

Enhancing Phenolic Content in Dried Broccoli: Insights from Ultrasonic Assisted Extraction and Response Surface Methodology

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Abstract – The research article investigated the impact of ultrasonic-assisted extraction on the total phenolic content of dried broccoli in comparison to traditional extraction methods. A meticulously designed experiment subjected dried broccoli samples to both ultrasonic and conventional extraction processes to assess their influence on the phenolic composition, a key determinant of the health benefits associated with broccoli consumption. The control group, which employed traditional solvent extraction, established a baseline for comparison, while the experimental groups utilized ultrasonic waves during extraction. Analysis of the extracts for total phenolic content using the spectrophotometer and the standard calibration curve revealed a significant disparity between the control and ultrasonic-assisted extracts. The study conducted a systematic investigation into the influence of control variables such as temperature (40-70 °C), solvent concentration (1:5-1:20 w/v), and extraction time (20-60 min) on the efficiency of the extraction process. Through the implementation of 17 experiments designed using response surface methodology (RSM), a detailed dataset was generated for statistical analysis. The outcomes of the study not only demonstrated the effects of ultrasonic-assisted extraction on total phenolic content but also allowed for the determination of the most favorable conditions for the process. The utilization of RSM models in this research provided valuable insights into the complex interactions among temperature, solvent concentration, and extraction time during the extraction of phenolic compounds from dried broccoli.

Keywords – Dried Broccoli, Extraction, Phenolic Content, Statistical Analysis, RSM

I. INTRODUCTION

Broccoli is a cruciferous vegetable known for its nutritional richness and has attracted attention for its potential health benefits due to its phenolic content. Phenolic compounds in broccoli are acknowledged for their antioxidant properties and their involvement in various biological activities that contribute to human well-being [1]. As consumers are increasingly looking for foods with enhanced health benefits, the extraction of phenolic compounds from broccoli has become crucial [2-3]. Broccoli is rich in phytochemicals, micronutrients, vitamins, and minerals, making it a valuable source of health-promoting substances [4]. Broccoli sprouts have been highlighted as functional foods with valuable health properties because of their bioactive compounds [5]. In addition, broccoli contains carotenoids, glucosinolate

minerals, phenols, polyphenols, selenium, and vitamins, further emphasizing its health-promoting potential [6]. Understanding and optimizing the extraction of phenolic compounds from broccoli is a key step in exploiting its health-promoting properties, in line with the growing consumer demand for functional foods with enhanced nutritional value.

The phenolic content of broccoli is essential for its health-promoting effects. Phenolic compounds, such as flavonoids and polyphenols, are known for their antioxidant properties that help to combat free radicals and reduce oxidative stress in the body. These compounds not only act as antioxidants but also have anti-inflammatory, anti-cancer, and cardiovascular health benefits. Therefore, it is essential to evaluate the phenolic content of broccoli to understand the potential impact of broccoli on human health and optimize its nutritional value. Studies have demonstrated a strong correlation between phenolic and antioxidant activity in broccoli, indicating that phenolic plays a significant role in the antioxidant capacity of broccoli [7]. Research has also emphasized the importance of phenolic compounds in broccoli for their health-promoting effects, underscoring the necessity of measuring and comprehending these compounds to maximize the nutritional benefits of broccoli [8]. Optimizing the extraction of phenolic compounds from broccoli to preserve its health-promoting properties and enhance its bioavailability is crucial [9].

Ultrasonic-assisted extraction (UAE) is a highly efficient and versatile extraction method that has gained prominence among various extraction methods. This process involves immersing dried broccoli in a solvent and applying ultrasonic waves, which induce cavitation and microstreaming. These mechanical actions disrupt plant cell structure and facilitate mass transfer and release of phenolic compounds into solvent [10]. Compared to conventional solvent extraction, UAE offers a faster extraction process without the need for high temperatures, which could degrade sensitive phenolic compounds. Ultrasound can expedite extraction while preserving the stability of these compounds, making UAE a valuable method for maintaining the integrity of phenolic compounds in broccoli. To optimize the UAE process, researchers often employ techniques such as response surface methodology (RSM) to systematically investigate crucial variables such as temperature, solvent concentration, and extraction time to efficiently maximize the phenolic yield [11-12]. RSM is a statistical and mathematical approach that enables the exploration of the interplay between multiple variables, providing a systematic way to understand and optimize complex processes [13].

In recent years, ultrasound-assisted extraction of plant materials using RSM has become a significant focus. This method has gained a lot of attention due to its effectiveness in extracting bioactive compounds from various plant sources. Ultrasound-assisted extraction combined with RSM is a valuable tool for optimizing the extraction of phenolic compounds [14], polysaccharides [15], flavonoids [16], antioxidants [17], and other bioactive components from plants.

The purpose of the present study was to investigate the impact of ultrasonic-assisted extraction on the TPC of dried broccoli in comparison with traditional extraction methods. This article delves into the importance of measuring the phenolic content of broccoli and highlights the role of RSM as a sophisticated method for optimizing the extraction process, contributing to the advancement of nutritional science and food technology.

II. MATERIAL AND METHOD

Sample Preparation

Fresh broccoli (*Brassica oleracea L.*) was obtained from a local market in Elazığ, Türkiye. Leaves and stems of broccoli were separated from the floret and discarded. In the conducted study, broccoli flowers underwent dehydration in a convection dryer set at 50 °C until a stable weight was attained. Following this drying process, the dried samples were subjected to grinding using a mechanical grinder and subsequently sieved for further analysis. The study did not explore the influence of drying

conditions on phenolic content. To maintain the quality of the samples, they were stored in airtight containers to prevent moisture absorption and degradation.

The powdered broccoli was mixed with a traditional solvent (ethanol) at a defined concentration. The mixture stood for a predetermined time (24 h) at a specific temperature (40 °C) for extraction. The solution was filtered to obtain the control extract.

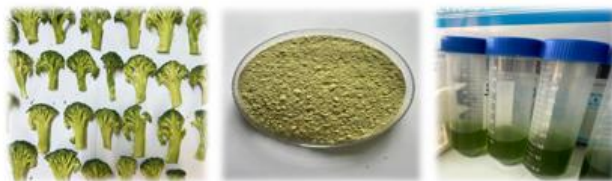


Fig. 1. Dried, ground, and extracted broccoli florets

Experimental Design

Temperature, solvent concentration, and extraction time were identified as critical control variables influencing the extraction process. The experiments included 17 RSM-designed runs, systematically manipulating the control variables. RSM was implemented to optimize the extraction conditions. A designed set of experiments, including a central composite design, allowed for the exploration of temperature, solvent concentration, and extraction time at multiple levels. The response variable, TPC, was measured for each experimental run. Each experiment within the RSM was conducted in triplicate to ensure reliability [18].

Total Phenolic Content (TPC) Analysis

Folin–Ciocalteu assay is a reliable and widely used method for measuring the TPC of plant extracts, including dried broccoli [19]. A 10% solution of Folin–Ciocalteu reagent was prepared by diluting it with distilled water according to the standard. A sample volume of 0.5 mL was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% (w/v) Na_2CO_3 . The reaction between the phenolic compounds in the extracts and the Folin–Ciocalteu reagent resulted in the formation of a blue complex. To allow full-color development, the reaction mixture was incubated in the dark for 30 min. Standard gallic acid solutions at known concentrations were subjected to the same Folin–Ciocalteu reaction and the absorbance values were recorded. After the incubation period, the absorbance of the reaction mixture was measured at 760 nm using a spectrophotometer. To determine the concentration of phenolic compounds in each extract, the absorbance values of the sample extracts were compared with the calibration curve. Results are expressed as milligrams of gallic acid equivalent (GAE) per gram of dried broccoli.

III. RESULTS AND DISCUSSION

The experimental design utilized in this study employs a central composite design (CCD) to investigate the effects of varying temperatures, extraction durations, and solute/solvent ratios on the extraction process. The detailed parameters and levels of these factors are outlined in Table 1, providing a structured framework for systematically exploring the interactions and influences of these variables on extraction efficiency and yield. This approach allows for a comprehensive evaluation of the key factors affecting the extraction process and facilitates the generation of meaningful insights into the optimal conditions for extracting the desired compounds from the plant material.

Table 1. Experimental conditions for central composite design and the corresponding results obtained for TPC

No	Temperature (°C)	Time (min)	Concentration (w/v)	TPC
1	46.08	28.11	8.04	95.47
2	55.0	40.0	12.5	138.93
3	63.92	28.11	16.96	116.50
4	55.0	60.0	12.5	235.65
5	55.0	40.0	12.5	137.52
6	63.92	51.89	8.04	291.73
7	55.0	40.0	20.0	165.56
8	70.0	40.0	12.5	141.73
9	40.0	40.0	12.5	221.64
10	55.0	40.0	5.0	130.51
11	63.92	51.89	16.96	172.57
12	55.0	40.0	12.5	138.22
13	46.08	51.89	16.96	179.58
14	55.0	20.0	12.5	88.46
15	46.08	28.11	16.96	74.44
16	46.08	51.89	8.04	193.60
17	63.92	28.11	8.04	96.87

The total phenolic content (TPC) levels observed in the study ranged from 74.44 to 291.73 mg GAE/100 g d.w. (mg of gallic acid equivalents per 100 g of dried weight). This variability in TPC values across different experimental conditions indicates the influence of the ultrasonic-assisted extraction process on the phenolic content of the dried broccoli samples. In comparison, the TPC value of the control extract, obtained through traditional solvent extraction, was approximately 120 mg GAE/100 g d.w., serving as a reference point for assessing the impact of ultrasonic-assisted extraction.

The wide range of TPC values observed under different extraction conditions suggests that certain parameters lead to significantly higher phenolic levels than others. Extracts falling within or exceeding the TPC range of the control extract imply that ultrasonic-assisted extraction may enhance the phenolic content compared to conventional methods. Further analysis, such as statistical tests or comparisons with recommended dietary intakes, could provide insights into the significance of these variations [20].

Effect of Time and Temperature on TPC Results According to RSM

Response surface methodology (RSM) helps optimize the independent variables to get the best result. This is done to determine the optimum conditions required to maximize or minimize the response. The results obtained are evaluated whether they are statistically validated and have practical significance. The application of RSM played a crucial role in systematically exploring the impact of control variables, such as temperature, solvent concentration, and extraction time, on the extraction process efficiency. RSM not only enhanced the understanding of the intricate interactions between these variables but also facilitated the identification of optimal conditions for maximizing phenolic extraction [21-24]. Figure 2 and Figure 3 show the effect of time and temperature on TPC results. According to RSM results, TPC values also increase as time and temperature rise.

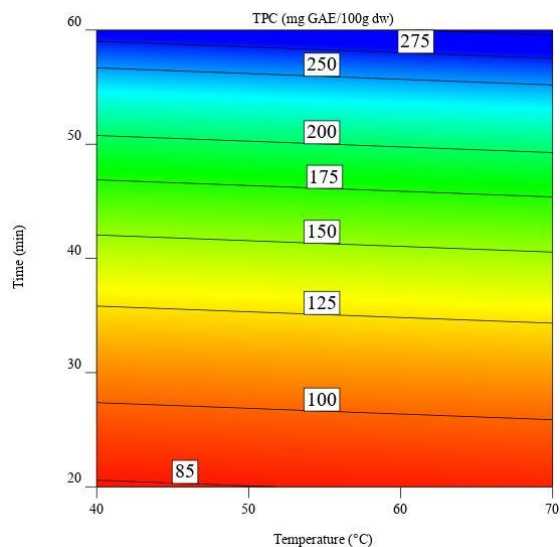


Fig. 2. Variation of TPC with temperature and time

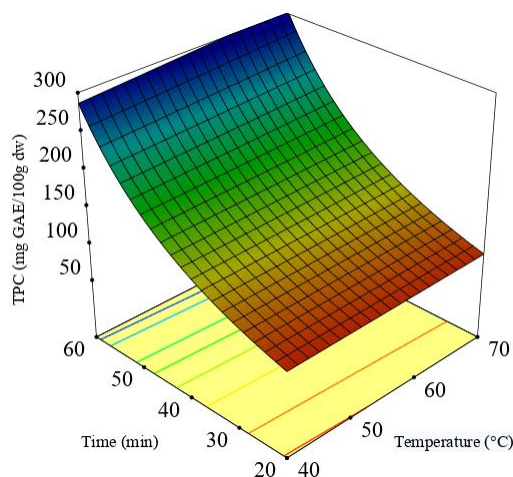


Fig. 3. Effect of temperature and time on TPC according to RSM

IV. CONCLUSIONS

In conclusion, this study systematically investigated the impact of ultrasonic-assisted extraction on the total phenolic content (TPC) of dried broccoli compared to traditional extraction methods. The TPC levels exhibited a significant range, varying from 74.44 to 291.73 mg GAE/100 g d.w. (mg of gallic acid equivalents per 100 g of dried weight), highlighting the variability in phenolic content under different extraction conditions. The TPC value of the control extract, approximately 120 mg GAE/100 g d.w., served as a reference point derived from traditional solvent extraction. The observed range in the experimental groups indicates that ultrasonic-assisted extraction has the potential to notably influence the phenolic composition of dried broccoli, with some conditions surpassing the TPC value of the control extract.

The findings of this study provide valuable insights into extraction techniques, particularly highlighting the effectiveness of ultrasonic-assisted extraction in boosting the phenolic content of dried broccoli. The observed variability within the TPC range underscores the importance of optimizing extraction conditions to fully exploit the health-promoting phenolic compounds in broccoli. These results are significant for industries involved in food processing and functional foods, offering a pathway to develop dried broccoli products with enhanced nutritional quality. Future research could delve deeper into the bioavailability and bioactivity of phenolic compounds extracted under optimized conditions, further elucidating the potential health benefits associated with consuming these innovative broccoli products. This study sets the stage for ongoing exploration and refinement of extraction methods to unlock the complete nutritional potential of dried broccoli for human consumption.

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