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Optimization of Ethanol Extraction Process for Phenolic Compounds from Watermelon Flesh and Rind by Response Surface Methodology and Evaluation of Their Functional Activities

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Abstract:

The study extracted phenolic compounds from watermelon flesh and rind, analyzed their antioxidant and functional activities, and assessed their impact on soybean oil stability. The Folin phenol method determined TPC, while the DPPH, assay evaluated free radical scavenging ability. The synthetic antioxidant watermelon sample showed the highest phenolic concentration (58.66), and the 100-ppm extract exhibited the highest phenolic content (26.22). Optimization analysis yielded conditions with a desirability rate of 0.894. The extract's antioxidant activity increased with higher concentrations, reaching 55.5 mg of gallic acid per liter at 400 ppm. Free radical scavenging activity increased from 60.86% (50 ppm) to 83.62% (400 ppm).The watermelon extract on the stability of soybean oil during storage. The watermelon extract effectively reduced soybean oil oxidation compared to the control (without antioxidants).Specifically, the peroxide number and thiobarbituric acid index were significantly lower in the oil samples containing 50 ppm, 100 ppm, 200 ppm, and 400 ppm of watermelon extract compared to the control sample.In conclusion, this study highlights the potential of watermelon fruit as a source of phenolic compounds with significant antioxidant and functional activities. The optimized extraction conditions demonstrated high efficiency in obtaining phenolic compounds. Moreover, the watermelon extract exhibited substantial antioxidant activity and contributed to enhancing the stability of soybean oil. These findings contribute to the understanding of the beneficial properties of watermelon and its potential application as a natural antioxidant in various industries. . This study demonstrated the significant impact of watermelon extract on the antioxidant and functional activities, as well as the stability of soybean oil. The antioxidant level of the extract increased from 26.22 mg to 55.5 mg of gallic acid per liter at concentrations of 100 ppm and 400 ppm, with a significant effect observed at a 95% probability level. The scavenging activity of the extract also showed a notable increase, ranging from 60.86% at 50 ppm to 83.62% at 400 ppm.In comparison to Butylated hydroxytoluene at a concentration of 100 ppm, concentrations below 400 ppm of watermelon extract exhibited lower antioxidant activity than the synthetic Butylated hydroxytoluene antioxidant. However, the watermelon extract still demonstrated a significant effect in controlling and reducing the oxidation rate of soybean oil during storage, as compared to the sample without antioxidants ($p<0.05$). In the control sample, the peroxide content of the oil increased from an initial value of 4.2 to 7.2 milliequivalents of oxygen per kilogram of oil after 66 to 70 hours of

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storage at 60-65°C. Conversely, in the oil samples containing 50 ppm, 100 ppm, 200 ppm, and 400 ppm of w.

Keywords: Watermelon extract, phenolic compounds, antioxidant activity, soybean oil stability, optimized extraction

INTRODUCTION

Essential components such vitamins, minerals, carbohydrates, crude protein, different amino acids, lipids, fatty acids, and crude fibre are abundant in watermelon seeds. Farooq, M 2021. The hunt for antioxidant and antibacterial chemicals from natural sources has recently drawn more research focus. The focus has shifted to natural sources as a result of infections developing a resistance to the effects of some manufactured medications. According to studies, the seeds contain the following phytochemicals: phytates, flavonoids, oxalates, phenols, and phytosterols. (H. R. Nadeem et al., 2021; M. Nadeem et al., 2021). They also contain alkaloids, tannins, glycosides, terpenoids, and steroids. (Zia-ur R., et al., 2004).The majority of these phytochemicals have antioxidative properties and offer potential biological substances. (Ranjha Kanwal, et al., 2021) The skin also has anti-ulcer, anti-inflammatory, antifungal, antibacterial, and Hepato protective properties in addition to their antioxidant effects. (Nagal S, Kaur C 2012)There are numerous ways to assess a substance's antioxidant activity, but spectrophotometric techniques like DPPH assays are the most popular (Galano, J et al., 2015. Farooq M et al., 2021). Foline-Ciolteau assays, among many others (Monton et al., 2022; Ballard ST. 2008). It is also possible to use cyclic voltammetry and bioamperometry. (10) Solvent extraction (ethanol, methanol, hexane, etc.) is a common technique for removing antioxidants from watermelon skin. In this study, two types of extraction—Soxhlet extraction and maceration—were carried out. (Monton et al., 2022). The antioxidant activity of watermelon-seed extracts was assessed by DPPH assay because the total antioxidant scavenging activity by this method can be investigated quickly and easily (M. Bimakret al., 2012).

Watermelon is a fruit crop commonly cultivated in high-elevated areas throughout the world. This crop is native to the tropical regions of West and Central Asia and its distribution extends from the Atlantic Ocean, Morocco, Pakistan, and Tunisia to the Black Sea, Armenia, the eastern regions of the Caspian Sea to Afghanistan, and India (Monton et al., 2022; Aksu et al., 2005, Wu N et al.,2012). The oxidation of oils and the alteration of oil and nutritional characteristics are detrimental to consumer health. (Wang L.2013. Wang L 2016).The generation of free radicals, sometimes, causes oxidative stress. (Monton et al., 2022). Antioxidants contain various free radicals and perform a crucial role in preventing the oxidation of lipids and food (Farooq and Azadfar et al., 2021; Tahami F, 2013).Bioactive substances enhance the nutritional value and overall quality of various products, including beverages, cosmetics, colors, and pharmaceuticals. These compounds contribute beneficial effects, such as antioxidant properties and health-promoting attributes, making the products more valuable and beneficial to consumers. (Farooq and Azadfar, 2021; Galano et al., 2015). Fruit skins and contain a significant quantity of naturally occurring antioxidants, primarily phenolic compounds (Alam et al., 2001; Takeuchi et al., 2010).

Optimize extraction efficiency of watermelon fruit extract using ultrasound under different intensity, time, and temperature. Measure phenols and free radical scavenging activity in extract concentrations (50, 100, 200, 400 ppm) using Folin and DPPH tests. Assess the antioxidant effect of the extracts on soybean oil by comparing peroxide and TBA indexes with a control sample and 200 ppm BHT.

In this chapter, phenolic compound was extracted by ethanol assisted by ultrasound waves from watermelon rind and flesh. The optimal extraction parameters were determined using response surface methodology. The functional components and activities of the extract such as phenolic compound, free radical scavenging activity, and thiobarbituric acid index were analyzed and evaluated. The peroxide and TBA index of the soybean oil added with the extract were compared with the control sample and soybean oil sample that has synthetic butylated hydroxytoluene.

Materials

Watermelon fruits from Peshawar, Pakistan, were thoroughly cleaned, and their rinds were extracted, washed, and dried at 35 to 40°C for 46 to 48 hours. The dried rinds were then ground to a powder and stored in airtight containers at 4°C until analysis. This meticulous process ensures top-quality watermelon rind samples for extracting valuable antioxidants with potential industrial applications.

MATERIALS AND METHODS

Materials

Watermelon fruits were procured from the city of Peshawar, KPK, Pakistan. To minimize respiratory and biological activity, the fruits were stored under refrigeration at 4°C until the examination time. Prior to use in our study, the whole watermelon fruit was thoroughly washed. Subsequently, the fruits were sun-dried, and the dried watermelon pieces were milled into a fine powder using an electric mill, passing through a 40-mesh sieve. The watermelon powder was then stored in a cool, dark, and dry area for subsequent tests.

Materials and reagents

A table listing the materials and reagents utilized in this study is provided below.

Table 1 the list of materials and reagents

Compound	Catalog Number	Purity
ABTS (2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)	A1888-2G	≥98%
Diammonium salt	-	-
DPPH (1, 1-diphenyl-2-picrylhydrazyl)	281689-1G	≥97%
Folin–Ciocalteu reagent	F9252-100 ML	1.9–2.1N
Gallic acid monohydrate	398225-100G	≥98%
Whatman Filter Papers No.1	WHA1001325	-
Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid)	391913-1G	purity98%
Acetone	179124-1L	≥99.5%
Sodium carbonate	222321-500G	≥99%
Potassium acetate	236497-100G	≥99%
Aluminum chloride hexahydrate	237078-100G	99%
Ethanol	459844-500 ML	≥99.8%

Equipment

A blender or food processor homogenizes watermelon fruit samples, facilitating extraction. Ultrasonic baths and sonicator enhance extraction efficiency. Centrifuges separate solid residue, rotary evaporators evaporate solvent, and HPLC (High-Performance Liquid Chromatography) quantify and characterize phenolic compounds. Spectrophotometers measure absorbance, determining total phenolic content and antioxidant activity. A drying oven or freeze dryer removes residual moisture, ensuring a dry and stable product for further analysis or storage.

Experimental design

In this study, the Box-Behnken design model was employed to optimize the ultrasound-assisted extraction parameters for watermelon rind powder. The relationship between the response data, including total phenolic content, total flavonoid content, DPPH radical scavenging activity, and $ABTS^{+}$ radical scavenging activity, and the independent variables was determined using a second-order polynomial model. The prediction error was calculated to compare the predicted values with the experimental results. The primary objective of the study was to identify the optimal conditions for extracting bioactive compounds from watermelon rind powder.

UAE extraction

Ultrasound-assisted extraction (UAE) is a technique used to enhance the extraction of bioactive compounds from watermelon skin and flesh. It involves applying ultrasonic waves to accelerate the extraction process. The steps include sample preparation, solvent selection, extraction setup, ultrasound application, and filtration. UAE offers advantages like increased efficiency, reduced extraction time, and improved yields of bioactive compounds. Watermelon contains valuable compounds with antioxidant, antimicrobial, anticancer, and properties. UAE makes these compounds more accessible for analysis and potential application in various industries. (Wang X et al., 2013).

Free radical scavenging activity (DPPH) measurement

Antioxidant activity was evaluated by measuring the inhibition of free radicals (DPPH). DPPH is a stable radical with a purple color, which turns yellow when reduced by hydrogen or electron donors (antioxidants). Different concentrations of the extract in methanol were added to a DPPH solution, and the absorbance was measured at 517 nm after 90 minutes of incubation. The percentage of DPPH inhibition was calculated using a formula. The IC50, the sample concentration providing 50% inhibition, was determined from the inhibition percentages plotted against the sample concentrations **(**Burits et al., 2009; Tanwer BS et al., 2010**).**

Measurement of peroxide value

Peroxide value is a common method used to assess oxidation in fats and oils. It measures peroxides formed during initial oxidation stages. The process involves sample preparation, solvent extraction, titration with sodium thiosulfate, and calculating the peroxide value. Prompt analysis is crucial to avoid further oxidation during storage. Variations exist in the method, but the underlying principle remains consistent. Specific standards may dictate the exact procedures and conditions based on the oil type and application **(**Dal Pra et al., 2013).

Calculation of thiobarbituric acid index (TBA)

The TBA index is a method to measure lipid peroxidation in fats and oils. It reacts with malondialdehyde (MDA), a product of lipid degradation, forming a pink-colored complex. The absorbance intensity at 532 nm is proportional to MDA concentration, reflecting the extent of lipid peroxidation. Higher TBA values indicate increased peroxidation and potential rancidity, while lower values indicate better oxidative stability. It is used in the food industry to evaluate oil quality and oxidative stability, expressed as mg/kg of MDA. Additional analyses may be needed for a comprehensive evaluation of oxidative status. (Tyug TS et al., 2010).

Determination of total phenol content

Utilizing the Folin–Ciocalteu test, the total phenolic content of watermelon extract was determined. Supercritical fluid and ultrasonic extraction were used to produce the extract. As a standard, gallic acid is used to plot a calibration curve. By dissolving 10 mg of watermelon peel in 10 mL of ethanol, a stock solution was created. From the stock solution, 250 µL was extracted for the test, and 750 µL of ethanol was added to form 1 mL solution. (Weremfo et al., 2020)To extract the solution, about 10 µL of fresh Folin–Ciocalteu reagent was added and stirred thereafter. Later, the solution was incubated for about 10 min under the conditions of light absence. Next, 100 µL of 7.5% Na_2CO_3 aqueous solution was added to the reaction solution, and the sample was incubated at 30°C for 30 min. The absorbance was then measured at 765 nm using a microplate reader with several detectors (Synergy HT, Bio-Tek). (Ballard ST., 2008).It was determined using the regression equation and expressed as l M/g equivalent gallic acid (Kumar et al., 2020; Johari MA et al.,2019).

Total Flavonoid Content

To determine the total flavonoid content in the watermelon extract, 10 mg of the extract was dissolved in ethanol to create a stock solution. Diluted samples were prepared and treated with $NaNO_2$, $AlCl_3$, and NaOH solutions to develop a color reaction. Absorbance was measured at 510 nm using a microplate reader. A calibration curve with known concentrations of gallic acid was used to quantify the flavonoid content in the sample, reported as l M/g equivalent Gallic acid **(**Kumar et al., 2020; Oliveira AS et al., 2018**).**

Determination of phosphorus

Phosphorous in the watermelon skin extract was detected as $PO^{4}{}_{3}$- using a spectrophotometric approach. The extract was mixed with sodium phosphomolybdate reagent, forming a yellow-orange complex. A calibration curve using standard phosphorus solutions was created. Absorbance at 430 nm was measured after storing the solution at 20°C for 20 minutes. Results were expressed as dry mass and averaged from three analyses. A control sample with 70% ethanol was used for comparison **(**AOAC, 2016).

Measurement of the metal elements

The described process is known as wet digestion, a sample preparation technique used for analyzing metal elements in complex matrices like watermelon rind using Atomic Absorption Spectroscopy (AAS). The method involves dissolving the rind in a combination of hydrochloric acid (Hcl) and nitric acid (HNO_3) to release the metal elements. After heating and filtration, the solution is diluted and subjected to AAS

analysis, providing accurate measurements of metal concentrations. Proper sample preparation is vital for reliable results in metal analysis of watermelon and other food samples. (AOAC et al. 2016).

Proximate Analysis

The proximate analyses of watermelon rind were conducted in accordance with the guidelines provided by the Association of Official Analytical Chemistry. The moisture content was determined by oven-drying the pectin samples at 105 °C. Ash content was measured by subjecting the dried pectin samples to a temperature of 600 °C for 3 hours in a furnace. The Kjeldahl method was used to analyze the crude protein content. Crude fiber content was determined using the gravimetric method, while the fat content was assessed using the Soxhlet method. The carbohydrate content (Ch %) was estimated using Equation (1):

$$Ch\ \% = (100 - Ms - Cp - Cf - As)\ \%$$

The proximate analyses of watermelon rind included the determination of carbohydrate (Ch), moisture (Ms), crude protein (Cp), crude fiber (Cf), and ash (As) contents. The specific methods used for analysis were oven-drying at 105 °C for moisture content, heating at 600 °C for 3 hours in a furnace for ash content, the Kjeldahl method for crude protein content, gravimetric method for crude fiber content, and the Soxhlet method for crude fat content.

Peroxide index

The peroxide index measures peroxide compounds in oils and fats to assess oxidation or rancidity. The sample is dissolved in a solvent and reacted with potassium iodide to produce iodine. After a specified time, iodine is titrated with sodium thiosulfate. The endpoint is determined using a starch indicator. The peroxide index is calculated based on titrant volume and sample weight, expressed as mEq O2/kg or mEq peroxide/kg of the sample. It is a crucial method for determining the freshness and quality of edible oils in the food industry.

Ultrasound-assisted extraction of optimal experiment using RSM

The study utilized Ultrasound-assisted extraction (UAE) in conjunction with Response Surface Methodology (RSM) to optimize the extraction of bioactive compounds from watermelon. RSM allows for the variation of extraction time, temperature, ultrasound power, solvent-to-sample ratio, and particle size to identify the optimal conditions. Statistical analysis with RSM software enabled the determination of the best combination of variables to achieve maximum extraction efficiency, reducing the number of tests and identifying significant factors and their interactions. Design Expert version 7 software was employed for the statistical design, with time, sound frequency, and temperature selected as independent variables and the percentage of extraction as the response. The study's findings contribute to the development of efficient extraction techniques for watermelon bioactive compounds, holding potential applications across various industries. (EsmaeilzadehKenari R et al., 2014).

Experiment design and results analysis

RSM is a collection of statistical techniques that are used in the optimization of processes where the desired response is affected by a number of variables. One of the advantages of this plan is to reduce the number of tests and obtain the most important

influencing factors in a process and possible interactions between them. Therefore, in this study, the statistical design of the response level was adopted using the Design Expert version 7 software. Time (A), sound intensity (B), and temperature (C) were selected as independent variables of the process and the percentage of extraction (Y) was evaluated as the response. Each of the independent variables have been examined at three levels, as shown in Table 2.

Table 2 Independent variables

Variable	Actual values		
	-1	-1	+1
Time (min)	20	20	60
Sound Frequency (kHz) (B)	30	30	75
Temperature (°C) (A)	55	55	100

Table 3 optimising the extraction efficiency table

Alignment	Time	Ultrasound Frequency	Temperature
1	30	120	65
2	20	80	65
3	30	80	55
4	20	80	45
5	40	80	65
6	30	80	55
7	30	40	45
8	30	80	55
9	20	120	55
10	30	80	55
11	30	80	55
12	40	40	55
13	40	120	55
14	30	40	65
15	30	120	45
16	20	40	55
17	40	80	45
18	30	80	55

Statistical analysis

Statistical analysis, Box-Benhken design including 18 experiments with 6 replications in central points was used. The number of treatments related to the extraction designed by the software as well as the obtained answers are shown in Table 3.

RESULTS AND DISCUSSION

Extraction efficiency

The analysis of variance of the central composite response surface model indicated that linear and quadratic effects significantly impacted the percentage of mountain watermelon fruit extraction. Among the quadratic effects and interactions, the parameter B2 had the greatest influence on extraction efficiency. Ultrasound application increased extraction efficiency by enhancing mass transfer and cell breakage through cavitation. Higher frequencies led to increased extraction efficiency.

Ultrasound-induced cavitation creates bubbles that collapse, causing tissue decomposition and cell membrane thinning, facilitating extraction (Vilkhu et al. 2008).

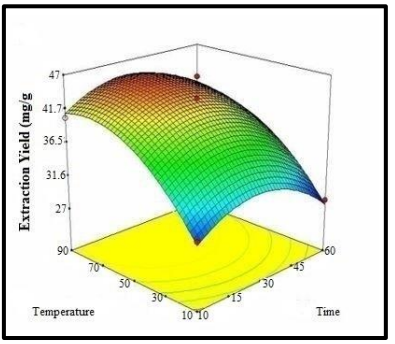

Fig. 1 Influence of Time and Sound Frequency on the Efficiency of Extraction Process

In Fig.1 we observe a 3D illustration of the factors influencing the extraction efficiency, namely the extraction time and temperature. Notably, as the temperature increased, the extraction efficiency demonstrated a consistent and steady improvement.

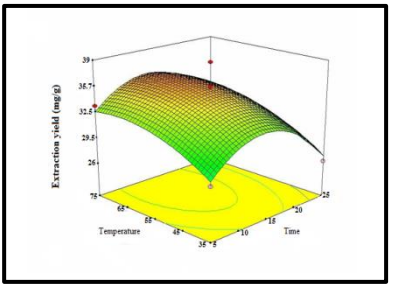

Fig. 2 Influence of Time and Temperature on the Extraction Process

Ultrasound-assisted extraction (UAE) causes bubble bursting, leading to increased mass transfer and high-speed liquid flow toward solid particles, breaking them. It disrupts cell walls and plant tissue, allowing bioactive compounds to enter the solvent, enhancing the nutritional value. Optimal extraction time and power improve diffusion and mass transfer, increasing the percentage of dry extract. UAE accelerates the extraction process, shortening extraction time compared to other methods. It improves extraction efficiency of colored compounds and grape extracts, and higher temperatures enhance extraction rates. UAE shows efficient plant tissue destruction and solvent penetration compared to other methods **(**Kamran et al. 2010).

Measurement of phenolic compounds

Based on the obtained results, it was observed that the maximum concentration of polyphenolic compounds, with a significant difference, was found in the mountain watermelon extract at 400 parts per million (ppm) (Fig.3). Conversely, the control sample showed the least amount of polyphenolic compounds. Notably, for the mountain watermelon extract, an increase in extract concentration from 100 to 400 ppm led to a proportional increase in the number of polyphenolic compounds, thereby enhancing its

antioxidant properties. The statistical analysis of the results indicated significance across all extract concentrations at the 95% probability level.

Comparatively, the antioxidant synthesized sample,(BHT) at 200 ppm concentration, exhibited a higher number of phenolic compounds than the samples containing mountain watermelon extract, and this difference was found to be statistically significant ($p<0.05$). Mean comparisons further revealed that the maximum concentration of phenolic compounds was observed in the BHT synthetic antioxidant sample, with a value of 58.66, while the lowest amount was associated with the 100-ppm extract sample, with a value of 26.22. These findings highlight the varying antioxidant potentials of different extract concentrations and the effectiveness of BHT as a synthetic antioxidant.

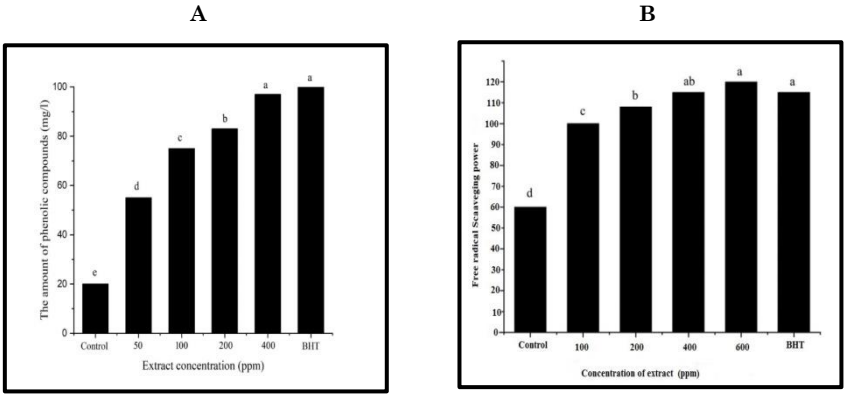

Fig. 3 The effect of different watermelon extract concentration levels on total polyphenolic quantities

Examining the extract's DPPH free radical scavenging activity

The study demonstrated that increasing the concentration of the extract resulted in higher Activity of DPPH in scavenging free radicals. The anti-radical activity improved with higher concentrations of the extract, showing a concentration-dependent nature. However, compared to the synthetic antioxidant BHT at 100 ppm concentration, other extract concentrations, except for 400 ppm, exhibited lower antioxidant activity. The increase in phenolic compound concentration enhances the extract's ability to scavenge free radicals due to the increased number of hydroxyl groups, resulting in higher antioxidant activity. These findings align with previous research that also observed a positive relationship between phenolic compound concentration and antioxidant capacity in various extracts.

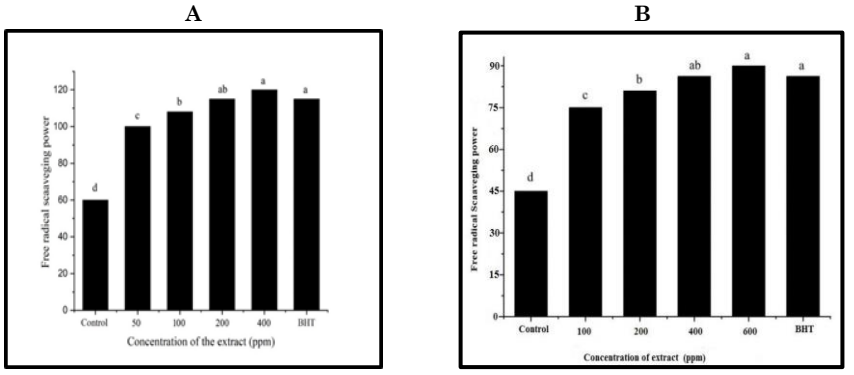

Fig. 4 DPPH free radical scavenging activity of various doses of watermelon extract

Proximate analyses

The watermelon rind's proximate analysis evaluated its composition, including ash, carbohydrate, protein, fiber, and fat contents, which impact pectin extraction quality and quantity. Moisture content was 5.98%, lower than reported values for red watermelon rinds (Table.5). Ash content was 6.97%, also lower, indicating suitability for pectin extraction with reduced ash content. Carbohydrate and crude protein contents were comparable to other watermelon rinds. Fiber and fat contents varied, likely due to differences in watermelon sample maturity.

Table 5 proximate analysis of watermelon rind

Moisture content (%)	5.98	10.03	11.01	11.01
Ash content (%)	6.97	16.92	12.89	11.96
Crude protein content (%)	10.99	11.02	10.96	10.94
Crude fiber content (%)	1.00	14.98	16.92	–
Crude fat content (%)	14.88	–	1.99	3.02
Carbohydrate content (%)	57.98	58.88	55.92	72.96

Suleiman et al. (2019) Sayed et al. (2013) Hoque et al. (2015)

Peroxide index

The results showed that the mountain watermelon extract concentration had a significant effect on reducing soybean oil oxidation during storage ($p<0.05$). Adding 400 ppm of extract to the oil decreased the peroxide value, indicating improved antioxidant activity. Other studies with natural antioxidants in oils also demonstrated enhanced oxidative stability. Overall, higher concentrations of phenolic compounds in the extract contributed to better antioxidant properties, enhancing soybean oil stability. Similar findings were observed in studies with other plant extracts and synthetic antioxidants like BHA and BHT. (Takeuchi TM ET AL. 2010**).** See in figure 5).

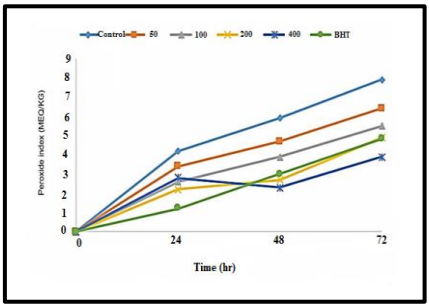

Fig. 5 Changes in the peroxide index of soybean oil samples were observed over varying storage durations at 65°C, employing different concentrations of watermelon extract

Thiobarbituric acid index (TBA)

The results showed that the variation in soybean oil's TBA index stored in an oven at 65°C was significantly influenced by mountain watermelon extract ($p<0.05$). The comparison of the mean index of thiobarbituric acid (TBA) resulting from the effects of various concentrations of watermelon extract is showed in (Fig.6) In the first days, the value of this index is very low because this index increases due to the decomposition of hydroperoxides formed in the first days and their conversion into aldehydes and ketones. As a result, the value of this index increases in the final days of the test. The control sample increased more than the treated samples in all stages of storage. The highest amount of thiobarbituric acid on all days belonged to the control sample. After storing the soybean oil at 65°C for 72 (h), the value of this index increased. Malondialdehyde is obtained from the decomposition of hydroperoxides. The highest ability to prevent the formation of secondary oxidation products was applied by the 400 ppm treatment of watermelon extract. So that the value of this index was 0.250 and 0.438 mg of malonaldehyde/kg, respectively, in the samples under this treatment during 72 h of storage (Fig.6). While the value of the thiobarbituric acid index (TBA) for 50, 100, and 200 ppm concentrations after 72 (h) of storage in an oven at 65°C was 0.737, 0.687, and 0.629 mg of malonaldehyde/kg, respectively. These results showed when the days were ending for this experiment the oxidation starts in the products at a faster rate and a large amount of peroxides formed in the early stages were decomposed and turned into malonaldehyde. See in figure 6).

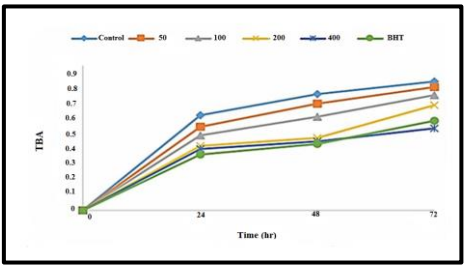

Fig. 6 Variation in the TBA index of the soybean oil samples after three days of being stored at 65°C

Sing et al. (2006**)** conducted a study to investigate the antioxidant activity of watermelon skin on soybean oil. The results demonstrated that the acetone extract exhibited superior antioxidant effects at a higher concentration compared to BHA and BHT synthetic antioxidants at a 0.2% concentration. In a study by Sing et al. (2006), the chemical composition of fennel volatile oil was examined, along with its anti-fungal and antioxidant properties. Additionally, the study evaluated the antioxidant activity of its acetone extract in linseed oil. The experiments revealed that both the volatile oil and acetone extract of fennel exhibited higher antioxidant activity when compared to BHA and BHT synthetic antioxidants. These findings highlight the potential of fennel extracts as effective natural antioxidants.

Effects of Extraction Parameters on Total Flavonoid Content

The yield of total flavonoids in watermelon extracts is influenced by factors like solvent composition, temperature, and extraction time. The study found that the highest flavonoid content was observed at 60°C and 90% ethanol concentration during a 120-minute extraction. The lowest yield was obtained at 40°C with 50% solvent concentration. The quadratic term of solvent concentration and temperature significantly influenced the total flavonoid content, emphasizing the importance of controlling solvent composition and temperature during the extraction process.

Table 6 Experimental findings were analyzed using multiple linear regressions and the second-order polynomial model (Eq. (1

Run	Ethanol concentration (%)	Temperature (°C)	Time (min)	Phenolic content	Flavonoid content	IC_{50} (mg/mL)
1	10 (−1)	20 (−1)	120 (0)	3.37	1.93	0.032
2	10 (−1)	40 (0)	60 (−1)	3.12	1.75	0.035
3	10 (−1)	60 (1)	120 (0)	3.44	1.68	0.031
4	10 (−1)	40 (0)	180 (1)	3.66	1.86	0.034
5	50 (0)	20 (−1)	60 (−1)	3.10	1.78	0.035
6	50 (0)	60 (1)	60 (−1)	3.45	1.76	0.041
7	90 (1)	40 (0)	60 (−1)	3.45	1.78	0.041
8	90 (1)	60 (1)	120 (0)	3.33	2.10	0.031
9	90 (1)	40 (0)	180 (1)	3.73	2.08	0.033
10	50 (0)	60 (1)	180 (1)	3.49	1.58	0.034
11	50 (0)	40 (0)	120 (0)	3.52	1.41	0.028
12	50 (0)	40 (0)	120 (0)	3.63	1.56	0.028
13	50 (0)	40 (0)	120 (0)	3.66	1.57	0.031
14	50 (0)	40 (0)	120 (0)	3.69	1.56	0.031
15	50 (0)	40 (0)	120 (0)	3.63	1.57	0.028
16	90 (1)	20 (−1)	120 (0)	3.25	2.05	0.028
17	50 (0)	20 (−1)	180 (1)	3.62	1.73	0.034

Table 7 Experimental design of phenolic compound extraction using ultrasonic-assisted Extraction and total phenolic compound concentrations

Independent variables TPC (µg GAE/mg dry sample)

No	Temperature (℃)	Time (min)	Ethanol concentration%	Flesh extract	Rind extract	Skin extract
1	30	30	35	3.18 ± 0.24	2.30 ± 0.17	4.34 ± 0.24
2	70	30	35	4.14 ± 0.14	2.95 ± 0.46	5.35 ± 0.01
3	30	60	35	2.95	2.11	4.15

				± 0.34	± 0.05	± 0.59
4	70	60	35	5.06	3.90	7.07
				± 0.17	± 0.36	± 0.23
5	30	45	0	1.67	1.08	2.96
				± 0.46	± 0.34	± 0.19
6	70	45	0	3.49	2.45	4.51
				± 0.44	± 0.15	± 0.60
7	30	45	70	2.58	2.08	3.96
				± 0.02	± 0.05	± 0.23
8	70	45	70	5.75	4.22	6.98
				± 1.93	± 0.01	± 1.33
9	50	30	0	2.51	1.42	3.87
				± 0.20	± 0.25	± 0.22
10	50	60	0	1.90	1.39	3.07
				± 0.57	± 0.24	± 0.03
11	50	30	70	3.91	2.48	4.77
				± 0.86	± 0.08	± 0.19
12	50	60	70	4.83	3.63	6.50
				± 0.65	± 0.25	± 0.35
13	50	45	35	4.11	2.92	5.35
				± 0.17	± 0.37	± 0.01
14	50	45	35	4.60	3.20	6.37
				± 0.22	± 0.64	± 0.48
15	50	45	35	4.21	3.09	5.63
				± 0.57	± 0.18	± 0.07
16	50	45	35	4.82	3.42	6.48
				± 0.24	± 0.22	± 0.34

Table 8 Physicochemical characteristics of watermelon rind (n=3) (results ± SD)

Parameter	Protein (g/100g)	Fiber (g/100g)	Ash (g/100g)	Fat (g/100g)	IC50 (mg/kg)	Total phenolic compound (g/100g)
Amount	6.75 ± 0.48	23.00 ± 1.42	12.90 ± 0.38	0.94 ± 0.08	146.80	2.57

Total Phenolic Content and Antioxidant Activity

Watermelon skin had the highest phenolic content (15.3 mg GAE/g) and antioxidant activity (DPPH: 31.1 µmol TE/g; FRAP: 240.0 µmol Fe2+/g), followed by flesh and rind. Watermelon skin exhibited significantly better phenolic content and antioxidant activity compared to both flesh and rind ($p < 0.05$).

Table 9 Total phenolic content and antioxidant activity

Sample	**Total Phenolic Content (mg GAE/g)**	**DPPH (µmol TE/g)**	**FRAP (µmol Fe^{2+}/g)**
Flesh	12.6 ± 0.3[b]	28.8 ± 3.4 [a]	182.2 ± 7.1 [b]
Rind	2.5 ± 0.3 [c]	7.0 ± 2.6 [b]	39.9 ± 9.0 [c]
Skin	15.3 ± 0.5 [a]	30.9 ± 6.3 [a]	239.9 ± 8.3 [a]

Values represent mean ± SD (n = 20 the total phenolic content and DPPH antioxidant activity were analyzed for 15 samples in each group. Different letters indicate significant differences between column means ($p<0.05$). DPPH stands for 2, 2-diphenyl-1-picrylhydrazyl, and FRAP stands for ferric reduction antioxidant potential. GAE represents gallic acid equivalent, and TE represents trolox equivalent. Watermelon skin showed the highest phenolic content and DPPH activity, while FRAP values were also significantly higher for watermelon skin compared to flesh and rind ($p< 0.05$). (Bouaziz M, 2008)

Heavy metals

The heavy metal concentrations (mg/l) in the watermelon rind were analyzed before dehydration, and the results are presented in (Table.10).Among the identified heavy metals, Ni had the highest content at 9.239 mg/l, while Cd had the lowest concentration. It is essential to note that heavy metals like Cd, As, Hg, and Pb can be highly toxic even at low levels and are biologically unnecessary. However, it is reassuring to find that the concentration of Ni in the watermelon rind was below the safe limit set by the World Health Organization (WHO limit in fruits = 10 ppm) This indicates that the watermelon rind does not pose a health risk concerning Ni content and can be considered safe for consumption or further processing.

Table 10 heavy metal content in the watermelon rind

Metal	Quantity (mg/l)
Pb	0.069>
Ni	9.239
As	1.011
Hg	<0.146
Cd	<0.003

Physicochemical properties

Watermelon rind contains 0.91 g/100 g crude fat, 23.00 g/100 g crude fiber, and 6.67 g/100 g crude protein by dry weight. The total phenolic content (TPC) extraction process with ultrasonic waves requires careful optimization to preserve and enhance phenolic compounds, as the interaction of sonication temperature and time can negatively affect TPC, while the interaction of sonication temperature and ethanol concentration has positive effects. Variations in physicochemical attributes between different studies may be due to differences in watermelon varieties. (Arabshahi DS et al. 2006)

Effect of ethanol concentration on the amount of total flavonoids

In this study, different concentrations of ethanol solution (20, 40, 60, 80, and 99.7%) were used to extract flavonoids from sorrel powder. TFC initially increased up to 60% ethanol, indicating good solubility and cell penetration. Beyond 60%, flavonoid extraction decreased due to increased dissolution of alcohol-soluble pigments and lipophilic components, reducing the binding of flavonoids with ethanol-water molecules (Li et al. 2019). The optimal ethanol concentration range for extraction was 40% to 80%.Regarding extraction time, TFC initially increased with time due to the concentration difference between raw material and extraction solution. After reaching a relative balance, the extraction rate stabilized. Additionally, longer extraction times also dissolved other alcohol-soluble substances, influencing flavonoid extraction. The optimized extraction time ranged from 30 to. 60 (min) to achieve efficient flavonoid extraction. (Delfanian. M et al., 2014)

CONCLUSIONS

This study focused on the ultrasound-assisted extraction of antioxidant-rich phenolics and flavonoids from watermelon rind powder. The researchers used a central composite response surface model to determine the impact of extraction parameters on extraction efficiency. They found that ultrasound application increased extraction efficiency by enhancing mass transfer and cell breakage through cavitation. Higher frequencies led

to increased extraction efficiency. The study revealed that watermelon skin had the highest phenolic content and antioxidant activity, outperforming both flesh and rind significantly. The extracted compounds demonstrated excellent antioxidant activity, particularly at a concentration of 400 ppm. The study suggested that these natural antioxidants could potentially replace synthetic ones in edible oils. In addition, the study highlighted the nutritional value of Citrullus lanatus seed flour, which is rich in protein, fat, and energy, but relatively low in calcium. It also reported the physicochemical characteristics of watermelon rind, including ash, carbohydrate, protein, fiber, and fat contents. Moreover, the researchers investigated the total phenolic content and antioxidant activity of watermelon extracts, as well as their effects on soybean oil oxidation. The results showed that increasing the concentration of the extract resulted in higher DPPH free radical scavenging activity. The extract demonstrated effective antioxidant properties, enhancing soybean oil stability. Furthermore, the study examined the yield of total flavonoids in watermelon extracts, with different extraction conditions tested. During a 120-minute extraction, the greatest flavonoid content was found at 60°C and 90% ethanol concentration. The study also analyzed the heavy metal content in the watermelon rind and found that the concentration of Ni was below the safe limit set by the World Health Organization, indicating the safety of watermelon rind for consumption or further processing. Overall, the research concluded that ultrasound-assisted extraction is a promising and eco-friendly method for obtaining valuable plant compounds with potential applications as natural antioxidants and for enhancing the nutritional value of food products.

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