INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 14, No. 1, p. 26-33, 2019
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Effects of roasting on the total phenolic contents and radical scavenging activity of fruits seeds

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Article published January 26, 2019

Key words: Phenolic content, Scavenging activity, *Prunus domestice, Prunus armeniace*, *Prunus persic*.

Abstract

The purpose of the present study was to explore the influences roasting on the radical scavenging activity and total Phenolic content on selected seeds. Fresh seeds of *Prunus domestice, Prunus armeniace* and *Prunus persica* were selected from the local market. The selected seeds were heated on the hotpot at a temperature 160 °C for 1 to 3 hours, respectively and one group were remain irrespective of any treatment (control). It was observed that roasting of fruit seeds produce different effects on total phenolic contents and radical scavenging activity. Antioxidant capacity was measured against the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) whereas the reducing capacity was evaluated with the Folin-Ciocalteu reagent (FCR). Total phenolic content in *Prunus domestica* was highest at 160 °C when heated for 1 hour (554 mg/100g), similarly the total phenolic content in the *Prunus armeniaca* was highest when heated for 2 hour (684 mg/100g) while the Total phenolic content in the *Prunus persica* was highest when heated for 2 hour (684 mg/100g). Radical scavenging activity in the *Prunus domestica* was highest when heated for 1 hour (48 %). Similarly radical scavenging activity in the *Prunus armeniaca* was highest during heated for 1 hour (86 %) while radical scavenging activity in the *Prunus persica* was at maximum (43 %) at 2 hour treatment. It is suggested that different effect were produced when different fruit seeds were roasted at a single temperature, Therefore different optimum temperature and conditions are required for roasting different seeds.

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Introduction

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radical. In turn, these radicals can start chain reaction (Helmut, 1997). Although oxidation reactions are crucial for life, they can also be damaging. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress appears to be an important part of many human diseases. The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Moreover, oxidative stress is both the cause and the consequence of disease. Antioxidants are widely used in dietary supplement and have been investigated for the prevention of diseases such as cancer, coronary heart diseases and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful (Prabhat *et al*., 1995; Bjelakovic *et al*., 2007; Baillie *et al*., 2009).

Phenolic are ubiquitous secondary metabolites in plants. They comprise a large group of biologically active ingredients (above 8000 compounds) from simple phenol to polymeric structure with molecular mass above 30,000 Da (Dexter *et al*., 1994). Phenolic acids are localized in cellular walls and vacuoles. Most of phenolic acids exist in a binding form which represents as much as 70 % in oats and wheat (Adom *et al*., 2002). Plant foods have phenolic compounds, which affect their: appearance, taste, odor and oxidative stability. In cereal grains, these compounds are located mainly in the pericarp (Naczk *et al*., 2004).

The major phenolic acids in cereals are ferulic and p-coumaric acids (Hahn *et al*., 1983; Zhou *et al*., 2004; Mattila *et al*., 2005; Holtekjolen *et al*., 2006). Anthocyanin are water-soluble pigments mostly studied in cereals (Yao *et al*., 2004).

The possible mechanism of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized (Mattill, 1947, Kishwar *et al*., 2018).

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasoline's to prevent the polymerization that leads to the formation of engine-fouling residues (Boozer *et al*., 1955). Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades (Aqil*et al*., 2006).

The study is to investigate the radical scavenging activity (RSA) and total phenolic antioxidants (TPA) in fruits seeds and also evaluate the effects of high temperature radical scavenging activity.

Materials and methods

Chemicals such as DPPH were purchased from Sigma Aldrich (Sigma, Germany). All chemical and reagents were of analytical grade or otherwise mentioned. Seeds of *Prunus Domestica, Prunus Armeniace, Prunus Persica* were purchased from local market Chakdara, Dir lower, Khyber Pakhtunkhwa, Pakistan. Seeds are removed and dried for 2 days. The internal embryo are removed and further dried. Three samples are selected i.e. *Prunus Domestica, Prunus Armeniace, Prunus Persica* and three replicates were selected for further study.

Roasting of Seeds

All seeds were first crushed and then placed in a hot spot which was heated up to 160 °C. First replicate were heated up to 1 hour, 2nd replicate were heated up to 2 hours and 3rd replicate are heated up to 3 hours. Each sample (1 gram) was macerated with 10 % ethanol and shake for 1 hour on shaker. The antioxidant activity of the ethanolic extracts was determined on the basis of their scavenging activity of the stable 2, 2- diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 56 µL of each solution of the extracts was added to 2 ml ethanolic DPPH freeradical solution. After 30 minutes the absorbance of the preparations were taken at 515 nm by a UV spectrophotometer which was compared with the corresponding absorbance of standard ethanolic concentrations. The molarity of DPPH is 0.1 mM or 0.0001M; volume was based on your requirement. The weighed amount was dissolved in ethanol or methanol and its absorbance was noted.

A solution of DPPH in methanol was freshly prepared at a concentration of 0.1mM and molecular weight of the DPPH is 394.32.Two milliliters DPPH solution was mixed with 56 µL of each extract samples. The samples were kept for 30 min in dark. The absorbance of the sample mixture was measured at 515 nm using (Shimadzu) UV/Vis-spectrophotometer (Shimadzu, Japan).The RSA toward DPPH radicals was estimated from the differences in absorbance of the DPPH solution with or without sample (control), and the percentage of RSA was calculated from the following equation.

$$\% \text{ RSA} = (Ac\text{-}As/Ac) \times 100$$

Where Ac is the absorbance of the control and as is the absorbance of the test sample. Absorbance of the control was also measured.

Total Phenolic Contents

Dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85 % phosphoric acid and 10 ml concentrated hydrochloric acid. Refluxed for 10 h, and then add 15 g lithium sulfate, 5 ml water and 1 drop bromine. Refluxed for 15 min. Cooled to room temperature and bring volume to 100 ml with water. One hexavalent phosphor molybdic/phosphor tungstic acid complexes are formed in solution. The solution was then diluted to required concentration.

The extracts for the total phenolic contents (TPC) assay were obtained by extracting one gram of homogenized seed with 20 ml of methanol at ambient temperature for 1 h with constant shaking. The TPC in *Prunus domestica, Prunus armeniace, Prunus persica* extracts was determined using Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a reference. The reagent was prepared by diluting a stock with distilled water (1/10, v/v). The samples (1.0 ml, three replicates) were introduced into the test cuvettes and mixed with 5.0 ml Folin-Ciocalteu's phenol reagent and 4.0 ml Na_2CO_3 (7.5 %). The absorbance was measured at 765 nm using Shimadzu UV/Vis spectrophotometer (Shimadzu, Japan) after incubation at ambient temperature for 1 h. The TPC was determined from the calibration curve and expressed in mg of gallic acid equivalents in 100 g of berries.

Results

Total Phenolic Contents

On the basis of calibration curve we can find out the absorbance of following samples using gallic acid as standard solution were measured. First the absorbance of standard solution were obtained (see in Table 1) and the calibration curve for these standard solutions were derived using Sigmaplot. In this way curve equation and R-square value was obtained through which the calculations were made.

The phenolic content of *prunus domestica* of the control were 708 mg/100g which reduced to 554 mg/100g and 150 mg/100g of the 1st and 2nd hour

treatment but later on in the 3rd hour treatment the phenolic content in the sample increase to 43 mg/100g.

Table 1. Absorbance of standard solution of gallic acid.

Conc. (mg/mL)	Absorbance
1	0.237
2	0.291
3	0.335
4	0.406
5	0.457

The phenolic content of *prunus armeniace* of the control were 299 mg/100g which reduced to 129 mg/100g in the 1st hour treatment and then increases to 882 in the second hour treatment and then again decreases to 548 mg/100g in 3rd hour treatment.

The phenolic content of *prunus persica* of the control was 153 mg/100g which increases to 163 mg/100g and 684 mg/100g in the 1st and 2nd hour treatment and then reduced to 255 mg/100g in the 3rd hour treatment (Table 2).

Radical Scavenging Activity (RSA)

DPPH is one of the free radicals widely used for testing radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extract of the selected seeds showed the radical scavenging activity. The antioxidant activity of the selected seeds depends upon on many compounds present in the seeds.

The mean % RSA for *prunus domestica* of control sample is 48 % which when subjected to heat for 1 hour the antioxidant activity reduced to 35 %. While when the extracts are again subjected to heat for 2 hour the antioxidant activity again raises to 48 %. While in the 3rd case when subjected to heat for 3 hour the antioxidant activity then reduced to 43 %.So the results suggested that the antioxidant activities are high in the control and 2nd hour treatment.

Table 2. Total phenolic contents of plant seeds.

Treatment	Seeds	Replicate	Absorbance	Conc. (mg/g)	Mean Conc. (mg/100 g)
Control	Prunusdomestica	I	0.203	0.438	708 ± 0.3
		II	0.215	0.654	
		III	0.236	1.032	
	Prunusarmeniaca	I	0.358	3.231	299 ± 0.2
		II	0.328	2.690	
		III	0.348	3.050	
	PrunusPersica	I	0.214	0.636	153.7 ± 0.7
		II	0.285	1.915	
		III	0.293	2.059	
1st hour Treatment	Prunusdomestica	I	0.158	0.373	554 ± 0.4
		II	0.193	0.258	
		III	0.236	1.032	
	Prunusarmeniaca	I	0.259	1.447	129.1 ± 0.2
		II	0.256	1.393	
		III	0.236	1.032	
	PrunusPersica	I	0.282	1.861	163.9 ± 0.5
		II	0.293	2.059	
		III	0.234	0.996	
2nd Hour Treatment		I	0.275	1.735	150.7 ± 0.3

	Prunusdomestica	II	0.273	1.699	
		III	0.239	1.086	
		I	0.202	0.420	882 ± 0.5
	Prunusarmeniaca	II	0.262	1.501	
		III	0.219	0.726	
		I	0.207	0.510	684 ± 0.4
	PrunusPersica	II	0.201	0.402	
		III	0.242	1.141	
		I	0.199	0.366	258 ± 0.1
	Prunusdomestica	II	0.197	0.330	
		III	0.183	0.077	
3rdHour Treatment		I	0.196	0.312	548 ± 0.6
	Prunusarmeniaca	II	0.183	0.077	
		III	0.109	1.256	
		I	0.185	0.114	255.2 ± 0.1
	PrunusPersica	II	0.189	0.186	
		III	0.155	0.427	

The mean % RSA for *prunus armeniaca* of control sample is 88 which when subjected to heat for 1 hour the antioxidant activity increases to 36. While when the extract is again subjected to heat for 2 hour the antioxidant activity reduced to 48. While in the 3rd case when subjected to heat for 3 hour the antioxidant activity then raises up to 77 %. So the results suggested that the antioxidant activity is high in the 1st hour and 3rd hour treatment.

Radical Scavenging Activity (RSA)

DPPH is one of the free radicals widely used for testing radical scavenging activity of a compound or a plant extract.

In the present study, ethanolic extract of the selected seeds showed the radical scavenging activity. The antioxidant activity of the selected seeds depends upon on many compounds present in the seeds.

Table 3. Radical scavenging activity of plant seeds.

Treatment	Seeds	Replicate	Absorbance	% RSA	Mean % RSA
		I	0.508	42.14	48.4 ± 10.1
	Prunusdomestica	II	0.365	60.13	
		III	0.496	43.08	
		I	0.131	85.18	
Control	Prunusarmeniaca	II	0.141	83.95	85.0 ± 1.0
		III	0.122	86.11	
		I	0.588	33.4	36.1 ± 6.4
	PrunusPersica	II	0.493	43.85	
		III	0.601	31.55	
		I	0.431	50.3	35.3 ± 14.9
1st hour Treatment	Prunusdomestica	II	0.559	35.33	
		III	0.694	20.35	
		I	0.116	86.79	86.5 ± 1.1

	Prunusarmeniaca	II	0.124	85.37	
		III	0.109	87.59	
		I	0.645	26.53	27.1 ± 1.9
	PrunusPersica	II	0.653	25.52	
		III	0.621	29.37	
		I	0.566	35.53	
	Prunusdomestica	II	0.438	50.11	48.3 ± 12.0
2nd Hour Treatment		III	0.356	59.45	
		I	0.325	62.38	
	Prunusarmeniaca	II	0.354	59.58	66.5 ± 9.6
		III	0.197	77.57	
		I	0.549	37.47	
	PrunusPersica	II	0.586	33.36	43.1 ± 13.5
		III	0.363	58.55	
		I	0.426	51.15	
	Prunusdomestica	II	0.415	52.15	43.2 ± 14.5
3rd Hour Treatment		III	0.645	26.53	
		I	0.176	79.35	
	Prunusarmeniaca	II	0.209	76.2	77.0 ± 2.0
		III	0.215	75.51	
		I	0.796	9.33	28.6 ± 17.4
	PrunusPersica	II	0.588	33.32	

The mean % RSA for *prunus persica* of control sample is 35 % which when subjected to heat for 1 hour the antioxidant activity reduced to 27 %. While when the extracts are again subjected to heat for 2 hour the antioxidant activity again raises to 43 %. While in the 3rd case when subjected to heat for 3 hour the antioxidant activity then again reduced to 28 %. So the results suggested that the antioxidant activities are high in the control and 2nd hour treatment (Table 3). Fig. 1 shows the correlation of total phenolic contents, radical scavenging activity and roasting time of the seeds.

It has been observed that by increasing heating time, a strong correlation was observed with total phenolic contents and radical scavenging activity.

Discussion

The purpose of this work was to find out the possible changes in the phenolic content and radical scavenging activity produced by the roasting at different time period. In *prunus domestica* the phenolic content were 708 mg/100g in the control.

The phenolic content decreases in the 1st and 2nd hour while increases in the 3rd hour. In *prunus armeniaca* the phenolic content were 299 mg/100g in the control which decreases in the 1st hour while increases in the 2nd hour which again decreases in the 3rd hour.

In *prunus persica* the phenolic content were 153 mg/100g in the control which increases in the 1st hour and 2nd hour respectively while decreases in the 3rd hour.

In *prunus domestica* the radical scavenging activity were 48 % in the control. The radical scavenging activity decreases in the 1st hour while increases in the 2nd hour and then again decreases in the 3rd hour. In *prunus armeniaca* the scavenging activity were 85 % in the control. The scavenging activity remains same in the 1st hour while decreases in the 2nd hour and

then again increases in the 3rd hour. In *prunus persica* the scavenging activity were 36 % in the control. The scavenging activity decreases in the 1st hour while increases in the 2nd hour and then again decreases in the 3rd hour.

It was observed that roasting of fruit seeds produce different effects on total phenolic contents and radical scavenging activity.

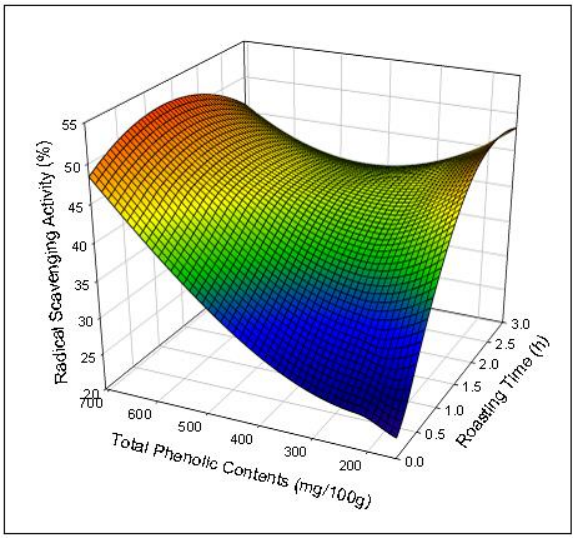

Fig. 1. Correlation of total phenolic contents, radical scavenging activity and roasting time.

It is suggested that different effect were produced when different fruit seeds were roasted at a single temperature, Therefore different optimum temperature and conditions are required for roasting different seeds.

Conclusion

The phenolic content decreases in the 1st and 2nd hour while increases in the 3rd hour. In *prunus armeniaca* the phenolic content were 299mg/100g in the control which decreases in the 1st hour while increases in the 2nd hour which again decreases in the 3rd hour. In *prunus persica* the phenolic content were 153mg/100g in the control which increases in the 1st hour and 2nd hour respectively while decreases in the 3rd hour. The radical scavenging activity decreases in the 1st hour while increases in the 2nd hour and then again decreases in the 3rd hour. In *prunus armeniaca* the scavenging activity remains same in the 1st hour while decreases in the 2nd hour and then again increases in the 3rd hour. In *prunus persica* the scavenging activity were 36 % in the control.

The scavenging activity decreases in the 1st hour while increases in the 2nd hour and then again decreases in the 3rd hour. It was observed that roasting of fruit seeds produce different effects on total phenolic contents and radical scavenging activity.

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