therefore require longer hydration and diffusion times for desorption and solubilization [35,36]. The negative power effect indicates that increased thermal severity can reduce the soluble tannin fraction through oxidative coupling, self-association, or complex formation [37,38], which lowers analytical recoverability. Curvature was

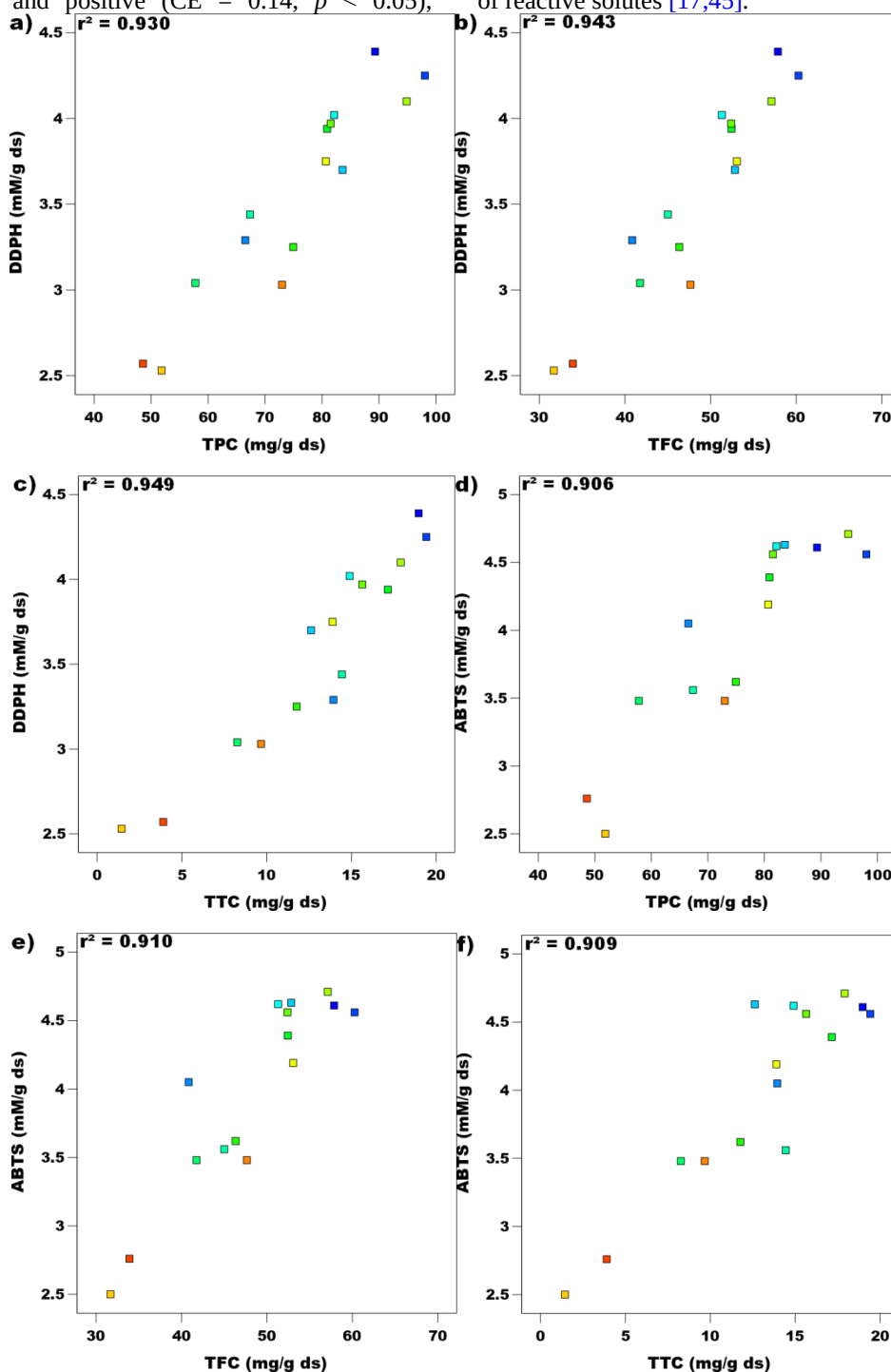
dominated by the time term. ET^2 was negative and highly significant (CE = -3.69, $p < 0.001$), whereas MP^2 and PS^2 were not significant ($p > 0.05$) (Table 4). This indicates an optimum at intermediate extraction time, beyond which additional exposure increasingly favors depletion mechanisms such as oxidation or precipitation over continued release [28,39]. Among interactions, only $MP \times ET$ was significant and positive (CE = 1.58, $p < 0.001$), while $MP \times PS$ and $ET \times PS$ were not significant ($p > 0.05$) (Table 4). As illustrated in Figure 1f, the positive $MP \times ET$ interaction shows that the influence of power on TTC depends on residence time. At short times, higher power mainly increases thermal stress with limited time for diffusion, whereas at longer times the same power input better translates into matrix permeabilization and net tannin transfer [37,40]. The absence of significant PS interactions suggests that, for TTC, particle size primarily acts as a baseline diffusion constraint rather than a strong moderator of the other factors.

3.2.4. Effect on DPPH Radical Scavenging Activity

The quadratic model for DPPH was highly significant ($p < 0.0001$) and the lack of fit was not significant ($p > 0.05$), indicating that the fitted response surface adequately represents DPPH variation within the experimental domain (Table 4). Model precision was supported by low residual dispersion (SD = 0.08 and CV = 2.21%), together with strong adequacy statistics, including adjusted $R^2 = 0.98$, predicted $R^2 = 0.91$, and adequate precision = 29.40, which support robust interpretation of factor effects. DPPH was closely aligned with the phytochemical indices quantified in this work. The regressions in Figure 2 show a strong relationship between DPPH and TPC (Figure 2a, $r^2 = 0.930$), TFC (Figure 2b, $r^2 = 0.943$), and TTC (Figure 2c, $r^2 = 0.949$). These high r^2 values indicate that most variation in DPPH is explained by changes in the extractable phenolic, flavonoid, and tannin pools as reported by Yu, Gouvinhas [41]. Among these classes, TTC exhibited the strongest relationship with DPPH, indicating that tannin abundance most closely tracks DPPH across the explored extraction domain. In line with this relationship, the significant DPPH effects reflect the same directional behavior as the phytochemical responses. ET increased DPPH (CE 0.57, $p < 0.0001$), whereas PS decreased it (CE -0.31, $p < 0.0001$) (Table 4). Longer ET supports continued solubilization and diffusion of phenolic type reducers from internal domains into the aqueous phase [33,42]. Smaller particles shorten diffusion paths and increase solvent access, which elevates the concentration of hydroxyl rich structures that contribute strongly to hydrogen and electron donation measured by DPPH [17]. In contrast, MP exerted a significant negative linear effect on DPPH (CE -0.27, $p < 0.0001$) (Table 4). This suggests that increasing power within the tested range reduces the recoverable fraction that remains highly reactive toward DPPH. Under higher power density, part of the phenolic pool can shift toward less reactive forms through oxidative conversion, coupling, or stronger association with co-extracted matrix components, which reduces effective quenching even when tissue disruption is intensified [12,43]. Curvature was also evident, as shown by significant negative quadratic terms for MP^2 ($p < 0.05$), ET^2 ($p = 0.001$), and PS^2 ($p < 0.05$), confirming

diminishing returns and an interior optimum for DPPH within the investigated ranges (Table 4). This pattern is consistent with progressive exhaustion of the most accessible fraction and a gradual reduction in marginal gain at higher MAAE severity [44]. Among interaction terms, $MP \times ET$ and $ET \times PS$ were not significant ($p > 0.05$), indicating that time did not show a meaningful joint effect with either power or particle size on DPPH within the studied domain. In contrast, the $MP \times PS$ interaction was significant and positive ($CE = 0.14$, $p < 0.05$),

showing that the effect of microwave power on DPPH depended on particle size (Figure 3a). At finer PS, phytochemicals are released more rapidly into the bulk solvent, which reduces localized degradation at the solid surface and favors preservation of DPPH active compounds [17,45]. Conversely, coarser particles impose higher internal diffusion resistance and promote less uniform microwave heating, thereby limiting the efficiency with which added power enhances the recovery of reactive solutes [17,45].



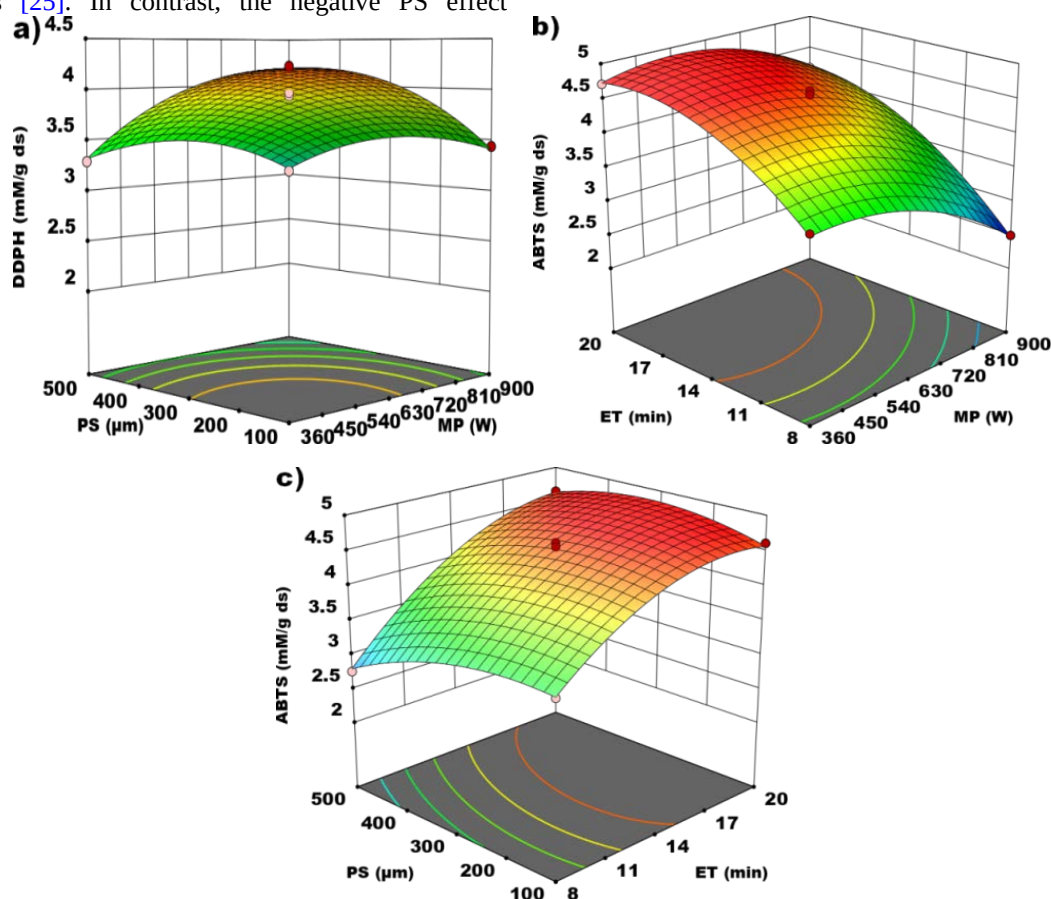
(a) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TPC-Total phenolic content, (b) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TFC-Total flavonoid content, (c) DPPH-1,1 diphenyl 2 picrylhydrazyl vs TTC-Total tannin content, (d) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TPC-Total phenolic content, (e) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TFC-Total flavonoid content, and (f) ABTS-2,2 azino bis (3 ethylbenzothiazoline 6 sulfonic acid) vs TTC-Total tannin content.

Figure 2. Pairwise relationships between antioxidant activity and phytochemicals *Sonchus oleraceus* leaf extract

3.2.5. Effect on ABTS Radical Cation Scavenging Activity

The quadratic model for ABTS was highly significant ($p < 0.0001$) and exhibited an excellent fit, as shown by a clearly non-significant lack of fit ($p > 0.05$) (Table 4). Low residual dispersion was also observed, with SD of 0.09 and CV of 2.15%, and the adequacy statistics were strong, with adjusted R^2 of 0.99, predicted R^2 of 0.97, and adequate precision of 32.15, confirming high explanatory and predictive performance across the design space. ABTS activity was also attributable to the extracted phytochemical pool. Figure 2 shows strong relationships between ABTS and TPC (Figure 2d, $r^2 = 0.906$), TFC (Figure 2e, $r^2 = 0.910$), and TTC (Figure 2f, $r^2 = 0.909$). The similarity among these r^2 values indicates that ABTS tracks the overall phenolic spectrum measured here, rather than being driven by only one class [41], and that most ABTS variability reflects changes in these indices. This phytochemical-driven pattern is also evident in the significant main effects for ABTS. ABTS increased with ET (CE 0.72, $p < 0.0001$) and decreased with MP (CE -0.40, $p < 0.0001$) and PS (CE -0.16, $p = 0.0031$) (Table 4). The strong positive time effect suggests that prolonged contact promotes accumulation of soluble reducing compounds [25]. In contrast, the negative PS effect

reflects diffusion limitation at larger particles [32]. The negative MP effect indicates that higher energy input shifts part of the extracted pool toward less reactive forms, consistent with the attribution of ABTS activity to both phytochemical abundance and redox quality [43]. All quadratic terms were negative and significant for ABTS, including MP^2 ($p < 0.001$), ET^2 ($p < 0.001$), and PS^2 ($p < 0.001$), confirming pronounced curvature and an interior optimum (Table 4). This indicates that antioxidant gains do not increase proportionally with process severity, because enrichment of the soluble pool competes with diminishing extraction returns and conversion losses at higher intensity [46,47]. Interaction effects further illustrate how variable combinations influence ABTS. Both $MP \times ET$ (CE 0.15, $p < 0.05$) and $ET \times PS$ (CE 0.19, $p < 0.05$) were significant and positive, whereas $MP \times PS$ was not significant ($p > 0.05$) (Table 4). The $MP \times ET$ interaction indicates that sufficient time is required for microwave assisted structural weakening to translate into net enrichment of ABTS active phytochemicals [48], as shown by the response surface in Figure 3b. Similarly, the $ET \times PS$ interaction indicates that longer time can partly compensates for coarse particles by allowing diffusion-controlled release to proceed despite higher internal resistance [49,50], as observed in Figure 3c.



(a) DPPH-1,1 diphenyl 2 picrylhydrazyl as function of MP-microwave power and PS-particle size, (b) ABTS-2,2 amino bis (3 ethylbenzothiazoline 6 sulfonic acid) as function of MP-microwave power and ET-extraction time, and (c) ABTS-2,2 casino bis (3 ethylbenzothiazoline 6 sulfonic acid) as function of ET-extraction time and PS-particle size.

Figure 3. 3D surface plots for the interactive effect of microwave power, extraction time and particle size on the antioxidant activity of *Sonchus oleraceus* leaf extract

3.3. Optimal Conditions for MAAE of Phytochemicals and Antioxidants from *Sonchus oleraceus* Leaf Extract

Numerical optimization was conducted using a desirability function, constraining MP, ET, and PS within the experimental ranges. Response goals were defined to balance extract potency and practical quality considerations. TPC, TFC, DPPH, and ABTS were set to maximize with the highest importance, whereas TTC was set to maximize with moderate importance. This weighting suits leafy matrices, where tannins add antioxidant value, but excessive levels can increase astringency and promote complexation with proteins and minerals, reducing nutritional quality and acceptability [51,52]. Under these criteria, the desirability solution predicted an optimum at 378.39 W, 18.81 min, and 174 μm , with predicted responses of TPC of 98.81 mg GAE/g ds, TFC of 60.47 mg RE/g ds, TTC of 19.80 mg TAE/g ds, DPPH of 4.43 mM TE/g ds, and ABTS of 4.73 mM TE/g ds. For validation, these values were adjusted to feasible setpoints aligned with equipment increments, namely 360 W, 20 min, and 180 μm . Such adjustments are standard in RSM verification, allowing direct experimental confirmation under implementable conditions. At these practical settings, the experimental values were TPC of 99.82 ± 0.06 , TFC of 59.78 ± 0.09 , TTC of 18.73 ± 0.04 , DPPH of 4.40 ± 0.04 , and ABTS of 4.70 ± 0.05 , in strong agreement with the predicted results within a 95% confidence interval. Overall, predicted and experimental values agreed closely for all targets, confirming good model validity and strong predictive performance ($R^2 > 0.95$) for phytochemical contents and antioxidant activities in MAAE extract. From a process perspective, the optimum corresponds to moderate MP, relatively long ET, and intermediate PS. This combination is well suited for aqueous microwave extraction of leaf tissues, as it enables effective volumetric heating and sufficient matrix disruption to enhance solvent access while avoiding excessive thermal stress that could degrade redox-active compounds [53,54]. The longer ET promotes diffusion-controlled transfer and equilibration of solubilized compounds [32], while the intermediate PS increases surface area compared with coarse milling but prevents overly fine powders that could compact, reduce porosity, and hinder solvent renewal and clarification [26].

4. Conclusion

The objective of this work was to determine the effects of microwave power, extraction time, and particle size on phytochemical recovery and antioxidant capacity during aqueous microwave assisted extraction. It also aimed to define response specific trends to support multi response optimization. The phytochemical responses followed a consistent mass transfer pattern. Recovery increased with longer extraction time and improved with smaller particles. However, the responses differed in their sensitivity to microwave power and in the onset of curvature. Total phenolic content reflected a broad phenolic pool governed mainly by matrix opening and diffusion. Curvature was

moderate and the benefit of higher power became more limited as severity increased. Total flavonoids showed stronger curvature and a richer interaction structure. This indicates a more sensitive fraction in which gains from release are offset earlier as conditions become more intense. Total tannin content was strongly governed by extraction time and by the time dependent expression of microwave effects. This trend is consistent with slower desorption and a greater tendency for secondary association during prolonged or intense processing. DPPH and ABTS followed a coherent antioxidant pattern driven by the extracted phytochemical pool. Even so, they differed in their response to microwave power and in interaction complexity. Overall, these outcomes support multi response optimization as the most defensible strategy for balancing phytochemical yield and antioxidant performance, rather than relying on a single compositional or radical scavenging endpoint.

Abbreviations

MP	Microwave power
ET	Extraction time
PS	Particle size
TPC	Total phenolic content
TFC	Total flavonoid content
TTC	Total tannin content
DPPH	1,1 diphenyl 2 picrylhydrazyl
ABTS	2,2 casino bis (3 ethylbenzothiazoline 6 sulfonic acid)

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

Ethical Approval

The research conducted is not related to either human or animal use.

Declaration of Competing Interest

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.

Data Availability

Data will be made available on request.

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