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Research Article

Effects of Different Cooking Methods on Improving Total Antioxidant Activity in Selected Vegetables

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Abstract: The present study compares Water-soluble Phenolic Content (WPC) and antioxidant activities in eggplant, kidney bean, bitter gourd and spinachprior to and after subjecting to boiling, microwaving and pressure cooking. The total antioxidant activity was increased in cooked spinach, eggplant and bitter gourd, estimated based on the ferric reducing antioxidant power, the Troloxequivalent antioxidant capacity and 2, 2-diphenyl-1-picryl-hydrazyl radical scavenging activity. Pressure cooking did not cause any significant decline in the antioxidant property. Boiling generally improved the overall antioxidant activity in all the vegetables. Correlation analysis suggests that WPC contributed to significant antioxidant activities in these vegetables. Thus, prudence in selecting an appropriate cooking method for different vegetables may improve or preserve their nutritional value.

Keywords: Antioxidant activity, cooking methods, phenolics, vegetables

INTRODUCTION

Antioxidants are compounds that inhibit the oxidation process via various mechanisms, such as by preventing the formation of reactive radical species or by scavenging them (Jimenez-Monreal *et al*., 2009). The levels of antioxidants and oxidants in the body are continuously maintained in homeostasis by dietary antioxidants and enzymatic antioxidant systems in the body. The imbalance of these systems has been postulated to cause chronic diseases over a sustained period, including cancer (Valko *et al*., 2007) and coronary heart disease (Adams *et al*., 1999). Dietary antioxidants from fruits and vegetables confer protection against harmful free radicals and they were associated with reduced risk of chronic diseases such as cardiovascular diseases, cancer, diabetes mellitus and degenerative diseases of aging (Hamidi and Tajerzadeh, 2003).

Generally, fruits are consumed raw but most vegetables are cooked by boiling, steaming, microwaving, stir-frying or pressure-cooking before consumption. The cooking process or introduction of a thermal treatment was reported to either increase, decrease or induce no significant changes in the antioxidant content and activity in vegetables (Chuah *et al*., 2008; Miglio *et al*., 2008; Wachtel-Galor *et al*., 2008). For example, boiling and baking did not alter the ascorbic acid, phenolic content, lycopene content and radical scavenging activity of tomatoes (Sahlin *et al*., 2004). Heavy loss of antioxidant components and antioxidant activity was observed in broccoli subjected to conventional cooking (Zhang and Hamauzu, 2004). Cooking by microwave heating and stir-frying was shown to retain antioxidant compounds in peppers (Chuah *et al*., 2008). Generally, most studies concurred that prolonged cooking decreases the antioxidant content and activity in vegetables (Faller and Fialho, 2009; Wachtel-Galor *et al*., 2008).

Alteration in the total antioxidant content and activities due to different processing methods is of scientific importance, especially its impact on dietary nutrition. Unfortunately, only a small number of reports are available, especially on pressure-cooked vegetables and are limited to a few selected vegetables only. The present study is aimed to address this by investigating the antioxidant levels and Water-soluble Phenolic Contents (WPC) in eggplant, kidney bean, bitter gourd and spanich, prior to and after subjecting to various cooking methods; namely, boiling, microwaving and pressure-cooking.

MATERIALS AND METHODS

Chemicals: $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and Trolox, 3- (2-pyridyl)-5, 6-bis (4-phenyl-sulfonic acid) -1, 2, 4-triazine (Ferrozine) were purchased from Sigma (Nanjing,

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China). Folin-Ciocalteu's reagent, glacial acetic acid, Hydrochloric acid (fuming) (HCl), iron (III) chloride hexahydrate ($FeCl_3$•$6H_2O$), potassiumpersulfate ($K_2O_8S_2$), sodium acetate trihydrateand sodium carbonate (Na_2CO_3) were obtained from Sigma (Shanghai, China). All other reagents used were of analytical grade.

Plant materials and processing: Eggplant, kidney bean, bitter gourd and spinach were purchased from Nanjing, China. The vegetables were washed and dab-dried with paper towel before weighing. They were divided into 150 g portions and were subjected to different cooking methods: for boiled samples, the vegetables were boiled in 100 mL boiling water for 5 min on a hot plate; for microwave-cooked samples, the vegetables were microwaved in 100 mL water with acommercial 1,000 W microwave oven for 5 min at high power; for pressure-cooked samples, the vegetables were autoclaved at 121°C under 2 MPa for 20 min with 100 mL water. The 800 mL beaker used for cooking was loosely covered with cooking cling-film to prevent evaporation during cooking. Cooked vegetables and the remaining liquid were cooled rapidly on ice to prevent further cooking from residual heat. They were homogenized with an electric blender. For raw (i.e., uncooked) samples, the vegetables were homogenized with 100 mL water. The homogenate was filtered and centrifuged at 2,400×g for 15 min to obtain a clear supernatant. The supernatant was freeze dried. The lyophilized extracts were kept in a freezer (-20°C) and were reconstituted with water on the day of experiment.

Assessment of antioxidant activity: For the Ferric reducing antioxidant power assay of all tissue extract, a modified method of Benzie and Strain (1996b) was adopted. The stock of FRAP solution included 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mMHCl and 20 mM $FeCl_3$•$6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $FeCl_3$•$6H_2O$. The temperature of solution was kept at 37°C before using it. 150 µL of different tissue extract were allowed to react with 2850 µL of FRAP solution for 30 min in the dark condition. Absorbance of colored product of different samples (ferrous tripyridyltriazine complex) was taken at 593 nm. The results were expressed as mM Fe (II) /g tissue basis and compared with that of ascorbic acid and (+) catechinhydrate.

Trolox equivalent antioxidant capacity assay: The Troloxequivalent Antioxidant Capacity (TEAC) assay was performed as described by Re *et al*. (1999). Trolox, a water soluble vitamin E analogue was used as the standard. TEAC values were expressed as mmol Trolox equivalent over 100 g lyophilized extract (mmol TE/100 g).

Diphenyl-1-picryl-hydrazyl radical scavenging assay: The DPPH radical scavenging activity of the different extracts of faba bean seed was measured according to the method of Yang *et al*. (2008) IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Water-soluble phenolic content determination: The total Water-soluble phenolic content of vegetables were determined according to Xu and Chang (2007) with slight modifications. After adding Folin-Ciocalteau reagent and sodium carbonate to aliquots of samples, the mixtures were set in a 40°C water bath for 20 min. The absorbance was measured at 740 nm using a spectrophotometer (Unico, Shanghai, China) and total phenolic contents were expressed as milligrams of garlic acid equivalents per grams of defatted sample.

Statistical analysis: Data were analyzed with SPSS 13.0 for windows. The mean and standard deviation of means were calculated. The data were analyzed by one-way Analysis of Variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at probability $p<0.05$.

RESULTS AND DISCUSSION

Cooking improves the total antioxidant activity: The FRAP assay has been reported to be well suited for biological fluids, such as plasma and plant extracts, to assess their total non-enzymatic 'antioxidant' activity (Benzie and Strain, 1996a). All cooking methods performed in this study improved the FRAP level of the vegetables (Table 1). Compared with the raw vegetable extracts, boiling improved the FRAP level of all the vegetables tested in this study. Interestingly, the order of FRAP level for each vegetable cooked in different methods remained the same, which was boiled>microwaved>pressure-cooked>uncooked. The data shows that uncooked vegetable extracts gave the lowest FRAP level compared with extracts from cooked vegetables. The decreasing FRAP level for uncooked vegetables was in the following order: kidney bean >spinach>eggplant>bitter gourd, ranging from 5.2 to 16.8 mmol FE/100 g (Table 1). All three cooking methods significantly ($p<0.05$) increased the FRAP level of spanich, with the highest level in the boiled sample. For bitter gourd and eggplant, boiling and microwaving significantly ($p<0.05$) increased the FRAP level. The FRAP level in uncooked kidney bean extract and those subjected to the various cooking methods were not significantly different. Microwaving and boiling for a short period of time had a negligible effect on the total antioxidant activity in peas but substantial losses occurred after prolonged boiling in water (Hunter and Fletcher, 2002). Similarly, the effect

Table 1: Extraction efficiency, antioxidant activities, water-soluble phenolic content of vegetable extracts under different cooking methods

	Extraction efficiency		Antioxidant activities			
Vegetable/processing	Lyophilized extract (g)	(%)[a]	FRAP (mmol FE/100 g)	TEAC (mmol TE/100 g)	DPPH (%)	WPC (mg GAE/100 g)
Eggplant						
Raw (uncooked)	3.6	2.4	8.0±0.4[a]	9.1±0.3[a]	10.5±1.1[a]	985.7±15.9[a]
Boiled	2.5	1.6	21.6±0.7[b]	10.3±0.5[a]	22.7±1.3[b]	1627.4±16.8[c]
Microwaved	1.7	1.1	21.5±0.8[b]	10.1±0.7[a]	10.9±1.2[a]	1677.1±15.4[c]
Pressure cooked	2.7	1.8	10.1±0.5[a]	9.6±0.8[a]	9.3±0.8[a]	1152.8±13.7[b]
Kidney bean						
Raw (uncooked)	2.4	1.6	16.8±1.2[a]	11.4±0.8[a]	41.4±1.8[c]	1724.1±32.6[b]
Boiled	4.2	2.8	19.2±1.7[a]	11.2±0.7[a]	29.3±1.5[a]	1752.1±33.1[b]
Microwaved	4.1	2.7	18.1±1.1[a]	10.2±0.5[a]	36.9±1.8[b]	1617.4±28.9[a]
Pressure cooked	4.6	3.1	17.1±1.4[a]	11.1±0.6[a]	26.7±1.7[a]	1635.6±27.6[a]
Bitter gourd						
Raw (uncooked)	2.7	1.6	5.2±0.3[a]	7.3±0.7[a]	5.8±1.1[a]	612.7±25.3[a]
Boiled	3.6	2.8	11.2±0.7[b]	7.5±0.6[a]	7.3±1.3[b]	805.6±32.1[b]
Microwaved	2.1	2.7	10.3±0.7[b]	8.4±0.8[a]	9.7±1.4[c]	801.4±28.4[b]
Pressure cooked	3.2	3.1	5.8±0.4[a]	7.9±0.6[a]	5.9±0.9[a]	612.5±26.4[a]
Spinach						
Raw (uncooked)	2.9	1.9	9.1±0.4[a]	8.5±0.7[a]	20.1±1.7[a]	1816.5±35.9[b]
Boiled	2.3	1.5	31.9±1.4[d]	10.1±0.8[a]	51.3±3.2[b]	3192.4±64.3[c]
Microwaved	2.1	1.4	14.3±1.1[b]	9.2±0.6[a]	23.6±2.2[a]	1618.7±34.2[a]
Pressure cooked	2.8	1.9	19.2±1.3[c]	9.3±0.5[a]	24.1±2.4[a]	1812.4±38.7[b]

For each vegetable, values within the same column followed by different uppercase superscript letters are significantly different ($p<0.05$); [a] The extraction efficiency percentage of lyophilized extract was calculated based on the amount of fresh sample used (150.0 g)

of prolonged heat treatment on the FRAP level was evident in our study as the FRAP level was lower in pressure-cooked than boiled and microwaved vegetables. However, the FRAP level in all of the pressure-cooked vegetables was significantly higher or the same as in raw (uncooked) vegetables.

The TEAC assay measures the total antioxidant capacity of the vegetables based on the ABTS radicals-cavenging-ability of both hydrophilic and hydrophobi cantioxidant compounds (Apak *et al*., 2007; Re *et al*., 1999). Table 1 shows that the order of increasing TEAC value in uncooked vegetables was similar to that of FRAP value: kidney bean>spinach>eggplant>bitter gourd. The TEAC value for uncooked vegetables ranged from 7.3 to 11.4 mmol TE/100 g (Table 1). For spanich, bitter gourd and eggplant, all three cooking methods caused only a mild increase in TEAC value. The TEAC level for kidney bean showed a mild decrease when subjected to the three cooking processes. However, these changes were not significantly different.

The DPPH radical reacts with proton-donating antioxidant compounds to reduce the DPPH radical concentration, which results in the change of the reagent color from deep violet to light yellow. Ascorbic acid was used as the positive control in this experiment and it scavenged 50% of the DPPH radical at 21.6 mM (data not shown). Similar to both FRAP and TEAC assays, the decreasing potency of DPPH radical scavenging among uncooked vegetable samples was as follows: kidney bean>spinach>egplant>bitter gourd (Table 1). Boiling significantly ($p<0.05$) improved the DPPH radical-scavenging activity of eggplant and spinach. Among the tested vegetables, all cooking methods used in this study either improved or did not cause significant changes to the DPPH radical scavenging activity of the extracts; except for kidney bean, which showed significant decrease of activity in the boiled and pressure cooked extracts compared with the uncooked samples (Table 1). Interestingly, the decrease of this activity was accompanied by the decrease of WPC, thus suggesting that the DPPH radical scavenging activity in kidney bean was contributed mainly by phenolic compounds.

The Folin-Ciocalteu reagent is a solution of complex polymeric ions formed from the combination of phosphomolybdic and phosphotungtic acids. The reagent oxidizes phenolates (ionized phenolics) present in the sample and reduces the acids to form a blue complex. The decreasing WPC level for raw (uncooked) vegetables was in the following order: spinach>kidney bean>eggplant>bittergourd, ranging from 612.7 to 1816.5 mg GAE/100 g (Table 1). Among the different cooking methods performed in this study, boiling increased the WPC in all vegetable extracts. Boiling significantly ($p<0.05$) increased WPC inspanich, eggplant and bittergourd. As for eggplant, all three cooking methods also caused significant ($p<0.05$) elevation of WPC: microwaving (70%), boiling (65%) and pressure-cooking (17%). Turkmen *et al*. (2005) reported that cooking caused loss of phenolic content in squash, peas and leeks but increased the level in green beans. Zhang and Hamauzu (2004) as well as Wachtel-Galor *et al*. (2008) reported a gradual decline of phenolic content in Brassica vegetables during cooking.

Conflicting reports have been cited on the effect of cooking or heat treatment on the antioxidant values of vegetables. Several studies have shown that heat

Table 2: Correlation analysis between various antioxidant parameters

Assay	R^2	r	Significance
Between WPC and antioxidant activities			
WPC and FRAP	0.8172	0.9040	***
WPC and TEAC	0.8246	0.9081	***
WPC and DPPH	0.8261	0.9089	***
Between reduction power and radical scavenging activity			
FRAP and TEAC	0.6127	0.7828	***
FRAP and DPPH	0.6578	0.8110	***
Between the two radical scavenging activities			
TEAC and DPPH	0.7242	0.8510	***

The R^2 value denotes the regression value and the r value denotes the Pearson's correlation value; The level of significance was expressed as *** ($p<0.001$)

attenuated antioxidant capacity in processed vegetable samples versus raw samples (Faller and Fialho, 2009; Podsedek, 2007). This was possibly due to the inactivation of antioxidant compounds (Faller and Fialho, 2009; Turkmen *et al*., 2005; Wachtel-Galor *et al*., 2008). Zhang and Hamauzu (2004) showed that boiling of broccoli florets for as brief as 5 min could decrease the vitamin C content by almost 70%, whereas Miglio *et al*. (2008) showed that boiling (48%), steaming (32%) and frying (87%) reduced vitamin C content in broccoli. However, others have attributed the increased antioxidant activity in cooked vegetables to matrix softening by heat, which could give rise to higher extractability (Miglio *et al*., 2008; Podsedek, 2007), production of redox-active secondary plant metabolites or polyphenols (Szeto *et al*., 2002; Wachtel-Galor *et al*., 2008) such as Millard compound (Nicoli *et al*., 1999) and release of active aglycones from flavonoid conjugates (Miglio *et al*., 2008; Turkmen *et al*., 2005). Munyaka *et al*. (2010) had shown that thermal treatment (15 min) above 70°C retained the reduced form of vitamin C (L-AA) in crushed vegetable products due to the inactivation of ascorbicoxidase in the vegetables, which prevented the oxidation of L-AA to dehydroascorbic acid. One should not rule out the possibility that the retention of antioxidant activity in the cooked vegetables in this study could also be contributed by L-AA. The factors that could possibly contribute to thermal stability of L-AA and the mechanisms involved deserve further investigation. It is evident that the various cooking techniques used for cooking different vegetables produce varying antioxidant potencies and effects.

Correlation analysis: Table 2 depicts the correlation analysis between the various antioxidant parameters. A strong correlation ($p<0.001$) between the WPC in the extracts with the level of antioxidant activity was present. This suggests that the water-soluble phenolic compounds present in these extracts contributed significantly to the observed antioxidant effect. The results depicted in Table 2 also show that the FRAP level of the vegetable extracts correlated with both the radical scavenging assays (TEAC and DPPH), indicating that the compounds present in the extracts were good reductants as well as radical scavengers. The data also show that the TEAC values correlated with the DPPH radical scavenging activity of the vegetable extracts, indicating the presence of a good association between these two parameters.

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