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Anti-Hyperglycaemic Activity and Antioxidant Properties of Pepper Waste and Fruit

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Abstract: *The objective of this study was to determine content of major antioxidants, antioxidant activity, and anti-hyperglycaemic activity in different fractions (seeds, peel, and fruit) of 7 pepper cultivars. Peppers were examined for their total phenolic (TP), total flavonoid (TF), total carotenoid (TC) contents, and antioxidant activity using a DPPH assay. The inhibitory activity of pepper fractions against yeast α-glucosidase and porcine pancreatic α-amylase were used to determine anti-hyperglycaemic activity. Red point pepper had the highest TP content and Kapya pepper had the highest TF content. Red peppers showed higher carotenoid content than green peppers, and the peel fraction of all cultivars had significantly ($P < 0.05$) higher TP levels. These results show that removal of pepper seeds during home cooking and processing, results in loss of antioxidant capacity; therefore, it is important to consume peppers along with their seeds in order to attain maximum health benefits.*

Keywords: *antioxidant capacity, anti-hyperglycaemic activity, pepper, total carotenoids, total flavonoids, total phenol*

I. Introduction

Recently, there has been renewed attention given to the antioxidant content of vegetables because many epidemiological studies suggested that regular consumption of fruits and vegetables can play an important role in preventing cancer, cardiovascular problems, and degenerative disease [1,2,3]. As a widely consumed vegetable, peppers have many beneficial biochemical and pharmacological properties including antioxidant, anti-inflammatory, antiallergenic, and anti-carcinogenic activities [4]. The health promoting activities are mainly due to its components like phenolics, flavonoids, vitamins C and E, provitamin A, and folates [5,6,7]. Carotenoids, fat-soluble antioxidants found in peppers, have received considerable research interest due to their antioxidant properties [8]. Dietary flavonoid intake has been reported to be inversely associated with the incidence of coronary artery disease, because of their reducing effect on oxidized LDL formation and growth of atherogenic plaques [9].

Inhibitors of α-glucosidase have been shown to be effective in suppressing postprandial hyperglycaemia by limiting glucose absorption and the resulting insulin response [10]. Recent studies suggest that intestinal α-glucosidase and α-amylase inhibitors from natural sources such as plant-based foods could be as effective as synthetic drugs with fewer side effects [11].

Capsicum is a genus of plants from the Solanaceae family that has a variety of names depending on location and type, and the most common pepper names are chili, bell, red, green, or just pepper. Peppers are fruits with a high importance in human diet due to their versatility to be consumed as fresh vegetables in salads, cooked meals, or dehydrated for spices. For instance, in Turkey, the Kapya pepper (red) is mostly used in pepper paste production and as an ingredient in different sauces, while green peppers are usually consumed fresh or cooked in meals.

While pepper is usually consumed with the skin, most consumers remove the seeds of pepper before eating because they are thought to be indigestible and contain low levels of nutrients. Conversely, there are studies demonstrating that fruit seeds and peels contain a significantly higher total antioxidant capacity and phenolic content than edible portions [12,13]. In addition, it has been reported that tomato seeds contain a higher phenolic content than the main fruit [14,15]. Recently, George et al. [16] reported that the tomato skin had 2.5 times higher lycopene levels than the pulp, and had significant amounts of phenolic compounds and ascorbic acid. The peels and seeds of various fruits and vegetables, which are waste products in the fruit and vegetable industry, may be a potential source of antioxidants.

In general, limited data are available on the antioxidant components and antioxidant activity of pepper seeds and peel. Therefore, it is difficult to determine if the waste from pepper products like paste and sauces have a rich nutritional value. To our knowledge, this is the first report on the effect of pepper waste on the inhibition of α-glucosidase and α-amylase activity. Therefore, objectives of this study were (1) to determine and compare the total phenol (TP), total flavonoid (TF), total carotenoid (TC) contents, and total antioxidant capacity, and (2) to evaluate anti-hyperglycaemic activity of both seed and peel fractions and the whole fruit of 7 pepper cultivars grown in Turkey.

II. Material And Methods

1.1. Fruit sampling

Pepper fruits from seven locally grown commercial pepper cultivars were collected from local markets. The peppers were separated into three different fractions: peel, seeds, and fruit. The peel of the pepper was carefully separated from the flesh using a sharp knife. The seed fraction of the pepper contained the seeds along with the part in the middle of the fruit that carries seeds (pith). The fruit fraction was the remaining portion of pepper without seeds or peel. All fractions were weighed before extraction.

1.2. Chemicals

Methanol 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Merck (Darmstadt, Germany), Folin–Ciocalteu reagent, gallic acid, aluminium nitrate, potassium acetate, and anhydrous sodium carbonate purchased from Sigma-Aldrich (Steinheim,Germany). α-Glucosidase from yeast and the substrates p-nitrophenyl a-D glucopyranoside (p-NPG) were purchased from Sigma, (St. Louis, MO, USA). p-Nitrophenyl-α-D-maltoheptaoside (PNPG7) was obtained from Megazyme (Bray, Co.,Wicklow, Ireland). HEPES was purchased from Lancaster (Mühlheim, Germany). α-amylase (EC 3.2.1.1) from porcine pancreas was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Dimethyl-sulfoxide (DMSO) was obtained from Merck KgaA (Darmstadt, Germany).

1.3. Determination of total carotenoid content

Fresh peppers, seeds, peel, and juice (5.0 g) were ground and extracted with a mixture of acetone and petroleum ether (1:1, v/v) repeatedly using a mortar and pestle until a colourless residue was obtained. The upper phase was collected and combined with crude extracts after being washed several times with water. Petroleum ether was added to the extracts to reach a known volume. Total carotenoid content was determined by recording the absorbance at 450 nm with a spectrophotometer [17].

1.4. Determination of total flavonoid content

The TF content was determined according with the aluminium chloride colorimetric method described by Chang et al., [18]. Briefly, 0.1 g aliquots of vegetable and fruit samples were dissolved in 1 mL deionized water. This solution (0.5 mL) was mixed with 1.5 mL of 95% alcohol, 0.1 mL of 10% aluminium chloride hexahydrate (AlCl3), 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionized water. After incubation at 25 °C for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionized water blank on a spectrophotometer (Hitachi, Model 100-20). Quercetin was used as a standard. Using a 7 point standard curve (0–50 mg/L), the TF levels of in fruits and vegetables were determined in triplicate. The data were expressed as milligram quercetin equivalents (QE)/g lyophilized powder. The data were then converted into milligram quercetin equivalents (QE)/100 g FW from fruit or vegetables based on the moisture content of lyophilized powder and fresh fruit and vegetable materials.

1.5. Determination of total phenolic contents

Fresh peppers (5.0 g) were extracted by stirring with 75 mL 80% ethanol at room temperature for 24 h, and filtered through a Whatman No. 1 filter paper. The filtrates were concentrated using a rotary vacuum evaporator at 40 °C. The resultant extracts were used to determine the TP content and antioxidant activities in vitro.

The TP content of extracts was determined according to a previous method [19]. In brief, phenolic content was estimated by mixing 0.2 mL of distilled water, 0.05 mL of diluted extracts, and 0.05 mL of Folin–Ciocalteu reagent. After 6 min, 0.5 mL of 7.5% sodium carbonate solution was added to the mixture, which was adjusted to 1.3 mL with distilled water and allowed to stand at room temperature for 60 min. Then, the absorbance was read at 765 nm. Gallic acid was read at 765 nm. Gallic acid was used to construct the calibration curve. The results were expressed as mg of Gallic acid equivalents (GAE)/100 g FW.

1.6. DPPH radicals scavenging activity assay

DPPH radical scavenging activity was determined according to Yu et al. [20]. This method is based on the ability of the antioxidant to scavenge the DPPH caution radical. Briefly, 100 mL of sample extract or standard was added to 0.9 mL buffer (0.02 M Tris-HCI in water) and 2 mL of DPPH reagent (0.0394 g in methanol) and the mixture was vortexed vigorously. It was incubated in the dark for 30 min at room temperature and the discolouration of DPPH radical was measured against blank at 517 nm. Ethanol (100%) was used as control. Inhibition (%) of DPPH absorbance = (Acontrol− Atest) × 100/Acontrol. ascorbic acid was used as reference standard, and the results were expressed as mg GAE/100 g FW of fruit. All determinations were performed in triplicate (n = 3).

1.7. Determination of alfa-glucosidase enzyme inhibition activity

First, 320 µL of 100 mM phosphate buffer (pH 6.8), 50 µL of 10 mM PNPG in the buffer, and 10 µL of extract in DMSO were mixed and incubated at 30 °C for 5 min, and then 20 µL of the buffer containing 0.01 mg/mL enzyme was added to the mixture. After incubation at 30 °C for 5 min, 3.0 mL of 50 mM sodium hydroxide was added to the mixture, and the absorbance at 410 nm of the liberated p-nitrophenol was measured.

1.8. Determination of porcine pancreatic alfa-amylase inhibition activity

A synthetic substrate, BPNPG7, was used as substrate in the assay of the porcine pancreatic α-amylase inhibitory activity. A reaction mixture containing 100 µL of Amylase HR Reagent and 40 µL of extract in the methanol was incubated at 37 °C for 5 min, and then 60 µL of 0.1 mg/mL enzyme in 0.1 M HEPES buffer (pH 6.9) was added to the reaction mixture. After further incubation at 37 °C for 10 min, the liberated p-nitrophenol was determined as described in the assay of the yeast α-glucosidase enzyme activity. Inhibition (%) was calculated as (A-B)/A*100, where A is the quantity of the reaction product in the absence of extract and B was that in the presence of extract.

1.9. Data analysis

All data is reported as mean ± standard error of the mean for three replicates. Non-parametric Kruskal-Wallis analysis was used to determine significant differences between the pepper cultivars and the different pepper fractions. Statistical significance was determined at $P < 0.05$. Data were analysed using SPSS for Windows version 16.0.

III. Results And Discussion

All cultivar names and biometrical characteristics of tested peppers can be found in Table 1. The TP content ranged from 198.5 to 758.9 mg GAE/100 g FW in seeds, from 69.5 to 153.2 mg GAE/100 g FW in fruit and from 107.8 to 441.7 mg GAE/100 g FW in peel in the 7 pepper cultivars (Table 2). Of all the pepper cultivars studied, RP pepper had the highest and C had the lowest TPC in total fruits. Among green peppers, P had the highest TP. The results indicated that pepper seeds (mean 373.47 mg GAE/100 g FW) displayed the highest amount of TP content followed by peel (mean 227.4 mg GAE/100 g FW) and fruit (mean 102.27 mg GAE/100 g FW). The results indicated significant differences in TP contents between the 7 cultivars (Table 2). Zhuang et al. [21] reported that pepper fruit contained TPs from 107.82 mg GAE/100 g FW to 499.24 mg GAE/100 g FW in 9 different cultivars. Lee et al. [22] found 178.0 to 384.9 mg chlorogenic acid equivalent/100 g FW TP in fresh peppers. The different values in different cultivars may be due to genetic factors and variation in the growing location.

In this study, significantly ($P < 0.05$) higher levels of TP and TF were detected in the seeds and peels of peppers than in the fruits. This could be explained by the accumulation of phenolic compounds in the dermal tissues of the fruit body because of their potential role in protection against ultraviolet radiation, pathogens, and predators [23]. Even though peels and seeds are a concentrated source of phenolic compounds, they have a lower mass than the pulp, for example seeds form 2.1–3.8% of fruit weight, while pulp forms 92.1–96.7% of fruit weight. By our measurements, the peels and seeds of the seven cultivars on average contributed 37% to the TP of whole pepper.

A similar trend in TF of the various pepper parts was observed to the trend seen in phenolic contents. The TF content in the seeds (mean 49.04 mg QE/100 g FW) was considerably higher than that in peel and fruit. The results of flavonoid content, expressed as milligrams of quercetin equivalents per 100 g, are presented in Table 2. In peppers tested, TF ranged from 45.1 to 102.8 mg QE/100 g FW in seeds, from 35.8 to 68.4 mg QE/100 g FW in peel, and from 11.9 to 33.8 mg QE/100 g FW in fruit. The highest TF was found in B pepper (33.8 ± 1.8 mg QE/100 g FW) fruit. Our results indicate that the level of flavonoids varies within different coloured peppers and cultivars. Red peppers showed significantly higher flavonoid values than green peppers. In addition, seeds and peels showed higher flavonoid values than the whole fruits. Main dietary sources of flavonoids are fruits and vegetables for humans, with tea and wine being secondary sources. Their biological effects are based on antioxidative activities, scavenging (chelating) capacities, and interaction with enzyme systems [24].

The TC contents of the seven peppers in this study are presented in Table 3, ranged from 3.5 to 42.6 mg/100 g FW for whole fruit, 0.8 to 2.8 mg/100 g FW for seeds and 1.8 to 67.3 mg/100 g FW for peel. Red peppers had a significantly higher carotenoid content than green peppers ($P < 0.05$). The highest carotenoid content was found in RP pepper (42.6 ± 9.5 mg/100 g FW), followed by and K and RC. Seeds had the lowest carotenoid levels of the fractions and C had the lowest carotenoid levels from the seven pepper cultivars examined. Our results suggest that pepper seeds are not rich in terms of carotenoids, compared with fruit and peel. The high or low carotenoid content for a given vegetable depends upon on various factors like cultivar, maturity, and processing conditions [25,26]. Carotenoid content in Capsicum annuum cultivars has been

extensively studied and characterized [27] for selecting high-carotenoid producing cultivars. Marin et al. [26] observed a high pro-vitamin A content in the mature (red) stage of peppers. In this study, red pepper showed significantly higher carotenoid values than green peppers.

Antioxidant capacity is the ability to prevent free-radical mediated oxidation of a substrate, and this capacity can be tested using a wide variety of methods [28]. In the present study, antioxidant capacity was measured by free radical (DPPH) scavenging assay. The total antioxidant capacity varied among the different pepper cultivars and in different parts: from 18.5 to 36.7 mg AAE/100 g FW in fruit, from 48.2 to 152.8 mg AAE/100 g FW in peel and from 90.5 to 287.3 mg AAE/100 g FW in seeds. The results showed that antioxidant capacity varied between different pepper cultivars and fractions (Fig. 1). The seeds of K pepper showed the highest antioxidant capacity while M contained the lowest. Green pepper seeds showed lower antioxidant capacity than the red pepper seeds, and peel antioxidant levels were the same.

Due to higher levels of all major antioxidant compounds, the seeds of peppers also had significantly ($P < 0.05$) higher antioxidant activity compared with that of the peels and whole fruits. Antioxidant capacity of C. annuum seeds from Sweet Italian, Reus long pepper, and jalapeno pepper have been reported [29,30]. The variability in their assay systems does not allow us to make suitable comparisons, but their results indicate that C. annuum seeds are a potential source of valuable bioactive compounds that could be used in the food industry. The peels and seeds of the seven cultivars on average contributed 37% to the TP, 32% to the TF, 4.1% to the TC, and 38% to the total antioxidant activity present in peppers. These results show that the seeds are an important contributor to the major antioxidants and overall antioxidant activity of peppers. Therefore, removal of pepper seeds during their fresh consumption, home cooking, and preparation of processed pepper products means a loss of antioxidants.

Fig. 2 shows the inhibitory activity of 7 pepper cultivars against yeast α-glucosidase and porcine pancreatic α-amylase. Seeds of RC had the highest α-amylase inhibition of 54% followed by seeds of RP and peel of RC, with an inhibition of 40 and 38%, respectively. The α-amylase inhibition capacity among different pepper cultivars and in different parts ranged from 9 to 36% in the fruits, from 8 to 38% in the peels, and from 15 to 54% in the seeds. The α-glucosidase inhibitory activity ranged from 15 to 48% in seeds, from 8 to 51% in fruit, and from 9 to 65% in peel, among the 7 cultivars of peppers (Fig. 2). In addition, α-glucosidase inhibitory activity of peel of cultivar K was higher than peel of RP, and the peel of RC showed the maximum activity.

Table 1 Biometrical characteristic of tested peppers.

Pepper type	Abbreviated name	Species	Colour	Weight (g)	Length (cm)	Seeds Weight (g)
Red Chili pepper (hot)	RC	Capsicum frurescens	Red	3-6	4-6	0.5-1.5
Red point pepper (hot)	RP	Capsicum annum	Red	13-25	20-22	2-4
Kapya pepper (sweet)	K	Capsicum annum	Red	70-90	10-18	7-9
Charliston pepper (sweet)	C	Capsicum annum	Green	25-35	15-20	3-5
Bell pepper (sweet)	B	Capsicum annum	Green	90-100	8-10	6-7
Point pepper (sweet)	P	Capsicum annum	Green	18-20	18-22	2-3
Mazamort pepper (sweet)	M	Capsicum annum	Green	20-25	10-15	3-4

Table 2 Total phenolic and flavonoid content in the skin, seed and fruit extracts in 7 cultivars of pepper

	Total phenolic content (mg GAE/100g FW)			**Total flavonoid content (mg QE/100g FW)**		
Pepper type	Seed	Peel	Fruit	Seed	Peel	Fruit
RC	389.6 ± 11.8	279.45 ± 3.7	153.25 ± 1.8	89.5 ± 5.3	35.8 ± 3.4	11.9 ± 0.5
RP	758.9 ± 15.7	441.71 ± 1.8	112.86 ± 1.3	45.1 ± 2.6	42.5 ± 2.7	17.8 ± 0.2
K	589.5 ± 12.3	318.54 ± 2.4	85.36 ± 1.1	92.5 ± 3.1	68.4 ± 2.4	13.7 ± 0.8
C	223.3 ± 9.7	107.85 ± 1.2	69.52 ± 0.8	85.3 ± 4.1	37.1 ± 2.8	19.6 ± 0.4
B	198.5 ± 10.8	109.59 ± 1.1	75.18 ± 0.9	102.8 ± 4.5	59.4 ± 4.1	33.8 ± 1.8
P	241.7 ± 15.1	285.41 ± 1.6	121.48 ± 1.5	68.5 ± 3.5	53.4 ± 3.6	27.8 ± 1.5
M	212.8 ± 18.0	149.26 ± 2.7	98.26 ± 1.2	88.2 ± 4.2	48.3 ± 2.7	21.5 ± 1.1

Table 3 Total Carotenoid content in the skin, seed, fruit and juice in 7 cultivars of pepper.

	Total Carotenoid content (mg/100g FW)		
Pepper type	Seed	Peel	Fruit
RC	2.2 ± 0.8	59.5 ± 5.1	28.4 ± 13.7
RP	2.8 ± 0.3	67.3 ± 8.7	42.6 ± 9.5
K	2.3 ± 0.7	55.1 ± 4.8	39.4 ± 12.4
C	0.8 ± 0.0	1.8 ± 0.0	3.5 ± 0.5
B	1.6 ± 0.9	5.2 ± 1.2	7.9 ± 0.8
P	1.8 ± 0.1	3.4 ± 0.9	8.1 ± 1.1
M	1.2 ± 0.2	2.8 ± 0.6	4.6 ± 0.7

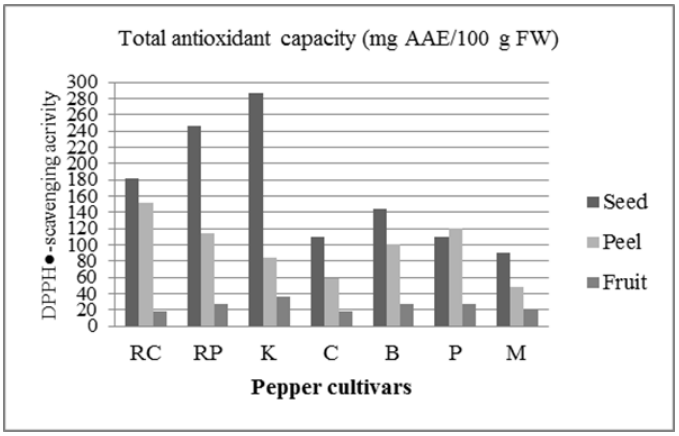

Fig. 1 Total antioxidant capacity of peel, seed and fruit of 7 pepper cultivars.

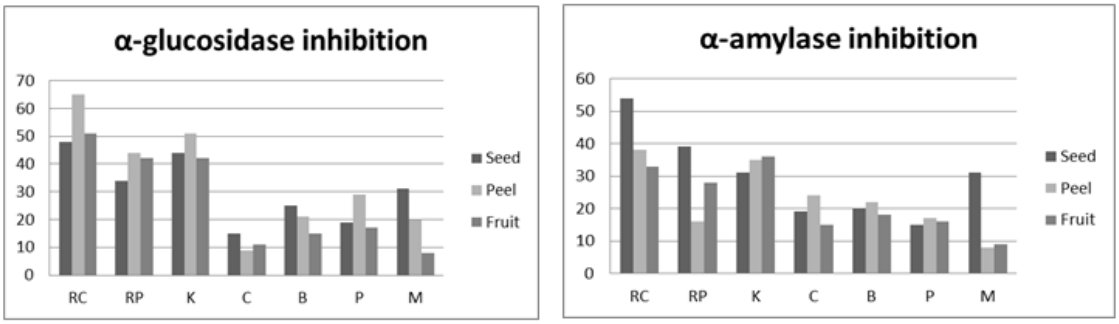

Fig. 2 Inhibitory activity of pepper seed, peel and fruit against yeast α-glycosidase and α-amylase.

IV. Conclusion

The results showed that the levels of TP, TF, and TC compounds changed depending on cultivar and part of the pepper. The whole fruit of pepper has valuable antioxidant capacity, with the largest amounts found in seeds. Pepper seeds also have considerably high TP and TF content. Considering that seeds are often thrown away as waste in the production of pepper products like pepper paste and sauce, the potential reported health benefits of pepper seeds should not be ignored. A conclusion section must be included and should indicate clearly the advantages, limitations, and possible applications of the paper. Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions.

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