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Research Article

Total Phenolic Contents, Antioxidant Activities and Antioxidant Capacities of Some Selected Pepper *(Capsicum Annuum L.)* Pulps and Seeds

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Abstract

Pepper *(Capsicum annuum L.)* contains a wide range of bioactive nutrients that have antioxidant properties. Recently, the waste of peppers, namely the seeds, has attracted interest due to their economical, biological, and chemical importance. Accordingly, the total phenolic content, antioxidant activity, and antioxidant capacity were determined in four different peppers and their seeds, as well as the effects of different extraction solvents. The total phenolic content of pepper pulp and seed extracts was detected by using the Folin–Ciocalteu method, the antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and the antioxidant capacity was implemented by phosphomolybdenum reduction method. The results indicated that the extraction solvent significantly affected the antioxidant properties, whereas water extraction showed the highest values. The highest values in phenolic content, antioxidant activity, and antioxidant capacity were observed in green pepper (515.85 mg GAE/g dry extract), charleston pepper (362.48 mg AAE/g dry extract), and capia pepper pulps (95.25 %), respectively. Also, the antioxidant activities of the examined pepper pulp and seeds showed a good correlation with their total phenolic content.

Keywords: Phenolic, antioxidant, *Capsicum annuum L.*, pulp, seed.

Bazı Biber (*Capsicum Annuum L.*) Posa ve Çekirdeklerinin Toplam Fenolik İçerik, Antioksidan Aktivite ve Antioksidan Kapasitelerinin Belirlenmesi

Öz

Biber (*Capsicum annuum L.*), antioksidan özelliklere sahip çeşitli biyoaktif maddeler ihtiva etmektedir. Son zamanlarda, atık olarak değerlendirilen biber çekirdeği ekonomik, biyolojik ve kimyasal önemi nedeniyle ilgi çekmektedir. Bu çalışma kapsamında dört farklı biber çeşidinin posa ve çekirdeklerinde toplam fenolik madde, antioksidan aktivite ve antioksidan kapasite analizleri ve farklı ekstraksiyon solventlerinin bu analizler üzerine etkisi incelenmiştir. Biber posa ve çekirdek ekstraktlarının toplam fenolik içeriği Folin-Ciocalteu yöntemi ile, antioksidan aktivitesi 2,2-difenil-1-pikrilhidrazil (DPPH) yöntemi ile ve antioksidan kapasitesi fosfomolibdenum indirgeme testi ile tayin edilmiştir. Elde edilen bulgular, ekstraksiyon solventinin antioksidan özellikleri önemli ölçüde etkilediğini, su ekstraksiyonunda ise en yüksek değerlerin gözlendiğini ortaya koymuştur. Fenolik içerik, antioksidan aktivite ve antioksidan kapasite açısından en yüksek değerler sırasıyla yeşil biber (515.85 mg GAE/g kuru ekstrakt), çarliston biber (362.48 mg AAE/g kuru ekstrakt) ve kapya biber posalarında (% 95.25) gözlenmiştir. Ayrıca, analiz edilen biber posa ve çekirdeklerinde antioksidan aktivite değerleri, toplam fenolik içerikleri ile iyi bir korelasyon göstermektedir.

Anahtar Kelimeler: Fenolik, antioksidan, *Capsicum annuum L.*, posa, çekirdek.

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1. Introduction

Pepper (*Capsicum spp*) is a member of the Solanaceae family and is an essential crop due to it is being an excellent source of vitamin C, carotenoids, and other biofunctional and antioxidant compounds. Pepper has been reported to have the highest content of vitamin C (Lee & Kader, 2000). Additionally, the different colours of the pepper derive from the carotenoid pigments produced during the ripening of fruit. They act as antioxidants and prevent the harmful effect of free radicals and molecular oxygen from promoting good health (Namiki, 1990). Peppers are a good source of polyphenols, especially flavonoids, quercetin, and luteolin (Chuah et al., 2008; Materska & Perucka, 2005). The antioxidant activity of phenolics is related to their role as a reducing agent, hydrogen donor, and singlet oxygen quencher (N Deepa et al., 2007). The delay or inhibit autoxidation by acting as radical scavengers (Namiki, 1990). The consumption of these compounds in foods has been recognized as beneficial for human health and reduces the risk of cancer, cardiovascular and other chronic diseases (Cui et al., 2007; Yahia & Ornelas-Paz, 2010).

As the chemical structures of the bioactive compounds found in peppers are different, the extraction solvent significantly alters the obtained compounds and their activities. In previous research, to measure antioxidant activity by extracting phenolics dichloromethane, acetone, methanol, ethanol, and 80 % methanol were used as solvents. (Daood et al., 2006; Marín et al., 2004; Materska & Perucka, 2005). Pepper has various polar compounds, and the antioxidant capacity of the extract changes according to the solvent used. Therefore, the proper extraction solvents should be used to detect the antioxidant capacity of pepper pulp and pepper seed.

Every year the food industry generates millions of tons of waste products from fruits and vegetables (492 million tons) (Gustavsson et al., 2011). These waste products can be considered as by-products. Nearly all of them consist of nutritional and functional constituents such as vitamins, pigments, antioxidants, and antimicrobials, etc. (Louli et al., 2004). One of these valuable waste products is pepper seed. Pepper seeds provide bioactive compounds and beneficial health effects (Luis R Silva et al., 2013). Studies of the antioxidant activities of different pepper pulp and seeds are more limited compared to other fruits and plants. Therefore, in this research, we investigated total phenolic contents, antiradical activities, and antioxidant capacities of four different types of pepper (Capia, Green, Charleston, and Mazamort) and their seed extracts by using ethanol, methanol, and water.

2. Material and Method

2.1. Plant Material

Four varieties of pepper (*Capsicum annuum L.*) pulps and pepper (*Capsicum annuum L.*) seeds, namely Capia Pepper Pulp (CPP), Green Pepper Pulp (GPP), Charleston Pepper Pulp (CPP), Mazamort Pepper Pulp (MPP), and these pepper seeds (CPS, GPS, CPS, MPS, respectively), were obtained from producers in Kayseri (Turkey).

2.2. Sample extraction for antioxidative tests

The dried samples were ground and mixed with ethanol, methanol, and water for 24 hours at 25ºC in the dark. The homogenates were centrifuged (Hettich, Universal 320, Deutschland) at 4100 rpm for 15 minutes then filtrated through Whatman 2 filter paper. After these treatments, the supernatants were evaporated using a rotary evaporator (Rotavapor R-200, Büchi, Switzerland) under vacuum at 45ºC. The dry extracts were obtained, and analyzes were immediately applied.

2.3. Methods

2.3.1. Evaluation of total phenolic content by Folin-Ciocalteu colourimetric method

The amount of phenolic compounds in 4 pepper pulps and seeds extracts was detected by the Folin-Ciocalteu colourimetric method. To 40 µL of each sample, 2400µL distilled water and 200µL Folin-Ciocalteu reagent were mixed and kept for 5 minutes. Then, 600µL sodium carbonate at 20% concentration and 760µL distilled water was added to the tube. The resulting mixture is homogenized and incubated for 2 hours at room temperature in the dark. Then, the absorbance of the blue complex was monitored at 765 nm (Yalcin & Kavucuoglu, 2014). The results were exhibited as gallic acid equivalents (GAE) in milligrams per gram dry extract.

2.3.2. Evaluation of antiradical activity by DPPH Radical Scavenging Activity

The DPPH (1.1-diphenyl-2- picrylhydrazyl) radical scavenging activity assay was measured using the method of Polat et al. (2014) with some modification. Pepper pulp and seed extracts (100µL) stirred with 3500µL DPPH methanol solution and extract react with DPPH solution for 30 minutes in the dark. Then the absorbance was monitored at 517 nm. Absorbance is zeroed with methanol.

2.3.3. Evaluation of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity by the phosphomolybdenum method was determined spectrophotometrically using the method of Polat et al. (2014). The reactive solution was prepared with 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, and 400 ml of extract was mixed with 4 ml of reagent solution rapidly. After homogenization, the solution was kept at 95°C for 90 minutes. Then, the absorbance was monitored at 695 nm. The results were exhibited as ascorbic acid equivalents (AAE) in milligrams per gram of dry extract.

2.3.4. Statistical analysis

Data evaluation was carried out by the two-way analysis of variance (ANOVA) procedure with statistical analysis software (SigmaPlot 11.0). The Tukey, multiple range test, was used for comparison of the means to find the effects of extraction solvents and pepper types and their interaction for various parameters in the different experiments.

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3. Results

3.1. Total phenolic content of pepper pulps and pepper seeds

There is excellent interest in phenolic compounds owing to their antioxidant activity. Because antioxidants have a protective effect against free radicals, which cause serious health problems such as cancer and cardiovascular diseases (Ghasemnezhad et al., 2011). Additionally, the effect of pepper extracts to prevent lipid oxidation was examined, and extracts were effective chiefly because of the presence of phenolic compounds (Alvarez-Parrilla et al., 2012).

The total phenolic contents (TPC) of the pepper pulp extracts (CPP, GPP, CPP, MPP) and these pepper seed extracts (CPS, GPS, CPS, MPS, respectively) with ethanol, methanol, and water were shown in Table 1. In general, the previous studies reported that the solvent and plant variety used in extraction had a significant ($p<0.05$) effect on the total phenolic content of the samples, statistically. When considering almost all samples, the total amounts of phenolic compounds were determined based on solvents with the following order: ethanol < methanol < water. Bae et al. (2012) found that the total phenolic content of extracts obtained with ethyl acetate was higher than methanol or aqueous methanol, ethyl acetate, and acetone extracts showed statistically similar results.

The phenolic contents ranged from 32.78 - 95.86 µg GAE/g in seeds and 75.97 - 124.87 µg GAE/g in pulps among ethanolic extracts, from 73.43 – 195.36 µg GAE/g in seeds and 117.45 – 243.83 µg GAE/g in pulps among methanolic extracts, 189.85 – 344.51 µg GAE/g in seeds and 323.82 – 515.85 µg GAE/g in pulps among water extracts. The total phenolics were expressed in the range of 33 – 250 mg GAE/100 g FW for different *Capsicum annuum L.* (Alvarez-Jubete et al., 2010; Oboh & Rocha 2006). In another study, the total phenolic contents were specified between 1078.26 - 4992.40 mg GAE/100 g FW of nine peppers (Zhuang et al., 2012). In comparison, all samples were significantly different in the seeds ($p<0.05$), ethanolic extracts of GPP and CPP were not different from one another, statistically ($p>0.05$). Significant differences were found between the pulp and seeds of the peppers except for capia peppers between ethanolic and methanolic extracts ($p<0.05$). GPP indicated significantly higher total phenolic contents than the other pepper pulp extracts ($p<0.05$).

In previous researches, the total phenolic contents of the diverse parts of the peppers were significantly different. For example, in a study, the amount of phenolic substance in the stalk extract was determined as 71.34 mg GAE/g dw, while it was determined as 52.27 mg GAE/g dw in pericarp and 64.07 mg GAE/g dw in the placenta (Chen & Kang, 2013). The total phenolic content was found as 1359 ± 148 g GAE/g in the fresh sample (Vega-Gálvez et al., 2009), 29.10 ± 0.18 mg g^{-1} as GAE in capia pepper seed extracts (Sim & Sil, 2008) and 186 - 1122 mg 100 g^{-1} as GAE in the different genotypes of sweet green peppers (N. Deepa et al., 2007). The results of previous research regarding total phenolic contents have been noticed in the literature for other *Capsicum* varieties. While some are similar, others are higher or lower than our results. These differences may be caused by product-based changes such as environmental conditions or maturity because of the activity of peroxides, being fresh or dehydrated, as well as the solvent used in extraction and method differences (Hervert-Hernandez et al., 2010).

Table 1. Total phenolic contents of four pepper pulps and seeds (mg GAE[a]/g dry extract)

Type	Ethanol	Methanol	Water	
CPP	$75.97^{Cd} \pm 2.45$	$194.67^{Be} \pm 2.86$	$402.98^{Ag} \pm 14.50$	**224.54^{y}**
GPP	$124.87^{Ca} \pm 1.18$	$233.29^{Bf} \pm 4.03$	$515.85^{Aa} \pm 0.34$	**291.34^{x}**
CPP	$116.40^{Ca} \pm 2.45$	$243.83^{Ba} \pm 1.96$	$468.80^{Ah} \pm 3.59$	**276.34^{x}**
MPP	$86.47^{Cde} \pm 3.24$	$117.45^{Bc} \pm 5.86$	$323.82^{Ae} \pm 2.93$	**175.91^{z}**
CPS	$81.65^{Cd} \pm 1.21$	$195.36^{Be} \pm 1.90$	$308.72^{Ad} \pm 2.29$	**195.29^{yz}**
GPS	$66.39^{Cc} \pm 2.94$	$125.46^{Bc} \pm 4.79$	$263.29^{Ac} \pm 2.82$	**151.71^{z}**
CPS	$95.86^{Ce} \pm 1.12$	$142.69^{Bd} \pm 1.24$	$344.51^{Af} \pm 1.56$	**194.35^{yz}**
MPS	$32.78^{Cb} \pm 2.60$	$73.43^{Bb} \pm 2.56$	$189.85^{Ab} \pm 5.39$	**98.69^{t}**
	85.05^{Z}	**165.77^{Y}**	**352.23^{X}**	

*Values in the column with different lowercase letters are significantly different at $p < 0.05$.
*Values in the row with different capital letters are significantly different at $p < 0.05$.
a GAE = gallic acid equivalents.

3.2. Antioxidant capacity of pepper pulps and pepper seeds

The antioxidant properties of fruits and vegetables have great importance in revealing the nutritional value (Ornelas-Paz et al., 2013). The antioxidant capacity (AC) of pepper pulps and pepper seeds was assessed using the phosphomolybdenum reduction method (Table 2). The extraction solvent had a significant effect on the AC of samples ($p<0.05$). Antioxidant capacity showed a comparatively moderate correlation with total phenolic components ($r = 0.430$). In our study, the antioxidant capacity of pepper pulps and pepper seeds ranged from 72.31 to 362.48 mg AAE/ g dry extract and from 51.83 to 183.41 mg AAE/ g dry extract, respectively.

Overall, regards total phenolic contents, water extracts were revealed to have better AC. Similar to our findings, Azeez et al. (2008) found the AC of black pepper as 443.67 moles ascorbic acid equivalents/ g for water extract and as 119.08 moles ascorbic acid equivalents/ g for ethanol extract. The methanol and ethanol extraction solvents obtained the second and third highest values. The highest values of antioxidant activity for ethanol, methanol, and water were 129.22 – 173.28 – 362.48 mg AAE/ g dry extract, respectively, while the lowest values were 80.95 – 51.83 – 105.82 mg AAE/g dry extract respectively.

Brito-Vega et al. (2014) determined the AC of *Capsicum annuum* and *Capsicum annuum var. glabriusculum* as 5.56±0.58 mg AAE/g FW and 6.79±0.43 mg AAE/g FW respectively. Biswas et al. (2011) evaluated 20 germplasm of pepper for AC and 23.59- 34.25 as vitamin C equivalents mg/g dry weight. This significant disparity in results may be due to differences in varieties.

Table 2. Antioxidant capacity of four pepper pulps and seeds (mg AAE[b]/g dry extract)

Type	Ethanol	Methanol	Water	
CPP	$91.14^{Bb} \pm 0.82$	$131.27^{Ae} \pm 11.66$	$137.00^{Ac} \pm 2.59$	**119.80^{YZ}**
GPP	$90.36^{Bb} \pm 2.47$	$102.59^{Bd} \pm 7.23$	$136.93^{Acd} \pm 5.19$	**109.96^{YZ}**
CPP	$80.95^{Cb} \pm 8.40$	$106.17^{Bd} \pm 7.78$	$362.48^{Aa} \pm 9.31$	**183.20^{X}**
MPP	$106.14^{Bb} \pm 3.07$	$72.31^{Cc} \pm 3.10$	$126.91^{Ac} \pm 1.44$	**101.79^{YZ}**
CPS	$129.22^{Ca} \pm 6.73$	$173.28^{Aa} \pm 17.64$	$146.45^{Bd} \pm 1.90$	**149.65^{XY}**
GPS	$84.38^{Ccb} \pm 1.73$	$113.56^{Bd} \pm 9.38$	$183.41^{Ae} \pm 2.53$	**127.11^{YZ}**
CPS	$90.42^{Bb} \pm 1.96$	$82.16^{Bc} \pm 0.70$	$133.12^{Acd} \pm 1.23$	**101.90^{YZ}**
MPS	$83.54^{Bcb} \pm 1.88$	$51.83^{Cb} \pm 1.97$	$105.82^{Ab} \pm 1.21$	**80.40^{Z}**
	94.52^{y}	**104.15^{y}**	**166.52^{x}**	

*Values in the column with different lowercase letters are significantly different at $p < 0.05$.
*Values in the row with different capital letters are significantly different at $p < 0.05$.
b AAC = ascorbic acid equivalents

3.3. Antioxidant activity of pepper pulps and pepper seeds

The antioxidant activity (AA) of pepper pulps and pepper seeds extracted by different solvents was tested by the DPPH free radical scavenging method, which displays a deep purple colour with absorption at 517 nm (Table 3.). Antioxidants can react with DPPH free radicals, turning them into colourless components. Also, the DPPH analysis, the dpph method, is widely used in antioxidant research due to its rapid results (Amarowicz et al., 2004). This method has commonly been implemented to evaluate the antioxidant capacity of peppers, and a concentration-dependent activity was noticed (L. R. Silva et al., 2013)

Pepper pulps and pepper seeds demonstrated the inhibition of DPPH radical scavenging activity between 28.59 – 95.25% and 10.54 – 41.18%, respectively. As a consequence, although all analyzed pepper pulps and pepper seeds indicated AA, the pepper pulps were a remarkably more robust scavenger of DPPH radicals. The water extract of CPP showed the highest DPPH-scavenging activity (95.25%), followed by the water extract of GPP and methanol extract of CPP with 85.90% and 85.16%. The inhibition of the water extract of MPS was the lowest at 10.54%. No statistical differences in AC were found between CPP and GPS ethanolic and methanolic extracts.

Nazzaro et al. (2009) examined the DPPH scavenging activity of two peppers and expressed them as 11.7% and 21.5%. N. Deepa et al. (2007) expressed that according to the results of DPPH analysis, the capia pepper showed between 20% and 72% inhibition compared to the control. The results obtained in our work agree with those found previously with several peppers aqueous extract. Unlike other studies, the effect of extraction solvent on the results was demonstrated.

Regression analysis between AA and TPC exhibit a linear correlation ($p<0.05$). The high correlation coefficient ($r = 0.731$) displays that phenolic amounts are associated with their antioxidant activity of pepper pulp and pepper seed. This statistically significant correlation was consistent with the results of other studies. (Bae et al., 2014; Ornelas-Paz et al., 2013; Sim & Sil, 2008).

Table 3. Radical scavenging activity of four pepper pulps and seeds (inhibition %)

Type	Ethanol	Methanol	Water	
CPP	$80.83^{Ca} \pm 0.31$	$85.16^{Ba} \pm 1.24$	$95.25^{Aa} \pm 0.06$	**87.08^{x}**
GPP	$40.79^{Be} \pm 0.92$	$38.07^{Cde} \pm 0.78$	$85.90^{Ag} \pm 1.62$	**54.92^{y}**
CPP	$31.64^{Bd} \pm 0.63$	$29.77^{Bc} \pm 0.56$	$46.06^{Ae} \pm 1.44$	**35.82^{z}**
MPP	$28.59^{Cd} \pm 4.62$	$41.01^{Be} \pm 0.88$	$54.29^{Af} \pm 2.50$	**41.30^{z}**
CPS	$32.82^{Bd} \pm 0.82$	$35.26^{Ad} \pm 1.48$	$24.06^{Cc} \pm 0.95$	**30.71^{zt}**
GPS	$29.67^{Bd} \pm 1.06$	$30.34^{Bc} \pm 0.47$	$41.18^{Ad} \pm 2.06$	**33.73^{z}**
CPS	$18.59^{Bc} \pm 0.31$	$17.83^{Bb} \pm 0.77$	$25.39^{Ac} \pm 1.37$	**20.60^{tq}**
MPS	$13.93^{Bb} \pm 0.56$	$19.80^{Ab} \pm 0.93$	$10.54^{Cb} \pm 0.18$	**14.75^{q}**
	34.61^{Y}	**37.16^{Y}**	**47.83^{X}**	

*Values in the column with different lowercase letters are significantly different at $p < 0.05$.
*Values in the row with different capital letters are significantly different at $p < 0.05$.

4. Conclusions and Recommendations

The antioxidant properties of four different pepper pulp and seed extracts were assessed with three different methods and three different solvents. Analysis indicated that all extracts showed antioxidant activity. When all is taken into consideration, the antioxidant properties of water extracts were better than extracts derived from ethanol and methanol. Generally, it was observed that the results obtained from the pulps of peppers were better than the results obtained from the seeds, except for the phosphomolybdenum reduction method. Overall, DPPH radical scavenging activity results in different pepper extracts were highly consistent with the total phenolic results. At the same time, antioxidant capacity with the phosphomolybdenum reduction method showed a comparatively moderate correlation with total phenolic components. The data can be used for further research to promote individual phenolic compounds in pepper extracts. On the other hand, in vivo studies should be conducted to reveal the mechanisms of action as antioxidants better.

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