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EFFECT OF HEAT TREATMENT ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF APPLE FRUITS

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Abstract:-The Effect of microwave and heat treatments on phenolic content and antioxidant activity of the apple (Malusdomesticavar. Balady)fruit was evaluated. The total phenolic content was enhanced with increasing the time (20 sec) of microwave from 14 to 16mg phenols/g apple peeland from 6.5 to 10.4 mg phenols/g apple flesh,but at 30 sec the total phenolic content was decreased to 6.8 mg.The DPPH assay IC_{50}value was increased with increasing the time of microwave from 3.68 to 7.9µg phenolic concentration at 30 s of microwave treatment of apple peel. In the apple flesh, the DPPH assay IC_{50}value was decreased with increasing the time of microwave from 8.6 to 4.9µg phenolicconcentration at 20 sec of microwave treatment.There is no remarkable effect of heat treatment (80ºC) on the phenolic content of apple peel and flesh. The DPPH assay IC_{50}value was decreased with heat treatment from 3.68 to < 2µg phenolic concentration of peel. In flesh, the DPPH assay IC_{50}value was increased with heat treatment from 8.6 to > 10µg phenolic concentration.The results indicated that microwave and heat treatments could be apple.

Keywords: Apple fruits, Phenolic compounds, antioxidant activity, DPPH.

INTRODUCTION

The oxidative stress imposed by reactive oxygen species (ROS) was reported to play an important role in many chronic and degenerative diseases, such as cardiovascular diseases, cancer, diabetes mellitus, ageing and neurodegenerative diseases (Azizova, 2002; Young and Woodside, 2001). The most common forms of ROS include superoxide radical, hydrogen peroxide, hydroxyl free radical, singlet oxygen and nitric oxide, which have significantly high biological activities in vivo and in vitro. They can directly lead to DNA mutation, alteration of gene expression, modification of cell signal transduction, cell apoptosis, lipid peroxidation and protein degradation (Nordberg and Arner, 2001). The scavenging of ROS is thought to be an effective measurement to depress the level of oxidative stress of organism for prevention and treatment of some chronic and degenerative diseases.

It has been reported that the intake of vegetables and fruits was inversely associated with the risk of many chronic diseases, such as cardiovascular diseases and cancer (Duthie*et al.*, 2000; Leifert and Abeywardena, 2008). Natural antioxidants in vegetables and fruits, such as vitamins and polyphenols, were considered to be responsible for theirhealth benefits (Leja*et al*., 2003). Apple (*Malusdomestica*Borkh) consumption contributed to the prevention and protection from several

degenerative diseases in humans, mainly due to their antioxidants against free-radicals (Boyer and Liu, 2004; Di Pietro*et al*., 2007). The phenolic compounds in apples are responsible for most of the antioxidant activity of the fruit (Lee *et al.*, 2003; Tsao*et al*., 2005). Less than 0.4% of the antioxidant activity (AOA) of apples was attributed to ascorbic acid content, indicating that other factors, such as phenolics, are the main contributors (Eberhardt*et al.,* 2000). More recent studies have shown that the content of phenolic compounds in apples varies considerably among different cultivars, and also within different parts of the same apple fruit (Lata*et al*., 2009; Leccese*et al*., 2009; Petkovsek*et al*., 2007).

Phenolic acids have attracted increasing attention for their antioxidant behaviour and beneficial health-promoting effects.They account for about one-third of the phenolic compounds in plant foods. It is assumed that many antioxidative phenolic compounds in plants are usually presented in a covalently-bound form (Xu*et al*., 2007). Therefore, reliable and practical methods for the liberation of natural antioxidants from plant materials are of considerable interest. Microwave energy can potentiate the bioavailability of free pharmacologically active natural compounds bypreventing the binding of polyphenols to the plant matrix (Gulatiet al., 2003). The internal temperature distribution of a material subjected to conventional-heating depends on its thermal conductivity, whereas microwave-heating results in the heating of all the individual elements of a material instantaneously. Consequently, heating time, using microwaves can be significantlyreduced as compared to conventional-heating methods (Robinson et al., 2009). Several methods, such as heat treatment, far-infrared radiation, electron-beam irradiation, fermentation and protease treatment were studied to liberate and activate low-molecular weight natural antioxidants (Jeong*et al*., 2004; Kim*et al*., 2008a; Kim *et al*., 2008b; Niwa*et al*., 1988; Xu*et al*., 2007; Ajila*et al*., 2011; Vega-Gvez*et al.*, 2012; Zhang *et al*., 2013; Zhang*et al*., 2014).

The aim of this study was to determine the influence of thermal processing techniques as microwaving (novel thermal processing), and roasting (conventional thermal processing) on phenolic contents and antioxidant activity of locally growing apple cultivar called Balady. This information would be useful for both nutritionists and consumers.

MATERIALS AND METHODS

Plant materials:

Freshly samples from locally growing apple cultivar called Balady were collected from Al-Taif region.

Thermal processing:

For microwave irradiation, peel or flesh of apple was placed in microwavable dishes and treated at 700 W for 10, 20, and 30 s. In case of heat treatment, peel or flesh of apple was placed in oven and heated at 80°C for 5 and 10 min. All the samples after the respective treatment were cooled to room temperature and extracted by shaking at 150 rpm and 25ºC for 24 h with 10 ml of 80% methanol and filtered using filter paper No. 1. The filtrate was designated as methanol extract. The extracted samples were taken for further biochemical analyses.

Determination of the total phenolic content:

Total phenolic content was measured according to Velioglu*et al*. (1998). Fifty µL of the methanol extract was mixed with 100 µL Folin-Ciocalteu reagent, 850 µL of methanol and allowed to stand for 5 min at ambient temperature. A 500 µL of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid. The results expressed as mg gallic acid equivalent/g tissues.

DPPH radical scavenging activity:

Free extract was (Ao*et al.*, 2008). A methanol solution (100 µL)

µL of freshly
Avolume amount of methanol was used as a control. After incubation
spectrophotometerically.

(OD control – OD sample)/OD control] x 100.

Theresults activity againstconcentration of phenolic contents. The inhibition concentration(IC_{50}) was defined as the amount of phenolic contents required for50% of free radical scavenging activity. The IC_{50} value wascalculated from the plots as the antioxidant concentrationrequired for providing 50% free radical scavenging activity.

RESULTS AND DISCUSSION

In the present study, the effect of microwave treatment at 700 W on phenolic content and antioxidant activity of local apple cultivar Balady was evaluated. The total phenolic content was increased with increasing the time (20 sec) of microwave from 14 to 16 mg phenolic/g peel and from 6.5 to 10.4 mg phenolic/g flesh. After 30 sec the total phenolic content was decreased to 10.8 and 6.8 mg phenolic/g peel and flesh, respectively (Table 1). Similarly, when Kinnow fruit peel powder was treated for 5 min at 125, 250 and 500 W, the total phenolicscontent was 5313.2, 5478.7 and 5808.5µg/g DW, respectively (Hayat et al., 2010). Anincrease in the phenolic content was observed with increase the irradiation time;however, when peel powder was treated at 250 W for 15 min therewas a sudden decrease in the content, which indicated that longerirradiation time was harmful to phenolics. This suggests that a shortertime is ideal for the release of phenolics under microwave irradiation.Dharman*et al*. (2009) reported that at prolonged irradiationtime, dielectric heating promoted the decomposition of ethylenecarbonate instead of converting it into the transesterified products.At longer irradiation time, the evaporative loss of dielectric species(water content) from the citrus peel powder may also contributeto the lower phenolics content to some extent. The highest content oftotal phenolics was obtained when peel powder was treated at 250 W for10 min. Gulati*et al*. (2003) found that the total phenols and catechinsof green tea manufacturingprocess, microwaveenergy theleaf matrix, which could increase catechin in green tea.Park *et al*.(2009) also found similar results for the use of far-infrared on green tealeaves.

The antioxidant capacity after microwave treatment was evaluated by DPPH assay. The correlation coefficient $(R)^2$ between phenolic concentration and DPPH scavenging activity was ranged from 0.959 to 0.997 for peel and flesh, respectively (Figs. 1 & 2).

Table 1.Phenolic content of local apple cultivar Balady after microwave treatment.

Treatment	Time (sec)	Tissue	phenolic content (mg/g tissues)
Control	-	Peel	14.0
Microwave 700 W	10		14
	20		16
	30		10.8
Control	-	Flesh	6.5
Microwave 700 W	10		8.6
	20		10.4
	30		6.8

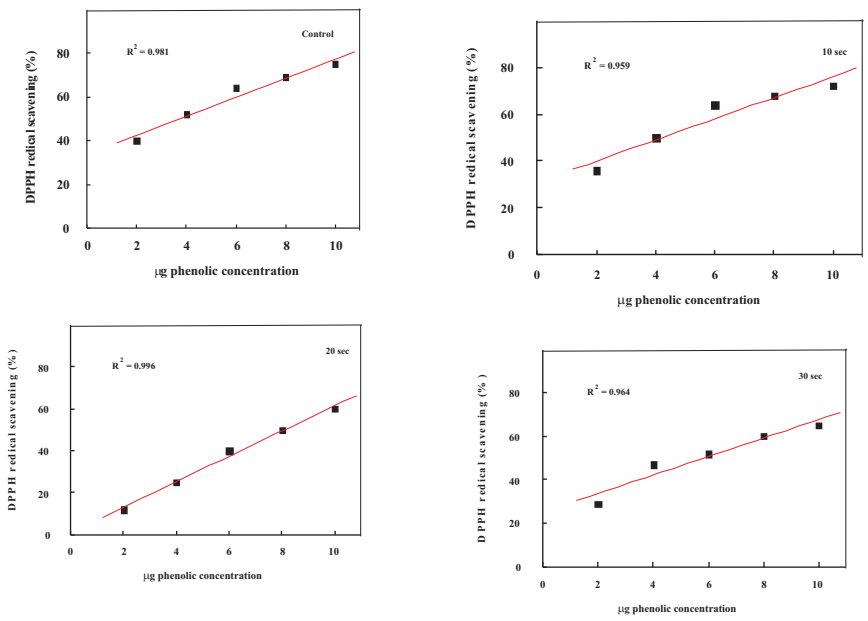


Fig. 1.Effect of microwave (700 W) on antioxidant activity of local apple cultivar Baladypeel as determined by DPPH assay.

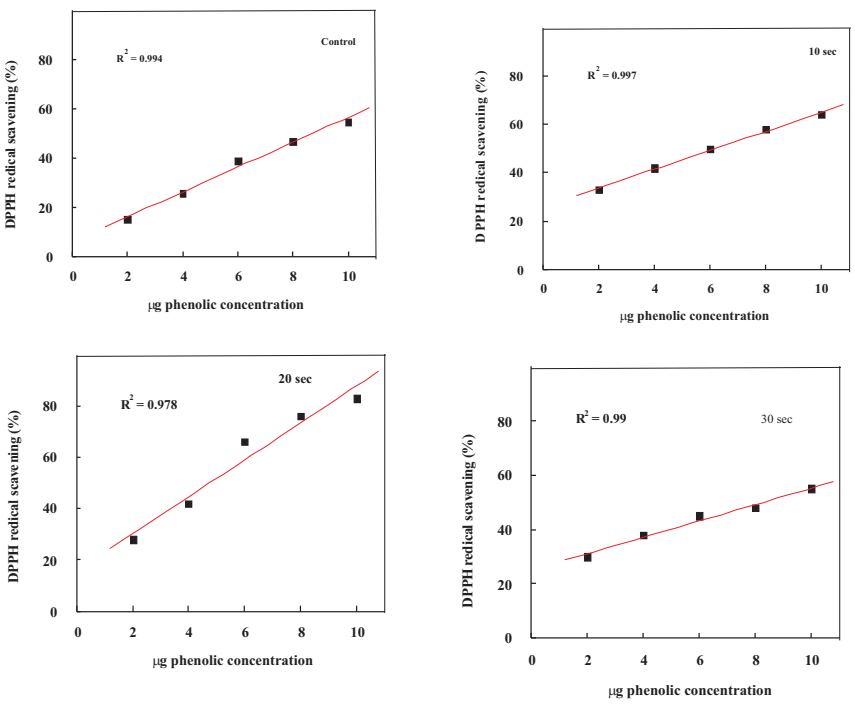


Fig. 2: Effect of microwave (700 W) on antioxidant activity of local apple cultivar Baladyflesh as determined by DPPH assay.

These high values of correlation coefficient (R^2) indicate the strong correlation. The DPPH assay Ic_{50} value was increased with increasing the time of microwave from 3.68 µg phenolic concentration (control) to 7.9 µg phenolic concentration at 30 s of microwave treatment of peel (Table 2). In flesh, the DPPH assay Ic_{50} value was decreased with increasing the time of microwave from 8.6 µg phenolic concentration (control) to 4.9µg phenolic concentration at 20 s of microwave treatment, where at 30 s the DPPH assay Ic_{50} value was increased to 8.3 µg phenolic concentration.Hayat et al. (2010) reported that the antioxidant capacity of the citrus mandarin peel extract increased with microwave power. For instance, after being heated for 5min at 125 and 500 W, the DPPH scavenging activity of peel powder was 17.96 and 26.30%, respectively. The antioxidant activity in all the systems was increased with treatment time; however, a slight decline was observed in the antioxidant activity of citrus peel powder treated at 250 W for 15 min. The sudden decrease in the total phenolic content could be the reason of lower antioxidant activity. Free phenolic compounds were shown to have greater antioxidant effect than the bound forms (Niwa*et al*., 1988). The increase in antioxidant activity withthe microwave power and time could be attributed to the increase in free fraction of phenolic compounds. Kim *et al*. (2008a) reported similar results that the antioxidant activity of citrus pomace was increased after electron-beam irradiation.

Table 2: Antioxidant effect of phenolic concentration of local apple cultivar Balady on reduction of DPPH radical scavengingafter microwave treatment.

Treatment	Time (sec)	Tissue	IC_{50} (µg phenolic)
Control Microwave 700 W	- 10 20 30	Peel	3.68 4.2 5.9 7.9
Control Microwave 700 W	- 10 20 30	Flesh	8.6 6.1 4.9 8.3

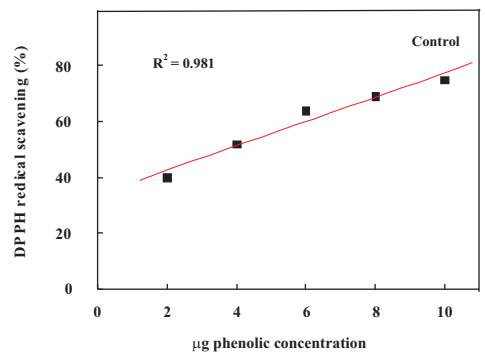
Table 3: Phenolic content of local apple cultivar Balady after heat treatment.

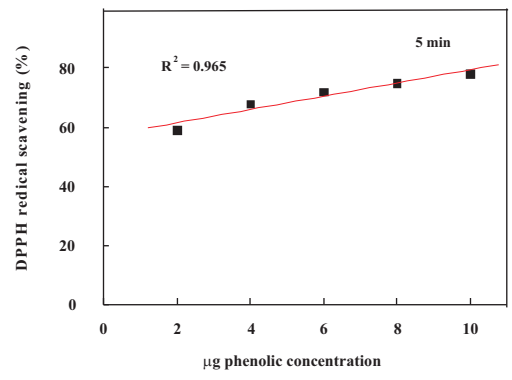
Treatment	Time (min)	Tissue	mg phenolic content/ g tissues
Control Heat at 80 ºC	- 5 10	Peel	14.0 14.5 14.9
Control Heat at 80 ºC	- 5 10	Flesh	6.5 6.5 6.7

Table 4: Antioxidant effect of phenolic concentration of local apple cultivar Baladyon reduction of DPPH radical scavengingafter heat treatment.

Treatment	Time (min)	Tissue	IC_{50} (µg phenolic)
Control	-	Peel	3.68
Heat at 80ºC	5		< 2
	10		<2
Control	-	Flesh	8.6
Heat at 80ºC	5		> 10
	10		> 10

The effect of heat treatment on phenolic content and antioxidant activity of local apple cultivar Balady was evaluated. There is no remarkable effect of heat treatment (80ºC) on the phenolic content of peel and flesh (Table 3). The correlation coefficient (R^2) between phenolic concentration and DPPH scavenging activity was ranged from 0.965 to 0.997 for peel and flesh, respectively (Figs. 3 &4). These high values of correlation coefficient (R^2) indicate the strong correlation. The DPPH assay Ic_{50} value was decreased with heat treatment from 3.68 µg phenolic concentration (control) to < 2 µg phenolic concentration of peel (Table 4). In flesh, the DPPH assay I_{50} value was increased with heat treatment from 8.6 µg phenolic (control) to > 10µg phenolic concentration. Previous study done Garcia-Barrera *et al.*(1998) have reported betalain degradation with increase in temperature.Roy *et al.* (2004) had shown that the extraction of betalainsfrom red beet was optimal at 40°C.





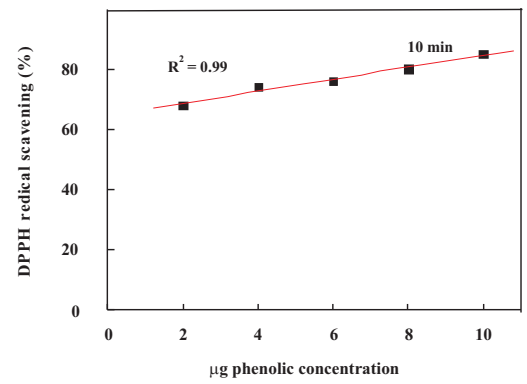
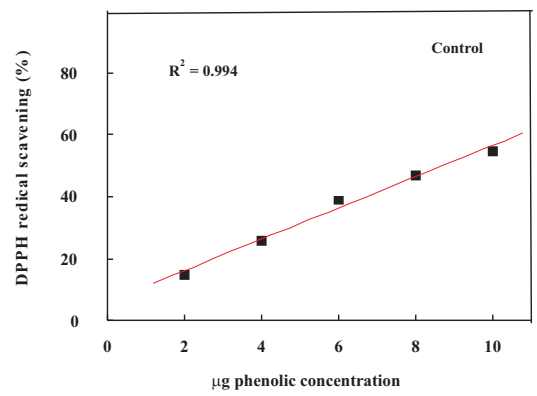
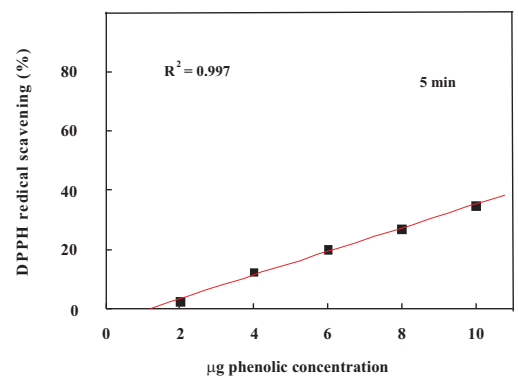


Fig. 3: Effect of heat (80ºC) on antioxidant activity of phenolic concentration in peel of local apple cultivar Baladyas determined by DPPH assay.





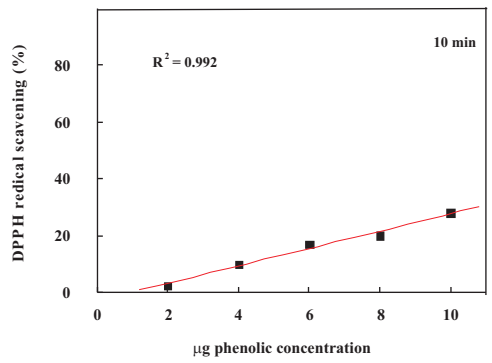


Fig. 4: Effect of heat (80ºC) on antioxidant capacity of local apple cultivar Balady flesh as determined by DPPH assay.

Boiling and roasting led todecrease in the yield of the obtained betacyanins and betaxanthins. Ravichandran*et al*. (2013) explained that thermal treatments decreased betalains stability was slight reduction in initial treatments,but with increased temperatures and time the antioxidant activity was increased. The results were in agreement with the results of Dewanto*et al*. (2002) who found that due to thermal processing there was an enhanced nutritional value of tomatoes and corn by increase of total antioxidant activity.In general, the increase of antioxidant activity occurring at least in the considered conditions. Also, according to Adefegha and Oboh (2009) cooking causes a significant increase in antioxidant activity in tropical green leafy vegetables. In conclusion, the results indicated that microwave and heat treatments could be apple.

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