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Stability of Dietary Phenolics and Antioxidant Properties of Vegetables Depends on Cooking Methodology

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ÖZET

Sebzelerin besinsel fenolik ve antioksidan özelliklerinin kararlılığı pişirme yöntemine bağlıdır

Amaç: Bu çalışmanın amacı; bol suda, az suda, buharlı pişiricide, düdüklü tencerede, mikrodalgada ve yağda kızartma olmak üzere altı farklı pişirme yönteminin, kabak, patlıcan ve patatesteki toplam fenolik madde ve antioksidan aktivite değerleri üzerindeki etkisini araştırmaktı.

Yöntemler: Çiğ ve pişmiş sebzelerde toplam fenolik madde ve antioksidan aktivite analizleri, UV-VIS spektrofotometre kullanılarak yapılmıştır.

Bulgular: Çiğ sebzelerde, kuru madde ağırlık üzerinden hesaplanmış toplam fenolik madde (TFM) içerikleri 173.09-912.64 mg/100 g (gallik asit eşdeğeri, GAE) olarak bulunurken, toplam antioksidan aktiviteleri (TAA) 3.12-51.20 µmol/100 g (trolox eşdeğeri, TE) olarak saptanmıştır. En az kayıp buharlı pişirici ile görülürken, en fazla kayıp kızartma ve bol suda pişirmede gözlenmiştir.

Sonuçlar: Araştırmadan elde edilen verilere göre, bütün sebzelerde tüm pişirme yöntemleri, genel olarak sebzelerin toplam fenolik madde ve toplam antioksidan aktivitelerini düşürmüştür ($p<0.0001$). Ancak, bol suda pişirme ve kızartma dışında uygulanan diğer pişirme yöntemlerinin, sebzedeki fenolik bileşikleri büyük ölçüde koruduğu saptanmıştır.

Anahtar sözcükler: Sebzeler, pişirme yöntemleri, toplam fenolik madde, toplam antioksidan aktivitesi

ABSTRACT

Stability of dietary phenolics and antioxidant properties of vegetables depends on cooking methodology

Objective: The objective of this study was to investigate the effects of six cooking methods, namely cooking in large and small volumes of water, steaming in a steam cooker, cooking in pressure cooker, microwaving and frying in oil, on total phenolic content and antioxidant activity of squash, eggplant and potato.

Methods: Determination of total phenolic content and antioxidant activity of raw and cooked vegetables was carried out by using UV-VIS Spectrophotometer.

Results: Total phenolic content of fresh vegetables ranged from 173.09 to 912.64 mg/100g (as gallic acid equivalent, GAE) on dry weight basis while total antioxidant activities ranged from 3.12 to 51.20 µmol/100 g (as trolox equivalent, TE). Minimum loss was acquired with steaming whereas maximum loss occurred with frying in oil and boiling in large volume of water.

Conclusions: For all vegetables, cooking methods in general caused somewhat loss in total phenolic content and antioxidant activities ($p<0.0001$). However, apart from frying and boiling in large volume of water, all other methods preserved the phenolics of the vegetables to a large extent.

Key words: Vegetables, cooking methods, total phenolic content, total antioxidant activity

INTRODUCTION

Reactive oxygen radicals produced in the human body as cellular byproducts during metabolic processes, are of significant importance since they affect tissues and cells adversely, by causing damage to DNA structure responsible for their maintenance, multiplication and growth (1-3).

Oxidative damage to the primary tissue of vital organs leads to progressive diseases such as coronary heart disease (CHD), stroke, cancer, and rheumatoid arthritis (4,5).

Antioxidant compounds like vitamin E, C, β-carotene and phenolics, present in various nutrients, especially in fruits and vegetables, protect the tissues from reactive oxygen radicals, through varied mechanisms (4,6). Studies have indicated a strong negative correlation between increased consumption of fruits and vegetables and risks of degenerative diseases like CHD, diabetes, Alzheimer's disease, cataract and some types of cancers (7-9).

It has long been perceived that phenolic content is affected by environmental factors, e.g., pedoclimatic (soil, sunshine, rain), agronomic (conventional/hydroponic, organic, greenhouse farming methods), harvesting methods, and time, storage conditions and processing techniques (10-13).

Most of the vegetables are processed and cooked prior to consumption. Studies have shown that cutting and peeling prior to cooking resulted in loss of phenolics (14,15).

Duration of cooking and methods also have been found to have a negative impact on phenolic content and antioxidant activity (15-19).

Other studies have indicated varied or no changes as a result of thermal treatment, cooking or processing (20-23).

This study was conducted to determine the impact of various cooking methods on total phenolic content and anti-oxidative activity of vegetables. The three most common vegetables in Turkish diet, namely, squash, eggplant and potato, were included in the study. The six cooking methods utilized were boiling in large and small volumes of water, steaming in a steam cooker, cooking in pressure cooker, microwaving and frying in oil.

MATERIALS AND METHODS

Vegetable materials

Fresh squash, eggplant and potato were purchased from several open markets in Ankara, Turkey and used as research material. Vegetables were randomly selected from the seller to make it to 1 kg each.

Preparation of vegetable samples

Vegetables were washed with tap water, dried on paper towels. Un-edible edges were taken off for eggplant and squash. Potatoes were peeled. All vegetables were cut into pieces approximately weighing 5-8 g. Seven portions, each weighing 100 g were separated. One portion was retained as raw, and the other six portions were subjected to cooking in six different methods as given below.

All cooking methods were repeated thrice at three different time intervals. Cooked samples were weighed to learn the weight loss during cooking methods. Samples in triplicates were used for each analysis.

Regular boiling in large volume of water (BL)

Two hundred and fifty ml (350 ml for eggplant) of boiling water was poured into a covered stainless steel pot (Tefal 4 L, with double-base) with a diameter of 20-22 cm. Vegetable samples (100 g) were added, and brought to boil. The pot was then covered and boiling was continued for 20 minutes at minimum flame (Arçelik, standard oven, with natural gas connection). The cooked sample was drained off and brought to room temperature.

Regular boiling in small volume of water (BS)

Fifty ml (75 ml for eggplant) of boiling water was poured into a covered stainless steel pot (Tefal 1 L, with double-base) with a diameter of 12-14 cm. Vegetable samples (100 g) were added, and brought to boil. The pot was then covered and boiling was continued for 20 minutes at minimum flame. The cooked sample were drained off and brought to room temperature.

Pressure boiling (P)

Fifty ml (75 ml for eggplant) of boiling water was poured into a pressure cooker (Tefal 6L Clipso-Vitamins, Model P4110769). Vegetable samples (100 g) were added and lid was closed. The cooker was first heated on high flame for 3 min to build maximum pressure, flame was brought to minimum and cooking was continued for 5 minutes. Pressure of the vessel was released, the lid removed, and cooked sample was brought to room temperature.

Regular steaming (S)

Vegetable samples (100g) were placed in the middle tray of 3 Tier Steamer (Tefal, Steam Cuisine, model VC 4002), the lid was placed and steamed as per instructions for 10 minutes. The lid was removed, tray was taken out and cooked sample was brought to room temperature.

Microwave cooking (M)

Fifty ml (75 ml for eggplant) of boiling water was poured in a microwaveable covered dish. Vegetable sample (100 g) were added to the dish and microwaved. (Arçelik, model

MD 594 Intellowave 720 W) at 720 W for 5 minutes. The dish was taken out, lid was taken off and sample was brought to room temperature.

Regular frying in oil (F)

One hundred g of sunflower oil was added to a fry-pan with a diameter of 32 cm (Tefal thermospot, 32 cm), heated to reach a temperature of 190ºC. One hundred g of cut vegetables were added and fried for 10 minutes at medium flame.

Analytical methods

Raw and cooked vegetables were homogenized in a blender (Waring, model 8011) for 30 seconds at high speed. Samples (0.5-1.5 g) were weighed from the homogenized mixture and used for analysis. Samples in triplicate were used for each vegetable.

Dry weight determination (DW)

Because of varying water content in vegetables, all calculations were based on their dry weight basis. For determination of dry matter, 2-3 g weighed homogenized samples (in triplicate) were taken, and smeared to a thin layer on the surface of a porcelain petri-dish used for drying. The samples were put to dry in a convection oven at 100º±2ºC until a constant weight was reached (24).

Determination of total phenolic content (TPC)

The amount of total phenolic content of raw and cooked vegetable samples were determined based on the principle of measurement of the blue colored molybdenum-tungstate complex, at 765 nm wavelength by UV-VIS spectrophotometer, formed as a result of oxidation of phosphotungstic and phosphomolybdic acid present in Folin-Ciocalteu reagent (25,26). Raw and cooked vegetable samples weighed (0.5-1.5 g) were extracted with 1 mL HCl solution (1.2 M HCl in 50% methanol) and kept in ultrasonic water bath (Bandelin Sonorex RK 100 H) at 90ºC for 2 hours. After making up the volume to 10 ml with deionized water, it was filtered through Whatman No: 1 filter paper. The clear extract was used for the determination of phenolic content as well as total antioxidant activity.

To 100 µl of the clear extract, 900 µl of deionized water was added. Five ml of 0.2 N Folin-Ciocalteu reactant and 4 ml saturated Na_2CO_3 was added and vortexed (Genie 2 Scientific Industries). After waiting in vibrating water bath (Memmert, model WB22) at 50±5ºC for 5 minutes, it was (? ya da the extract was?) cooled to room temperature and absorbance measured at 765 nm by UV-VIS spectrophotometer (ATI UNICAM model 8700).

Determination of total antioxidant activity (TAA)

Antioxidant activity of raw and cooked vegetable samples was determined based on the principle of comparison of inhibitory action, due to oxidation of antioxidants present in the aqua-phase of the nutrient with ABTS.+ [2,2'-azinobis(3-ethylbenzotiazolin-6-sulfonic acid)] cation radical, with Trolox (26). Ferrylmyoglobin obtained due to the reaction between H_2O_2 and methmyoglobin (MMb), further reacts with ABTS in the medium to form blue-green colored ABTS.+ radical. Antioxidant of the nutrient sample inhibits this reaction and the degree of inhibition is directly related to the antioxidant activity of the sample. For comparison, standard curve is obtained with Trolox solution of 0.5, 1.0, 1.5, 2.0, and 2.5 mM strength which is accepted as possessing 100% antioxidant activity.

The sample extract prepared for the determination of total phenolic contact was also used for this analysis.

To an aliquot of 21µl of sample, 2126.5 µl of phosphate buffer, 90 µl (70 mM) MMb and 75 µl ABTS (5.0 mM) were added. After bringing it to a temperature of 30ºC in a water bath, reaction was triggered by adding 375 µl of H_2O_2 (1 mM). Absorbance is measured at the end of the lag phase of the reaction, determined by 2.5 mM Trolox standard (approx. 6 min).

Maximum absorbance of ABTS.+ cation radical is obtained at 417, 645, 734, and 815 nm. However, in order to minimize the interference of non-specific reductants, spectrophotometric measurements were taken at 734 nm by UV-VIS (ATI UNICAM model 8700). A mixture of 2147.5 µl of phosphate buffer, 90 µl MMb and 75 µl ABTS was used as blank sample. Analysis was done in triplicates for each vegetable/cooked sample and results expresses as TEAC (Trolox Equivalent Antioxidant Capacity).

Calculation of total phenolic content (TPC) and total antioxidant activity (TAA)

Total phenolic content of the samples was calculated based on the Gallic Acid standard curve and the results were expressed as Gallic Acid Equivalent (GAE)/100 g dry weight (25,26). The analyses were repeated thrice for each of the fresh and cooked samples and mean value was used. For every sample extract with phenolic content ranging between 1-4 mM, obtained absorbance value (antioxidant activity) was compared against 2.5 mM Trolox antioxidant activity standard curve to determine the antioxidant activity corresponding to the specific phenolic content. Total antioxidant activity was calculated as the mean of the samples having 3 different concentrations (27).

Statistical Analysis

Data were recorded as arithmetic means±standard deviation and coefficient of variation. One way ANOVA was carried out to test whether the loss of phenolic content of vegetables during different cooking techniques was significant. For the significant results, Tukey HSD tests were performed to determine the source of this difference. All experiments were repeated at least three times independently.

SPSS for windows (version 10.0) statistical packet was used for the analyses.

RESULTS AND DISCUSSION

The effect of cooking methods on total phenolic content of vegetables

On analyzing total phenolic content of raw vegetables, variation was found among different vegetables and also within each other. Cooking affected the phenolic content of all vegetables, however variation was noticed based on the vegetable type and cooking method used (Table 1).

Total phenolic content of raw eggplant was found to be the highest (912.64±29.67 mg/GAE/100g dry wt) followed by potato (349.76±30.89 mg/GAE/100g dry wt) and squash (173.09±10.15 mg/GAE/100g dry wt). Two different studies have reported total phenolic content of varieties of eggplant S.melongena ranging between 671-4165 µmol/100g and 740-1330 mg/100g, respectively (28,29). Phenolic content of potato has been found to range within 100-781 mg/100 g in different studies that were conducted (16,30-33) Total phenolic content of squash was reported to be 133 mg/100g by Andlauer and Stumpf (18) However, phenolic content in squash was found to be very high (833 mg GAE/100g KMA) by Turkman et al. (22) as compared to other studies and the authors concluded, that may have been resulted due to the analysis method used, variety of the vegetable, cultivar and stage of maturity (16).

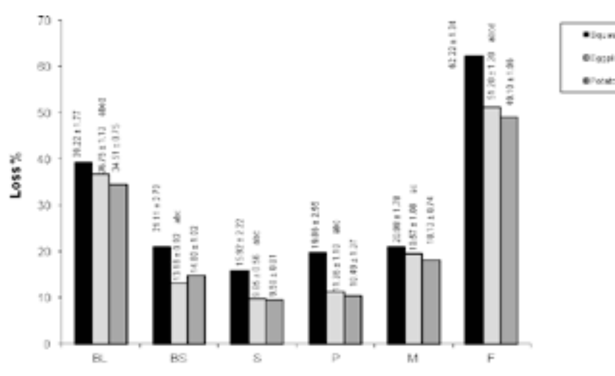

Figure 1: Effect of cooking methods on percent loss of phenolic content in vegetables.
%loss was calculated using raw vegetables as starting material.
a: p<0.0001, b: p<0.0001 (squash-eggplant), c: p<0.0001 (squash-potato), d: p<0.0001 (potato-eggplant).

Table 1: Effect of cooking methods on total phenolic content of vegetables

		Squash	Potato	Eggplant
	Processing time	**Total phenolics (mg GAE/100 g DW)**	**Total phenolics (mg GAE/100 g DW)**	**Total phenolics (mg GAE/100 g DW**
Fresh	-	173.09±10.15	349.76±30.89	912.64±29.67
BL	20 min	105.03±9.25	228.99±18.97	597.04±13.49
BS	20 min	136.30±9.73	298.08±28.24	792.26±20.41
S	10 min	145.50±8.25	316.03±25.90	823.84±28.63
P	3 min+5 min	138.84±10.97	312.82±23.80	809.13±28.89
M	5 min	136.90±7.26	286.50±27.61	734.05±25.70
F	10 min	65.47±5.31	178.63±15.15	445.94±15.34

BL: regular boiling in large volume (250 ml) water, BS: regular boiling in small volume (50 ml) water, S: regular steaming, P: pressure boiling, M: microwave (720 W), F: regular frying (190ºC). Data expressed as means values (x±S) of triplicate experiments (on dry basis).

Table 2: Effect of cooking methods on antioxidant activity of vegetables

		Squash	Potato	Eggplant
	Processing time	Antioxidant activity (μmol TE/100 g DW)	Antioxidant activity (μmol TE/100 g DW)	Antioxidant activity (μmol TE/100 g DW)
Fresh	-	5.01±0.61	3.12±0.32	51.20±4.51
BL	20 min	3.03±0.47	2.02±0.24	33.03±2.89
BS	20 min	3.82±0.46	2.62±0.30	43.20±3.49
S	10 min	4.41±0.60	2.78±0.29	45.45±4.32
P	3 min+5 min	4.14±0.57	2.74±0.29	43.85±4.07
M	5 min	3.93±0.49	2.54±0.28	39.80±4.07
F	10 min	1.93±0.37	1.60±0.19	25.63±2.01

BL: regular boiling in large volume (250 ml) water, BS: regular boiling in small volume (50 ml) water, S: regular steaming, P: pressure boiling, M: microwave (720 W), F: regular frying (190ºC). Data expressed as means values (x± S) of triplicate experiments (on dry basis).

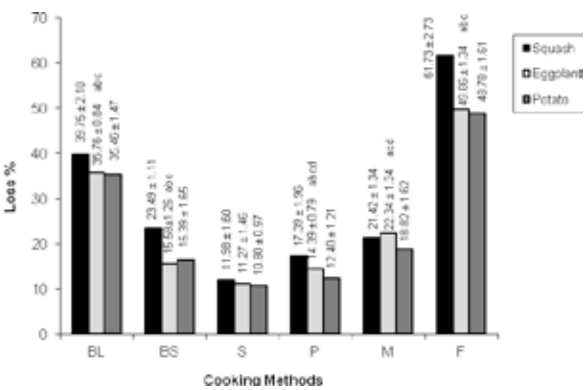

Figure 2: Effect of cooking methods on percent loss of antioxidant activity in vegetables.
% loss was calculated using raw vegetables as starting material.
a: p<0.0001, b: p<0.0001 (squash-eggplant), c: p<0.0001 (squash-potato), d: p<0.0001 (potato-eggplant)

Frying resulted in maximum loss and steaming caused minimum loss of phenolics content of all vegetables (Table 1, Figure 1). During frying, loss of phenolics was as high as 62.22% in squash, similar to that found by Tudela et al., (34) who reported up to 71% loss of chlorogenic acid in potatoes when fried for 4 minutes. Studies by Ewald (14) and Crozier et al. (35) reported similar losses indicating breakdown of phenolics at high temperatures. Cutting and peeling of vegetables, cooking time have also been reported to affect the breakdown of phenolics by Hirota et al. (36). On the contrary, Velioğlu et al. (37) indicated marginal rise of phenolics possibly due to the release of free flavonols. The second most highest loss in phenolics for vegetables was obtained during boiling in large volume of water possibly due to its running off into the boiling medium, as suggested by Zhang et al. (38). The loss was in the order of squash>eggplant>potato (Table 1) and were found to be statistically significant (F=30.261, p<0.0001). During boiling in small volume of water, phenolics loss was in the order of squash>potato>eggplant, and it was significant between squash and potato, and squash and eggplant (F=50.963, p<0.0001). Andlauer and Stumpf (18) reported 30% loss of rutin in squash and 42% loss in chlorogenic acid in potato during boiling. Several factors as boiling time, volume of water and size of vegetables and stirring have been reported to possibly affect (?) the loss (14,18,34). In this study, the best results from the aspect of loss in phenolics, were obtained by steaming, pressure cooking and microwave (Table 1). Xu and Chang (39) have also reported smaller losses in total phenolics, antioxidant activitity during pressure and steam cooking as compared to regular boiling of cool season food legumes. Among the vegetables included in this study, the loss was the highest in squash in the order of 15.92%, 19.85%, and 20.98%, respectively (Figure 2). These results were lower than 22.9-39.7% of loss in spinach during microwave cooking reported by Kuti and Konuru (37) and Tudela and Cantos, (34) who reported 40-50% loss of chlorogenic acid in potato while cooking in pressure cooker. On the other hand, some other studies have indicated that pressure cooking and radiation treatment of plant materials cleaved and liberated the bound phenolic compounds, therefore resulting in the increase of free phenolic componds and enhancement of anti-oxidant capacity of the extracts. However, higher power and longer treatment time also could result in degradation of some phenolic componds (38-41). It was concluded that cooking time, volume of water used, power, type of microwaveable dish or pressure cooker used could affect the loss.

The effect of cooking methods on antioxidant activity of vegetables

Similar to the phenolic content, variation among vegetables was reported for antioxidant activity. Cooking methods used affected the antioxidant activity of vegetables parallel to its phenolic content (Table 2). Anthocyanidin present in eggplant is primarily responsible for its high antioxidant activity (51.20±4.51 μmol TE/00 g DW), followed by squash (5.01±0.61 μmol TE/100 g DW) and potato (3.12±0.32 μmol TE/100 g DW). Red wine, red cabbage, black grapes, red onion and turnips, potatoes containing this red pigment has been reported to have high antioxidant activities (42,43).

Losses during frying were as high as 61.73%±2.39 in squash, followed by eggplant and potato (Table 2, Figure 2). Franke et al. (44) reported more than one third loss of hydroxylcinnamic acid and antioxidant activity in eggplant, when fried and boiled in large volume of water. During quick stir-frying of bamboo shoots, an increase in the antioxidant activity in bamboo shoots, inspite of the decrease of its phenolic content was reported by Zhang et al. (38). The authors suggested that high temperatures during stir-frying could lead to the Maillard reaction which promoted generation of antioxidant substances, however, long term exposure to high temperatures could cause decomposition of phenolic compounds in the extract and affect its antioxidant activity negatively (38).

In this study, antioxidant activity loss during boiling in large volume of water for vegetables were in the order of squash>eggplant>potato, parallel to their loss of phenolic content (Table 1). Loss of antioxidant activity for boiling in large ($F=21.220$; $p<0.0001$) and small volumes ($F=92.023$; $p<0.0001$) of water was statistically significant for squash and eggplant and squash and potato. Significant loss in antioxidant activity in spinach, cabbage, celery and black beans during boiling was demonstrated in various studies (41,45-46), the loss being proportional to the cooking time. It has been suggested that the thermal treatment affected semi-permeability of cellular membrane, resulting in loss of phenolics from the vegetable tissues into the cooking medium. Mass balance analysis showed that boiling caused more dry solid loss as compared to steaming (45).

Minimum loss of antioxidant activity parallel to phenolics was obtained with the steaming followed by pressure cooker and microwave in the order of squash>eggplant>potato (Figure 2); the loss was however insignificant between the vegetables ($F=1.718$, $p>0.1$). Ewald et al. (14) in his study reported minimum loss of phenolics and antioxidant activity during steaming in green beans, onions and green peas as compared to boiling, microwave and frying. Xu and Chang indicated greater retention of phenolic content and antioxidant activity during steaming and pressure boiling as compared to regular boiling in eclipse black beans (39). On the other hand, studies by Rong et al. (38) and Hayat et al. (40,41) have indicated that steam, pressure cooking and microwave treatment of plant materials enhanced the anti-oxidant capacity of the extract by liberating the bound phenolic compounds into its free form. However, the loss was greater during boiling due to the increased contact area of the sample and running off of the antioxidant substances in the boiling medium (38). Therefore, in general, the difference in results could be attributed to several factors as the type of vegetables used, cultivars, cooking medium and vessels, cooking time and energy, etc.

Correlation between total phenolic content and antioxidant activity of vegetables

Phenolic compounds, phenolic acids and flavonoids present in plant materials are known to be strong antioxidants and a positive correlation between the two has been reported in several recent studies (30,45-46). In this study, a correlation between total phenolic content and antioxidant activity was determined for all raw and cooked vegetable samples. A strong correlation was found between the two for all samples analyzed. Correlation coefficient ranked highest for potato ($r=0.957$), followed by eggplant ($r=0.8995$) and squash ($r=0.8729$) (Figure 3).

In a recent study performed by Zhuang et al. on pomegranate juices and wines (45), the authors have reported strong correlation between total polyphenols and antioxidant activities. In a study by Kahkönen et al. on berries (48) showed a higher correlation between antioxidant activity expressed as TEAC (trolox equivalent antioxidant capacity) and total polyphenols ($r=0.98$), as compared to anthocyanin ($r=0.60$), indicating … (indicating what?). In another study, Sellappan et al. (49) also reported

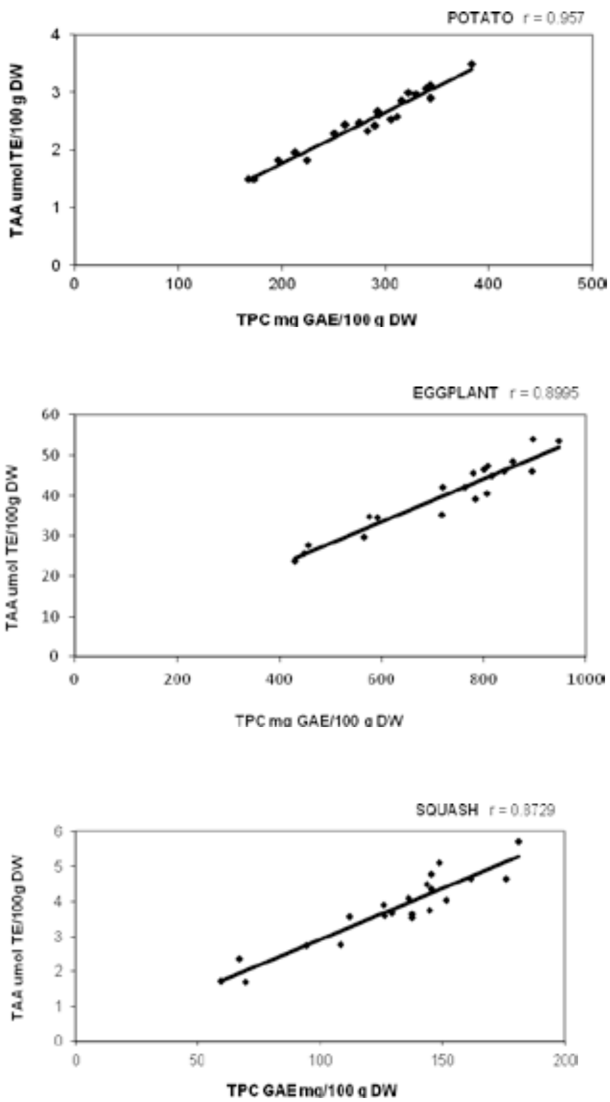

Figure 3: Correlation between phenolic content and antioxidant activity in potato, eggplant and squash.

a strong correlation (r=1) between antioxidant activity and total polyphenol as well as anthocyanin content of blackberries and grapes. Similarly, Javanmardi et al. (50) reported a fairly high correlation (r=0.71) between total phenolic content and antioxidative activity in basil, indicating that the rest of the antioxidative activity may have resulted due to the presence of other antioxidant compounds in the plant material.

On the other hand, this correlation was found to be weak in some recent studies performed in in vitro and in vivo samples of cultivated mushrooms (51), edible seaweeds from southern coast of Thailand (52), and some grains and cotton seeds (30), reflecting the presence of other active components in addition to phenolic compounds in the extracts that may have been the cause of this non-conformity. Among studies performed on vegetables, İsmail et al. (53) reported no correlation between the phenolic content and the antioxidant activity in cabbage, leek and spinach. Velioğlu et al. (30) also reported poor correlation between the phenolic content and the antioxidant activity in vegetables with high anthocyanin content as red onion, red potatoes and blackberries, although the authors found this correlation to be strong in grains and cotton seeds.

Plant materials contain different phenolic compounds with varied antioxidative properties (6,8,10-12). Researchers have suggested that the poor correlation between the phenolic content and the antioxidant activity in nutrients may have resulted not only due to the presence of phenolic compounds with varied antioxidant activities, but also due to the other antioxidant compounds in the material like α-tocopherol, β-carotene, vitamin C, and selenium, etc (30,32,54-55).

CONCLUSION

In all the vegetables, frying and boiling in large volume of water resulted in a significant loss of total phenolic content and antioxidant activity. Other cooking procedures showed results similar to or different from each other based on the vegetable type. It was concluded that, other than frying and boiling in large volume of water, the other four cooking methods more or less retained the phenolics and antioxidant activity of the vegetables to a large extent. A correlation between total phenolic content and antioxidant activity was observed for all raw and cooked samples. However, changes during cooking affected both indices differently.

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