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Comparison of bioactive compounds extracted from pumpkin skin using supercritical fluid and subcritical water methods

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ABSTRACT

One of the most important problems in the food industry is food oxidation. Pumpkin skin has been noted for having phenolic and carotenoid compounds with antioxidant properties. In this research, phenolic and carotenoid extracts of pumpkin peel were obtained using sub-critical water extraction and supercritical fluid extraction methods and then phenolic and carotenoid compounds were measured. The combined extracts had the highest antioxidant properties due to the synergistic properties of the extracts. In general, in all the antioxidant activity evaluation methods, the combined extract extracted using supercritical fluid and then the combined extract extracted using subcritical water showed the highest antioxidant activity. Also, the extraction method with subcritical water was more effective for extracting carotenoids and the extraction method with supercritical fluid was more effective for extracting phenolic compounds. There was a relationship between the amount of phenolic and carotenoidal compounds of the extracts and the antioxidant activity. In all three methods antioxidant activities of extracts increased by increasing in extract concentration. The results showed that the combined phenolic-carotenoid extract of pumpkin skin can be a suitable alternative to synthetic antioxidants as a natural antioxidant.

1. Introduction

Oxidation reactions not only alter the organoleptic properties of food products but also reduce their nutritional value and shelf life. Moreover, due to the production of undesirable compounds that adversely affect consumer health, they contribute to aging, cardiovascular diseases, mutations, and cancer. Therefore, stabilizing food products under thermal and storage conditions is inevitable. Various methods are used for food stabilization, among which the use of antioxidants is one of the most important approaches [1]. Although synthetic antioxidants perform effectively during thermal processing and storage conditions, their use raises concerns regarding food safety and potential toxicity. The most potent synthetic antioxidant, TBHQ, is not approved for use in Japan, Canada, and Europe, and BHA has been removed from the GRAS (Generally Recognized as Safe) list. Consequently, the search for natural antioxidants as alternatives to synthetic ones has gained significant importance [2].

Studies have shown that the antioxidant activity of certain fruits and vegetables depends on their total phenolic content [15]. Phenolic compounds are a group of aromatic secondary plant metabolites that are widely distributed throughout plants and possess multiple biological activities, such as antioxidant and antibacterial effects [3]. Today, edible food waste has gained significant attention and value and can be considered rich sources of antioxidant polyphenol. Utilizing these wastes as a source of polyphenols can offer considerable economic benefits for food producers [4]. Pumpkin, scientifically known as *Cucurbita pepo* from the *Cucurbitaceae* family, has high nutritional value. It contains 2 to 10 mg of vitamin C and 9 to 10 mg of vitamin E per 100 grams. It serves as an excellent source of carotenoids and phenolic compounds for human consumption. In addition to lycopene, beta-carotene, and alpha carotene, it also contains lutein, which is abundant in the peel of the fruit and possesses properties such as provitamin activity, antioxidant effects, and health benefits [5,6,7].

Extraction is a critical step in obtaining valuable compounds such as phenolics and carotenoids from plant tissues. The choice of an appropriate extraction method depends on the type of target compounds, the structural features of the plant matrix (fruit, stem, seed, leaf, root, flower, etc.), the required extract quality and yield, process conditions (temperature, pressure, etc.), and the economic feasibility of the process [8].Among modern extraction techniques for antioxidants, supercritical fluid extraction (SFE) and subcritical water extraction (SWE) are currently prominent. SFE offers many advantages, such as reduced extraction time and environmental friendliness. Its high solubility enhances mass transfer. SFE exhibits properties between those of a gas and a liquid, with its key feature being high density. Supercritical fluids have high permeability and lower viscosity compared to liquid solvents. The best solvent for this method in extracting natural plant compounds is CO_2, as it is inexpensive, inert, accessible, tasteless, and GRAS-listed [9]. SWE is defined as water heated between 100°C and 300°C. It is an effective solvent for both polar and non-polar compounds. It maintains its liquid state between 100°C (boiling point of water) and 374°C (critical point of water) under a pressure below 22 MPa. Moreover, water is a widely available, non-toxic, and low-cost solvent, making SWE an ideal option as a solvent in pharmaceutical and food applications [10].Considering the impact of extraction method and conditions on the efficiency and composition of extracts, and aiming to improve system performance and process efficiency without increasing costs, this study investigates and compares the extraction of phenolic and carotenoid compounds from pumpkin peel using two non-thermal methods: Supercritical Fluid Extraction (SFE) and Subcritical Water Extraction (SWE).

2. Materials and Methods

2.1. Materials

Pumpkin (*Cucurbita pepo*, var. *Styarica*), which has a more intense orange color compared to other varieties, was selected. After thorough washing, the peels were separated. The thickness of the removed peel was 1 ± 0.2 cm. The peels were then dried in a vacuum oven at 40°C and ground into a powder with a particle size of 2 mm using a grinder [11].

2.2. Methods

2.2.1. Supercritical Fluid Extraction (SFE)

To begin the extraction, 12 grams of pumpkin peel along with glass beads were placed in the extractor. The extractor was mounted on a heater. Carbon dioxide, used as the solvent, was

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pumped at a flow rate of 15 mL/min using an HPLC pump to reach the desired pressure. Subsequently, ethanol: water (80:20) was introduced at a rate of 0.25 mL/min using another HPLC pump. The mixture of carbon dioxide and ethanol: water (80:20) was fed into the extractor. Pressure was regulated by a thermal regulator. Both the CO_2 and the ethanol: water co-solvent was pre-heated using a pre-heater to reach the operating temperature. To ensure that the desired temperature was reached, the inlet and outlet temperatures of the extractor were measured. The operating conditions were temperature 60°C, duration 3 hours, and pressure 25 MPa. The extract was collected in vials every 30 minutes and stored in a refrigerator until further use [12].

For extraction of bioactive compounds, initially 20 grams of sample were mixed with 100 mL of ethanol as a co-solvent. The extraction conditions for phenolic compounds were set at temperatures ranging from 308 to 385 K, pressures between 0.03 to 0.25 MPa, and a duration of 30 minutes. For carotenoids, the conditions were: temperature between 40 to 70 K, pressure between 25 to 35 MPa, and a duration of 30 minutes. After extraction, the phenolic and carotenoid compounds were stored at −18°C until analysis.

2.2.2. Subcritical Water Extraction (SWE)

For total extract preparation, 12 grams of pumpkin peel along with glass beads were placed in the extractor. The extractor was mounted on a heater. Water was used as a solvent and pumped at a flow rate of 1 mL/min using an HPLC pump to reach the required pressure. Pressure was controlled using a thermal regulator. Water was pre-heated with a pre-heater to reach the operating temperature. To ensure the target temperature was achieved, the inlet and outlet temperatures of the extractor were monitored. The operating conditions were temperature 120°C, duration 3 hours, and pressure 5 MPa. The extract was collected every 30 minutes in vials and stored in a refrigerator until use [12].

For extraction of bioactive compounds using SWE, phenolic compounds were extracted under the following conditions: temperature range 100–200 K, pressure 6.89 MPa, and duration 10–50 minutes. For carotenoids, the conditions were: temperature range 160–200 K, pressure 6.89 MPa, and duration 30 minutes. The extracted materials were stored at −18°C until further analysis.

Following individual extraction of pumpkin peel using both SFE and SWE methods, total extracts were obtained. Finally, the extracts obtained from these two innovative and combined extraction methods were compared in terms of phenolic content and antioxidant capacity, with regard to oxidative stability of canola oil.

Table 1:Treatments examined in extract tests

Treatment Code	Type of Antioxidant Used
CON	No antioxidant (control)
TBHQ	100 ppm of synthetic antioxidant TBHQ
PHSP	Phenolic extract obtained by supercritical fluid extraction
CASP	Carotenoid extract obtained by supercritical fluid extraction
PHSB	Phenolic extract obtained by subcritical water extraction
CASB	Carotenoid extract obtained by subcritical water extraction
MIXSP	Combination of phenolic and carotenoid extracts obtained by supercritical fluid extraction
MIXSB	Combination of phenolic and carotenoid extracts obtained by subcritical water extraction

2-3.Analysis

2-3-1. Quantitative and Qualitative Determination of Total Phenolic, Flavonoid, and Carotenoid Contents

To determine the quantitative and qualitative levels of phenolic compounds, flavonoids, and carotenoids in the extracts, high-performance liquid chromatography (HPLC) was used. The carotenoid extracts were dissolved in ethyl acetate and analyzed using an HPLC system equipped with an XDB-C18 column (5 µm; 4.6×150 mm) and a UV-vis detector. Chromatographic separation was carried out at a constant flow rate of 1.5 mL/min at a

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wavelength of 470 nm using a solvent mixture of acetonitrile: dichloromethane (75:25 v/v). Carotenoids in the extracts were identified and quantified by comparing the retention times and peak areas with standard samples. Each extract was analyzed twice.

2-3-2. Antioxidant Activity of the Extracts

2-3-2-1. DPPH Free Radical Scavenging Assay

A volume of 2.7 mL of freshly prepared DPPH solution (6×10^{-5} M) was mixed with 0.3 mL of extract at different concentrations (100, 200, 300, and 400 ppm) as well as with 100 ppm of the synthetic antioxidant TBHQ as the positive control. The mixture was vigorously stirred and kept in the dark for 1 hour. Absorbance was then measured at 517 nm, and the percentage of DPPH scavenging was calculated using the formula:

$$\%\text{DPPH quenched} = [1 - (A_{sample})/(A_{DPPH})] \times 100$$

where A_{sample} and A_{DPPH} are the absorbance values of the sample and the control, respectively.

2-3-2-2. Ferric Reducing Antioxidant Power (FRAP)

A volume of 2.5 mL extract solution was mixed with 2.5 mL of sodium phosphate buffer (200 mM) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 116.272g for 8 minutes. Afterwards, 5 mL of the supernatant was mixed with 5 mL of deionized water and 1 mL of 0.1% ferric chloride. The absorbance of the resulting solution was measured at 700 nm. TBHQ at 100 ppm was used as the positive control.

2-3-3. β-Carotene–Linoleic Acid Bleaching Assay

First, a stock solution was prepared by dissolving 5 mg of β-carotene in 10 mL of chloroform. Then, 600 µL of this solution was added to a mixture of 40 mg linoleic acid and 400 mg Tween 40. Chloroform was removed via rotary evaporation, and 100 mL of oxygenated distilled water was added and vigorously stirred to form an emulsion. Then, 5 mL of this emulsion was transferred to test tubes and 200 µL of each extract was added. The absorbance of samples was measured at 470 nm at time zero and after 120 minutes in a 50°C water bath. The antioxidant capacity was calculated as a percentage of inhibition using the following formula:

$$\text{Antioxidant Activity (\%)} = (DRc - DRs) \times 100 / DRc$$

where DRc and DRs are the degradation rates of the control and sample, respectively, calculated as:

DRc and DRs calculated as ln (a/b)

(a) being the absorbance at time zero and(b) being the absorbance after 120 minutes.

3. Results and Discussion

3-1. Total Phenolic, Flavonoid, and Carotenoid Contents of the Extracts

Extraction is a key step in recovering and separating bioactive compounds from plant samples [15]. The results of phenolic, flavonoid, and carotenoid contents of the extracts are presented in Table 2. Phenolic compounds are responsible for scavenging free radicals in plant samples, and the antioxidant activity of a plant depends on both the type and amount of its phenolic compounds. The highest total phenolic content was observed in the PHSP extract, while the lowest was found in CASB. Supercritical fluid extraction was more effective in extracting phenolic compounds compared to subcritical water extraction [16,17]. One study reported the total phenolic content in pumpkin peel as 160.23 mg/g dry weight [18]. Another study calculated the total phenolic content in pumpkin extract as 672.19 mg gallic acid/100 g of extract, which is higher than the value obtained in this study, likely due to differences in extraction method. The highest total carotenoid content was observed in the CASB extract. The total carotenoid content obtained via subcritical water extraction was higher than that of supercritical fluid extraction. Simultaneous application of heat and pressure

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in supercritical fluid extraction enhances the solubility of CO_2 and significantly improves the extraction of phytochemicals and nutrients from pumpkin peel [19]. Mala et al. (2016) reported β-carotene content in pumpkin peel as 11.89 mg/100 g. Singh et al. (2016), who investigated phenolic and flavonoid contents of pumpkin peel extracts using different solvents, reported total phenolics ranging from 13.92 to 33.48 mg GA/100 g and flavonoids from 3.79 to 11.72 mg QE/100 g, which are lower than the values obtained in this study. Since they used a solvent-shaking method for extraction, it can be concluded that both subcritical water and supercritical fluid extractions were more effective in extracting phenolic compounds.

Both phenolic and carotenoid compounds can scavenge free radicals or singlet oxygen, donate hydrogen atoms, and degrade free radicals [17].

Table 2 Amount of phenolic, flavonoids and carotenoid compounds of the extracts

total carotene (mg ß-carot/100 g E)	Total flavonoids (mg QE/100 g E)	Total phenol (mg GA/100 g E)	Type of extract
3.88±0.90[d]	218.72±3.48[b]	203.8±0.87[b]	PHSB
12.16±2.47[b]	2.03±0.36c	9.82±1.68[c]	CASB
8.04±3.36[a]	1.11±0.04[d]	10.29±1.43[d]	CASP
3.22±2.35[c]	276.21±2.19[a]	343.5±8.84[a]	PHSP

Non-identical lowercase letters in each column indicate statistically significant differences at the 5% level.

2-3. Antioxidant Activity of Extracts

The DPPH free radical scavenging method is a rapid assay used to determine the hydrogen-donating capacity of chemical substances, thereby assessing their antioxidant activity. When DPPH molecules encounter a proton-donating radical, their violet color rapidly fades [19]. The antioxidant activity evaluation via DPPH is based on the ability of DPPH, a stable free radical, to undergo discoloration in the presence of antioxidants. Therefore, a lower absorbance value indicates a higher radical scavenging capacity of the extract. In the ferric reducing antioxidant power (FRAP) assay, free radicals are neutralized via electron transfer or hydrogen atom donation. These methods are simple, cost-effective, and suitable for industrial applications [20]. Linoleic acid oxidation accelerates the formation of free radicals, which ultimately leads to the bleaching of highly unsaturated β-carotene molecules. Thus, the addition of an antioxidant to a β-carotene: linoleic acid emulsion may prevent β-carotene discoloration by neutralizing linoleate free radicals [21,22].

The polarity of radical-scavenging compounds is a key parameter influencing their antioxidant activity [16]. The results of antioxidant activity assays using DPPH radical scavenging, ferric ion reduction, and β-carotene–linoleic acid bleaching are presented in Figures 1, 2, and 3, respectively. As shown, increasing the extract concentration significantly enhanced DPPH scavenging, ferric ion reduction, and β-carotene protection, with statistically significant differences observed.

Reference [16] demonstrated that an increase in phenolic compound content led to enhanced antioxidant activity, as measured by DPPH scavenging, which is consistent with the findings of the present study. Several other researchers have similarly reported a positive correlation between antioxidant activity and total phenolic content [23,24].

Abootalebian et al. (2016) used the DPPH assay as a rapid method to evaluate the antioxidant activity of various Mentha extracts. Their results showed that increasing the extract concentration from 50 to 500 ppm led to a significant increase in DPPH radical scavenging activity. Notably, the antioxidant activity of the extract at 500 ppm was higher than that of the synthetic antioxidant BHA, which aligns with the findings of the present study at 400 ppm extract concentration.

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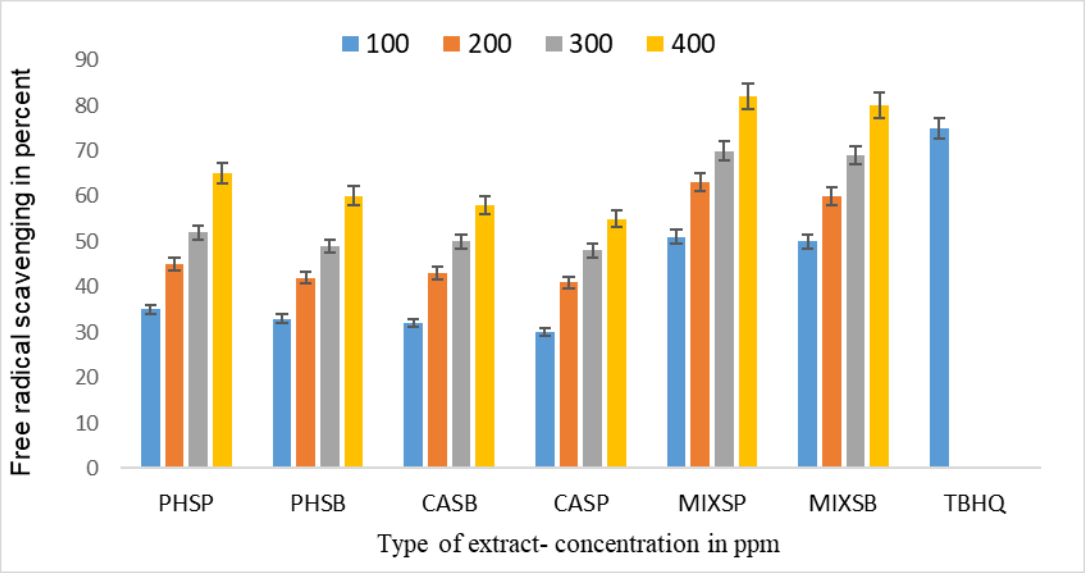

Fig1 DPPH free radical inhibition rate of different concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

Agregán et al. (2017) reported a concentration-dependent antioxidant activity of plant extracts in the ferric reducing antioxidant power (FRAP) assay, with increased extract concentrations leading to enhanced radical scavenging. The specific types of compounds present in the extracts significantly influence their antioxidant activity. Mala et al. (2016) investigated the antioxidant activity of pumpkin peel and pulp extracts using three different assays: DPPH radical scavenging, FRAP, and ABTS radical scavenging. In all methods, an increase in extract concentration led to enhanced antioxidant properties, consistent with the findings of the present study. As observed, the quality and composition of phenolic compounds varied depending on the extraction method. It appears that non-phenolic compounds, such as low-molecular-weight proteins and carbohydrates, may also contribute to radical scavenging [24].

Since the antioxidant activity of extracts is related to both the number and position of hydroxyl groups on phenolic compounds, for instance, caffeic acid (with two hydroxyl groups) exhibits greater antioxidant activity than p-coumaric acid (with one hydroxyl group). In this regard, the levels of both compounds were higher in subcritical water extracts compared to supercritical fluid extracts. Carotenoids present in the carotenoid-rich extracts can inhibit peroxidation. They could quench singlet oxygen, which otherwise leads to free radical formation and subsequent cellular damage. Extracts are considered bioactive compounds utilized to manage oxidative reactions. Both carotenoids and phenolic compounds in the extracts act as radical scavengers and play a key role in preventing oxidation.

Furthermore, phenolic extracts obtained through subcritical water extraction showed stronger inhibitory effects and higher antioxidant activity in all three evaluation methods. Phenolic extracts demonstrated stronger antioxidant effects compared to carotenoid-rich extracts. This may be attributed to the presence of conjugated double bonds in carotenoids, which are more prone to oxidation, especially during isomerization and in the presence of oxygen [25]. In other words, carotenoids are relatively stable in the cellular matrix of pumpkin but become highly sensitive to light, heat, oxygen, and acidic conditions [26].

Avila et al. (2018) evaluated the antioxidant activity of pumpkin peel flour and reported noticeable activity in the FRAP assay, attributed to the presence of bioactive compounds in the pumpkin peel. John et al. (2014) also confirmed a direct relationship between the phenolic content and antioxidant activity. Similarly, Maizura et al. (2011) reported a positive correlation

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between total phenolic content and ferric reducing ability in turmeric and ginger extracts, aligning with the findings of the current study. J. Singh et al. (2016), using DPPH, ABTS, and FRAP assays, demonstrated a significant correlation between phenolic content and antioxidant activity in pumpkin peel extracts, further confirming that higher phenolic concentrations enhance antioxidant capacity. Maghsoudlou et al. (2017) investigated the antioxidant activity of peel and pulp extracts from two fig cultivars using DPPH and FRAP assays and found that antioxidant activity increased with extract concentration, which is in agreement with the results of the present study.

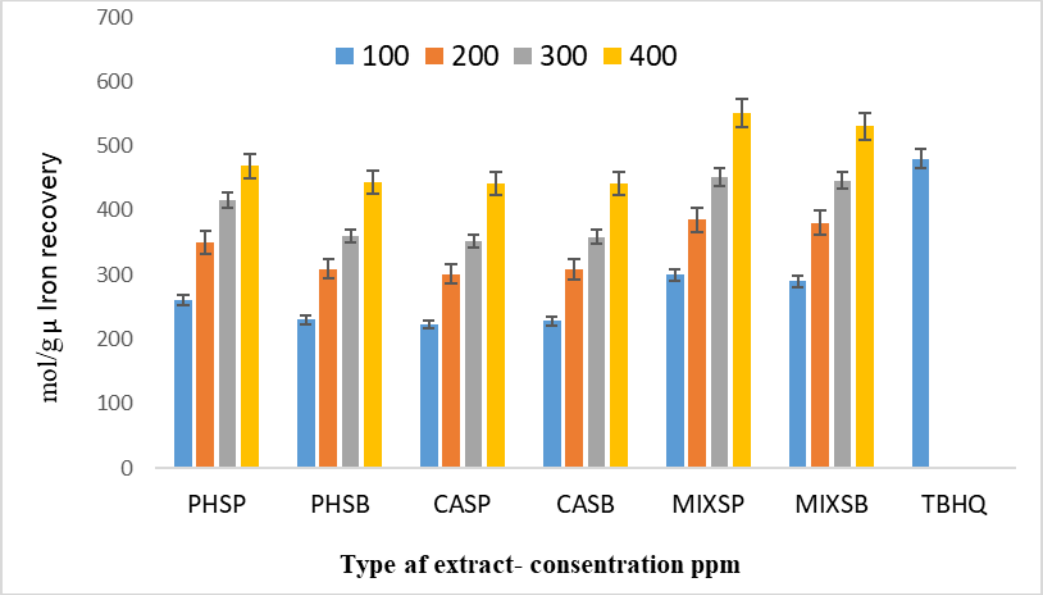

Fig 2 Iron reduction rate of different concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

As observed, in all extracts, an increase in extract concentration led to enhanced antioxidant activity. Specifically, the extract obtained via subcritical water extraction exhibited higher antioxidant capacity, attributed to its elevated levels of phenolic and flavonoid ompounds. Moreover, the antioxidant potential of the extracts is closely related to the chemical structure of the bioactive compounds present in the crude extract [27]. The observed differences in ntioxidant activities between extracts obtained by different extraction methods can be attributed to variations in the type and content of phenolic or carotenoid compounds. Abootalebian et al. (2016) assessed the antioxidant activity of various concentrations of Mentha family extracts and demonstrated that increasing the extract concentration enhanced antioxidant activity in the β-carotene-linoleic acid bleaching assay—findings that are consistent with the results of the present study. It was also observed that in all three antioxidant evaluation methods, the highest antioxidant activity was associated with the combined phenolic and carotenoid extract samples. This indicates a synergistic effect between phenolic and carotenoid compounds in contributing to antioxidant properties.

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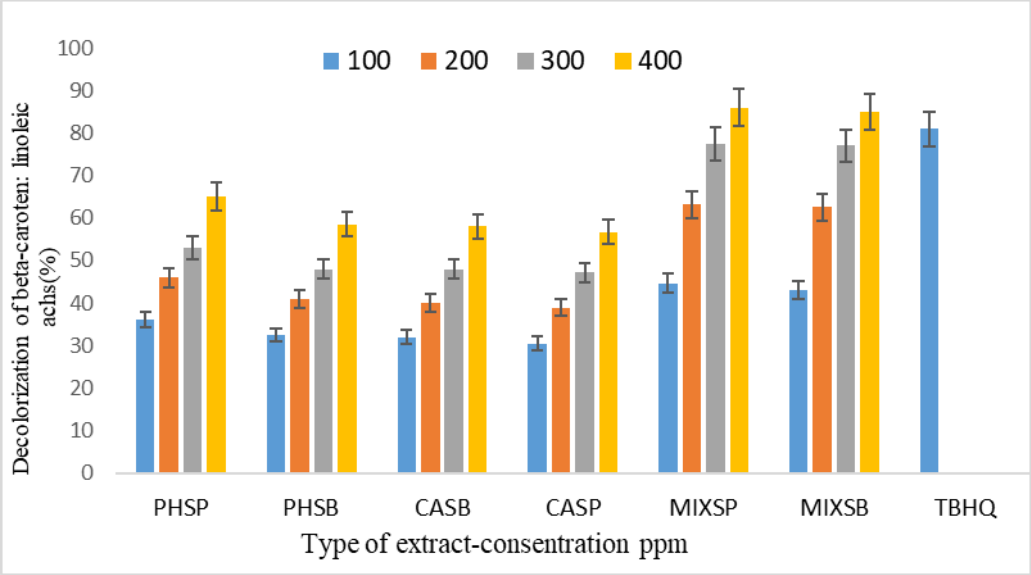

Fig 3 The amount of decolorization of beta-carotene: linoleic acid of different concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

The comparison of the mean content of phenolic and carotenoid compounds in the extracts obtained by supercritical fluid extraction (SFE) and subcritical water extraction (SWE) is presented in Table 3. The highest concentration of phenolic compounds was observed in the extract obtained through the SFE method. The total phenolic content extracted by different solvents varies due to differences in solvent polarity and their solubilization capabilities [28]. In supercritical conditions, carbon dioxide—being a molecule with limited polarity—can exhibit increased polarity when used in combination with a co-solvent polar. This enhances the extraction efficiency of carbon dioxide and improves the solubility of polar organic compounds [18]. Carotenoids, being water-insoluble compounds, were found in higher amounts in the pumpkin peel extract obtained by the subcritical water method.

Table 3 Amount of phenolic and carotenoid compounds of extracted extracts

Samples	Total phenol (mg GA/100 g E)	total carotene (mg/100 g E)
SWE	213.6 ± 5.87^{b}	15.2 ± 2.35^{a}
SFE	353.5 ± 8.84^{a}	11.48±0.90^{b}

(SWE: extract from subcritical water, SFE: extract from supercritical fluid)

3.3. Antioxidant Activity of Extracts

The results of antioxidant activity of the extracts, evaluated using the DPPH radical scavenging method, ferric reducing antioxidant power (FRAP), and β-carotene–linoleic acid bleaching assay, are shown in Figures 4, 5, and 6. As observed, increasing the extract concentration led to enhanced radical scavenging, iron reduction, and inhibition of β-carotene bleaching, with statistically significant differences. Konrade and Klava (2017) demonstrated that an increase in phenolic content is associated with enhanced antioxidant activity as measured by DPPH radical scavenging, which aligns with the findings of

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the present study. Other researchers have also consistently reported an increase in antioxidant activity with higher phenolic compound levels in the extracts [24,25]. At equal concentrations, the extract obtained by supercritical fluid extraction (SFE) showed higher antioxidant activity than that from subcritical water extraction (SWE), due to its higher phenolic content. The type of compounds present in the extract significantly influences its antioxidant capacity [20]. Abootalebian et al. (2016) reported a strong correlation between total phenolic content and antioxidant activity. Since phenolics are heat-sensitive compounds, the lower phenolic concentration observed in the subcritical water extract in this study may be attributed to the degradation of some phenolic compounds during the high-temperature SWE process. Rangsriwong et al. (2009) also associated the reduction of phenolic content at higher SWE temperatures with thermal degradation.

Interestingly, the antioxidant activity of a mixed extract composed of SFE and SWE (MIX (SFE-SWE)) was higher than that of either SFE or SWE alone. At 400 ppm concentration, the MIX extract even exhibited greater antioxidant activity compared to the synthetic antioxidant TBHQ.

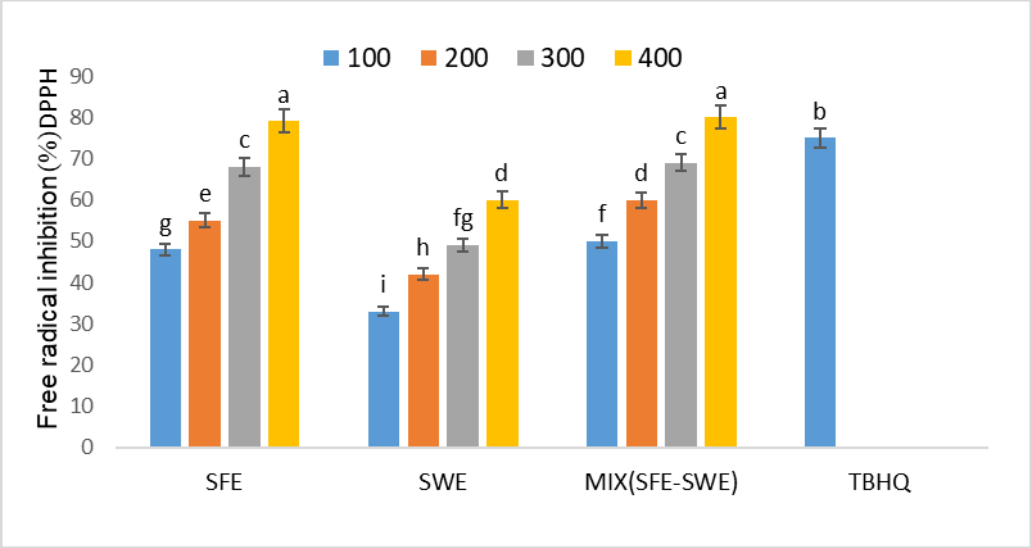

Fig 4 free radical inhibition rate of different DPPH concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

Avila et al. (2018) measured the antioxidant activity of pumpkin skin flour and found that it exhibited antioxidant activity in the ferric reducing antioxidant power (FRAP) assay, which was attributed to the presence of bioactive compounds in pumpkin skin. John et al. (2014) also reported a direct relationship between the phenolic content and antioxidant properties.

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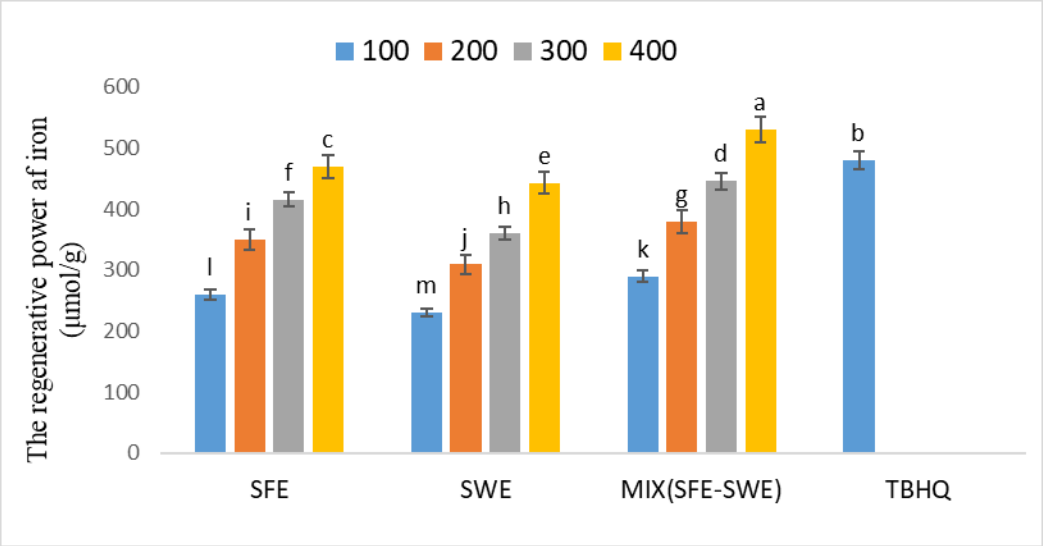

Fig 5 The amount of iron reduction power in different concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

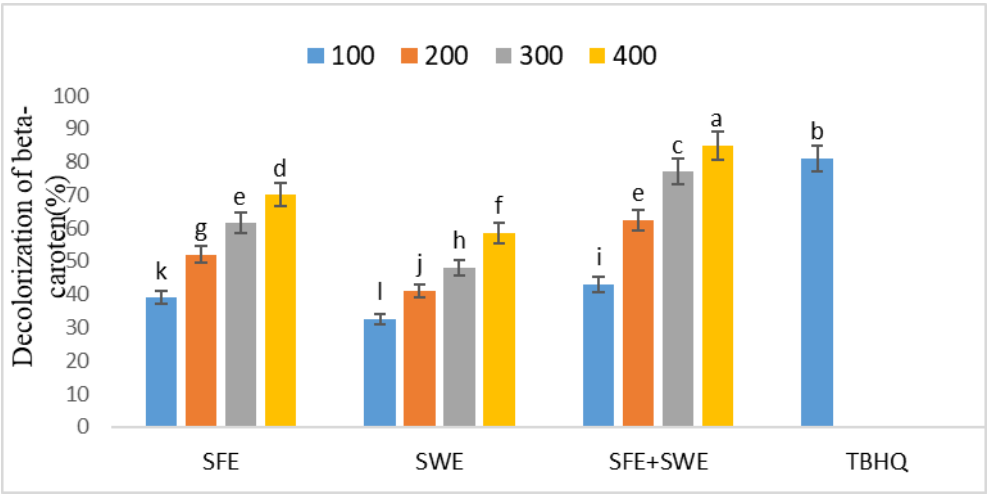

Fig 6 The amount of decolorization of beta-carotene: linoleic acid in different concentrations of extract (100-400 ppm) and synthetic antioxidant TBHQ (100 ppm)

4- Conclusion

In this study, phenolic and carotenoid extracts of pumpkin peel were obtained using supercritical fluid extraction and subcritical water extraction methods. The antioxidant properties of the extracts at various concentrations were measured and compared with the synthetic antioxidant TBHQ. The results showed that the combined extracts, due to their synergistic properties, exhibited the highest antioxidant activity in all three methods tested. Overall, in all antioxidant activity assays, the combined extract obtained by supercritical fluid extraction and then by subcritical water extraction showed the highest antioxidant activity. Furthermore, subcritical water extraction was more effective in extracting carotenoids, while supercritical fluid extraction was more effective for phenolic compounds. A strong correlation was observed between the antioxidant activity of the extracts and their phenolic and carotenoid content. Additionally, the combined extract, which exhibited the highest antioxidant activity in all three methods of radical scavenging, iron reduction, and β-carotene: linoleic acid bleaching, contained more linoleic acid compared to individual extracts. Pumpkin peel, a by-product of pumpkin processing factories, is a rich source of phenolic, carotenoid, and other bioactive compounds. The findings of this study suggest that the natural antioxidant of the combined pumpkin peel extract, due to its

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phenolic and carotenoid content, can be used as a substitute for synthetic antioxidants in food industries.

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مقاله علمی_پژوهشی

مقایسه ترکیبات زیست فعال مستخرج از پوست کدو حلوایی با استفاده از روش های سیال فوق بحرانی و آب زیر بحرانی

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چکیده

یکی از مهترین مشکلات صنعت غذا اکسیداسیون مواد غذایی است که منجر به کاهش ارزش تغذیه ای و عمر ماندگاری می شود. پوست کدو به دلیل دارا بودن ترکیبات فنولی و کاروتنوئیدی با خاصیت آنتی اکسیدانی مورد توجه قرار گرفته است .در این پژوهش عصاره فنولی و کاروتنوئیدی پوست کدو حلوایی با استفاده از دو روش استخراج با آب زیر بحرانی و استخراج با سیال فوق بحرانی استحصال و اندازه گیری شد. عصاره های ترکیبی به دلیل خاصیت سینرژیستی دارای بالاترین خاصیت آنتی اکسیدانی بودند. بطور کلی در تمام روش های ارزیابی فعالیت آنتی اکسیدانی عصاره ترکیبی استخراج شده با استفاده از سیال فوق بحرانی و پس از آن عصاره ترکیبی استخراج شده به روش آب زیر بحرانی بالاترین فعالیت آنتی اکسیدانی را نشان دادند. همچنین روش استخراج با آب زیر بحرانی برای استخراج کاروتنوئیدها و روش استخراج با سیال فوق بحرانی برای استخراج ترکیبات فنولی موثرتر عمل نمود. بین میزان ترکیبات فنولی و کاروتنوئیدی عصاره ها و خاصیت آنتی اکسیدانی رابطه وجود داشت و با افزایش غلظت فعالیت آنتی اکسیدانی عصاره ها در هر سه روش خاصیت آنتی اکسیدانی افزایش یافت. نتایج نشان داد عصاره ترکیبی فنولی-کاروتنوئیدی پوست کدو حلوایی می تواند به عنوان یک آنتی اکسیدان طبیعی جایگزین مناسبی برای آنتی اکسیدان‌های سنتزی باشد.

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