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DPPH, ABTS, AND APX ANTIOXIDANT IN RAW AND COOKED BROWNISH YELLOW AND PURPLE POTATO TUBERS

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ABSTRACT

This study was to evaluate and compare the antioxidant of extracts from common brownish yellow potato and purple potato cultivars during cooking methods. The colored skin potatoes generally had slightly higher antioxidant activity in raw states than common brownish yellow cultivars. Three antioxidant properties were analyzed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ascorbate peroxidase (APX) scavenging activity. The DPPH, ABTS, and APX activity of the raw potatoes was high, but not when boiled or baked. DPPH scavenging activity of the raw purple potato was evaluated at 1.0 mg/ml was 53.5%, that of boiled potato was 45.8% at same concentration. Although purple potatoes were slightly higher in DPPH activity than common potatoes, there was no significant difference ($p > 0.05$). ABTS scavenging activity of raw of common brownish yellow potato was evaluated at 1.0 mg/ml of water extracts was 33.0%, those of boiled and roasted potatoes at same concentration were 8.2% and 11.9%, respectively. APX scavenging activity of raw common potato was evaluated at 1.0 mg/ml was 43.3%, those of boiled and roasted potatoes were 18.1% and 17.4% at same concentration, DPPH, ABTS, and APX scavenging activities were shown a significant difference between raw and cooked (boiled and roasted) potatoes ($p < 0.05$). Boiling and roasting treatments showed reducing effects on the antioxidant properties of common brownish yellow potato and purple potato cultivars. Namely, antioxidant capacity of potatoes were significantly lost during common cooking practices such as boiling and roasting.

KEYWORDS: ABTS, APX, DPPH, yellow potato and purple potatoes.

INTRODUCTION

Reactive oxygen species (ROS) are produced by several endogenous and exogenous processes, and their negative effects are neutralized by antioxidant defenses.[1] Antioxidants are compounds that inhibit oxidation. Antioxidants are a group of compounds that provide protection against the harmful effects of free radicals and other reactive oxidants. Antioxidants have received a lot of press for their possible role in the prevention of many degenerative diseases of ageing such as cancer, arthritis, neurodegenerative diseases, and heart disease.[2]

Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Numerous methods have been used to evaluate antioxidant activities of natural compounds in foods or organisms with varying results.[3] Among many antioxidants, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were commonly used to assess antioxidant activity in vitro. Hydrogen peroxide (H_2O_2), an important relatively stable non-radical reactive oxygen species is produced by normal aerobic metabolism in plants.[4] At low concentrations, H_2O_2 acts as a signal molecule involved in the regulation of specific biological/physiological processes (photosynthetic functions, cell cycle, growth and development, plant responses to biotic and abiotic stresses).[4] Ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor.[5]

The potato (*Solanum tuberosum* L.) is the third most important food crop in the world after rice and wheat in terms of human consumption. Potatoes have been cultivated for thousands of years and are still a very popular food source. Potato is a crop of highland origin and has been domesticated in the high Andes of South America and has become a major food crop in the cool

highland areas of South America, Asia, and Central and Eastern Africa.[6] Potatoes provide significant amounts of carbohydrates, potassium, and ascorbic acid in the diet.[7] There are about 100 or more varieties of edible potatoes. They range in size, shape, color, starch content and flavor. The skin of potatoes is generally brown, red or yellow, and may be smooth or rough, while the flesh is yellow or white. Potatoes also contain a variety of phytonutrients that have antioxidant activity. Among these important health-promoting compounds are carotenoids, flavonoids, and caffeic acid, as well as unique tuber storage proteins, such as patatin, which exhibit activity against free radicals.

There were some large variations in antioxidant components and activity between the cultivars examined, mainly due to colour variations.[8]

Most foods are consumed after a cooking procedure that may involve boiling, microwaving, steaming or baking.[9] Cooking can lead to many physical and chemical changes in plant structure. The concentration of phytochemical compounds in food materials may be enhanced through the matrix softening effect, cell wall or membrane broking and improved extractability. Conversely, heat treatment can cause thermal degradation of these chemical compounds.[10] Antioxidants are used in food to protect it from deleterious effects of oxidation and are also employed as dietary supplements to neutralize the adverse effects of oxidative stress.[11] The antioxidant activity of vegetables is mainly based on their phytochemical compounds, like polyphenols. Cooking can trigger not only oxidation of these compounds, but also leakage of water-soluble compounds.

The purpose of the present study is to evaluate common brownish yellow potato and purple potato cultivars during cooking methods as sources of antioxidants for DPPH, ABTS, and APX radical to examine whether they are losing significant antioxidant activity or not for raw or cooking.

MATERIALS AND METHODS

Sample extract

Potatoes (200g) were added to 1,000 ml of water that had just reached the boil in a stainless steel pan and cooked for 20 min. The samples were drained off and cooled rapidly on plenty of ice.

Potatoes (200g) were baked in an oven for 20 min and rapidly cooled on ice.

The plant materials were ground using a Retsch GM 200 mill (Fisher Bioblock, France). Ground plant material (100 g) with distilled water at 50°C under agitation. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature and left in the dark at room temperature for 20 min. After filtration, the water was removed in a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at 70°C. To get dry powder, samples placed in a low temperature vacuum chamber. These powders were then used to determine antioxidant activities. All analyses were realized as much as possible in an area protected against light.

DPPH free radical

The antioxidant activity of the seaweed extracts was measured based on the scavenging activity of DPPH free radical according to the method described by Brand-Williams et al.[12] with slight modifications. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[13]

The transformation results in the formation of colour change from purple to yellow. Each sample stock solution (1.0 mg/ml) was diluted to final concentrations of 0.1, 0.5, and 1.0 mg/ml, in water or ethanol. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of 80% ethanol. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. DPPH (final concentration 300 µM) was added to the solutions prepared with sample extracts and standard antioxidant substances. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The radical scavenging reaction was carried out at 37 ºC in dark for 30 min. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) at the wavelength 515 nm. Corresponding blank sample was prepared and L-Ascorbic acid (0.1, 0.5, and 1.0 mg/ml) was used as reference standard (positive control). Inhibition of free radical scavenging activity was calculated using the following equation.

ABTS radical scavenging activity

ABTS was dissolved in water to a 7 mM concentration, and 2.45 mM potassium persulfate was prepared. Two stock solutions were mixed and kept in the dark at room temperature for 16 h before use. The ABTS solution was diluted with methanol to an absorbance of 0.700 at 734 nm and equilibrated at 30°C. After the addition of 200 µl of the diluted ABTS solution to 40 µl of the sample extracts (2.5 and 5.0 mg/mL concentration), the decrease in absorbance was measured for 1 min after mixing the solution, and the final absorbance reading was monitored for 33 min by absorbance at 734 nm using the Microplate Reader (VersaMax, California, USA). Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner.

Ascorbate peroxidase (APX) scavenging activity

The activity of APX (EC 1.11.1.1) was determined according to Nakano and Asada[14] by monitoring the oxidation rate of ascorbate at 290 nm. The reaction

medium was incubated at 28°C. It consisted of 100 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid and 0.1 mM H2O2. The decrease in absorbance was monitored for 2 min from the start of the reaction and the activity of APX was expressed in lmol ASC min-1 mg-1 of protein.

Statistical analysis

Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance ($p < 0.05$). The Spearman correlation coefficient was applied to evaluate the degree of correlation between the different antioxidant activity categories, DPPH, ABTS, and APX. The analysis was carried out at least in triplicate. The results were expressed as the mean±SD. Significance and confidence level were estimated at $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula.

Inhibition (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

EC_{50} is defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction of a maximum scavenging capacity. To determine the EC_{50} value of the active component, the technique using 96-well microplates was employed.[15] Regression analysis by a dose response curve was plotted to determine the EC_{50} values.

RESULTS

Table 1 was shown the antioxidant activities for DPPH radical of common brownish yellow potato and purple potato cultivars during cooking methods or a raw state. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research potato extracts in the assay mixture. The DPPH activity of the raw potatoes was high, but not when boiled or baked. Both common brownish yellow and purple potatoes had radical scavenaging effects at all concentration. However, their effects were not great levels. DPPH scavenging activity of raw extract (common brownish yellow potato) evaluated at 1.0 mg/ml was 50.9% and that of boiled potatoes was 38.9% at same concentration. DPPH scavenging activity of the roasted potato evaluated 40.8%. There was no significant difference between boiled and roasted potatoes ($p > 0.05$). DPPH scavenging activity of the raw purple potato was evaluated at 1.0 mg/ml was 53.5%, that of boiled potato was 45.8% at same concentration. DPPH scavenging activity of roasted potatoes evaluated 47.3%. Although purple potatoes were slightly higher in DPPH activity than common potatoes, there was no significant difference ($p > 0.05$). Figure 1 was shown the rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for potatoes on 1.0 M. The values for raw, boiled, and roasted states of common yellow potatoes were 84.0%, 63.7%, and 66.7%, respectively. The values for raw, boiled, and roasted states of purple potatoes were 85.0%, 70.7%, and 73.3%, respectively. An EC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. EC_{50} value was inversely related to the antioxidant activity of crude extracts. The values of EC_{50} for raw, boiled, and roasted states of common yellow potatoes were 201.6 µg/ml, 231.7 µg/ml, and 224.2 µg/ml, respectively (Table 3). Those of EC_{50} for purple potatoes were 198.6 µg/ml, 225.1 µg/ml, and 220.4 µg/ml, respectively.

Using ABTS radical scavenging method, for the determination of antioxidant activity, the obtained results recorded in Table 2. The rates of antioxidant activities of the water extracts for potatoes were also dependent on concentrations. Various concentrations of raw potatoes were higher than those of the boiled and roasted states. ABTS scavenging activity of raw of common brownish yellow potato was evaluated at 1.0 mg/ml of water extracts was 33.0%, those of boiled and roasted potatoes at same concentration were 8.2% and 11.9%, respectively. The all groups did not show a statistically significant difference ($p > 0.05$). However, there was a significant difference between raw and cooked (boiled and roasted) potatoes ($p < 0.05$). The values for raw, boiled, and roasted states of purple potatoes were 34.2%, 9.0%, and 14.1%, respectively. The three groups (raw, boiled, and roasted) did not show a statistically significant difference ($p > 0.05$). However, there was a significant difference between raw and cooked (boiled and roasted) potatoes ($p < 0.05$). Figure 2 was shown the rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for potatoes on 1.0 M. The values for raw, boiled, and roasted states of common yellow potatoes were 64.3%, 23.2%, and 26.0%, respectively. The values for raw, boiled, and roasted states of purple potatoes were 66.6%, 24.5%, and 27.6%, respectively. The values of EC_{50} for raw, boiled, and roasted states of common yellow potatoes were 190.5 µg/ml, 197.2 µg/ml, and 197.6 µg/ml, respectively (Table 3). Those of EC_{50} for purple potatoes were 187.2 µg/ml, 197.5 µg/ml, and 195.3 µg/ml, respectively.

APX scavenging activity of raw common potato was evaluated at 1.0 mg/ml was 43.3%, those of boiled and roasted potatoes were 18.1% and 17.4% at same concentration, respectively. The boiled and roasted extract values of APX scavenging activity were lower than that of raw potato. The all groups did not show a statistically significant difference ($p > 0.05$). However, there was a significant difference between raw and cooked (boiled and roasted) potatoes ($p < 0.05$). Figure 3

was shown the rate of APX inhibitory of H_2O_2 (positive control) and relative inhibitory rate for potatoes on 1.0 M. The values for raw, boiled, and roasted states of common yellow potatoes were 47.2%, 19.7%, and 18.9%, respectively. The values for raw, boiled, and roasted states of purple potatoes were 49.0%, 19.2%, and 21.4%, respectively. The values of EC_{50} for raw, boiled, and roasted states of common yellow potatoes were 241.9 µg/ml, 273.9 µg/ml, and 270.7 µg/ml, respectively (Table 3). Those of EC_{50} for purple potatoes were 214.9 µg/ml, 271.4 µg/ml, and 268.5 µg/ml, respectively.

Table 1. The degree of inhibition (%) of DPPH by raw and cooked brownish yellow and purple potato tubers at different concentrations.

Type	Concentration (mg/ml)	Potato		*t*-test
		Brownish yellow	Purple	
Raw tubers	0.25	11.67±4.40	18.91±3.67	0.002
	0.50	29.87±1.06	32.11±3.76	
	0.75	44.05±2.70	46.53±4.74	
	1.00	50.91±4.69	53.53±4.38	
Boiled tubers	0.25	7.84±4.86	12.23±4.30	0.590
	0.50	21.72±2.24	23.97±4.41	
	0.75	29.73±4.22	35.56±4.39	
	1.00	38.91±3.06	45.79±3.34	
Roasted tubers	0.25	9.41±3.67	16.21±4.11	0.749
	0.50	23.15±4.13	30.05±4.69	
	0.75	30.44±4.19	37.59±5.57	
	1.00	40.84±4.83	47.28±3.77	
F-test		0.040	0.014	

Data represented the mean ± SD from three replicates.

Table 2. The degree of inhibition (%) of ABTS by raw and cooked brownish yellow and purple potato tubers at different concentrations

Type	Concentration (mg/ml)	Potato		*t*-test
		Brownish yellow	Purple	
Raw tubers	0.25	13.17±1.21	14.01±0.80	0.113
	0.50	19.50±1.11	20.02±1.17	
	0.75	27.32±0.78	27.56±0.91	
	1.00	32.96±0.42	34.17±1.18	
Boiled tubers	0.25	1.05±0.94	1.34±1.07	0.224
	0.50	3.05±1.22	4.34±1.36	
	0.75	8.19±0.79	8.97±0.78	
	1.00	11.85±2.07	12.55±2.37	
Roasted tubers	0.25	1.33±1.27	2.06±1.37	0.195
	0.50	4.80±0.84	5.63±0.84	
	0.75	10.29±1.50	10.93±1.72	
	1.00	13.34±1.57	14.11±1.68	
F-test		0.506	0.462	

Data represented the mean ± SD from three replicates.

Table 3: The degree of inhibition (%) of APX by raw and cooked brownish yellow and purple potato tubers at different concentrations.

Type	Concentration (mg/ml)	Potato		*t*-test
		Brownish yellow	Purple	
Raw tubers	0.25	7.03±2.33	14.45±0.44	0.308
	0.50	23.08±5.46	25.24±1.94	
	0.75	33.22±3.70	34.45±3.39	
	1.00	43.32±3.79	44.98±3.07	
Boiled tubers	0.25	1.36±0.60	2.36±1.54	0.073
	0.50	6.15±0.62	6.75±1.64	
	0.75	12.61±1.95	12.91±1.38	
	1.00	18.06±1.46	17.61±1.29	
Roasted tubers	0.25	1.02±0.02	2.68±1.54	0.242
	0.50	8.12±4.49	8.20±2.77	
	0.75	13.95±1.07	14.92±2.91	
	1.00	19.58±2.95	19.67±0.50	
F-test		0.366	0.385	

Data represented the mean ± SD from three replicates.

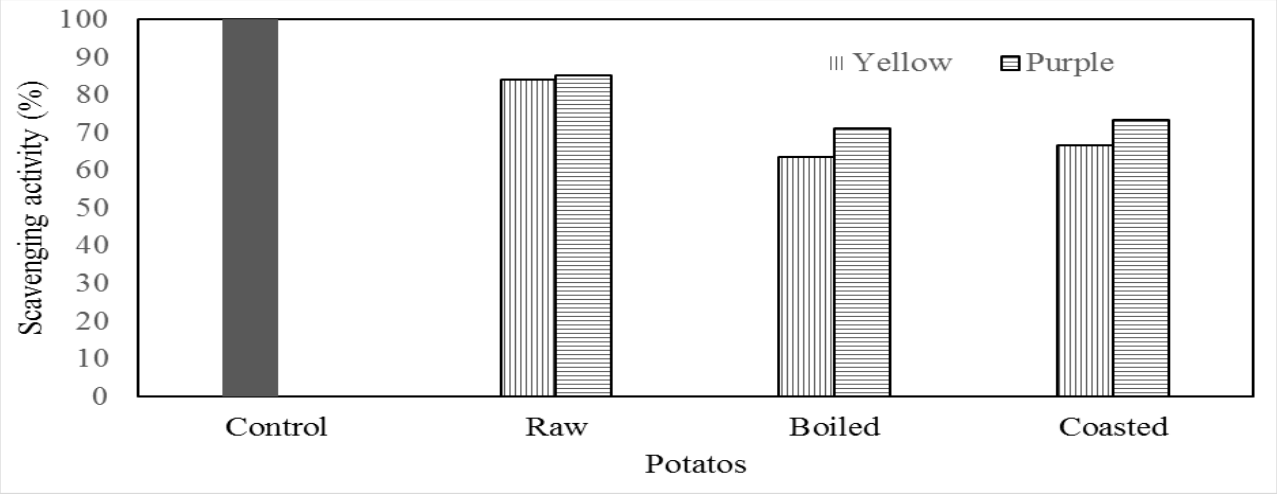

Figure 1. The rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for raw and cooked brownish yellow and purple potato on 1.0 M.

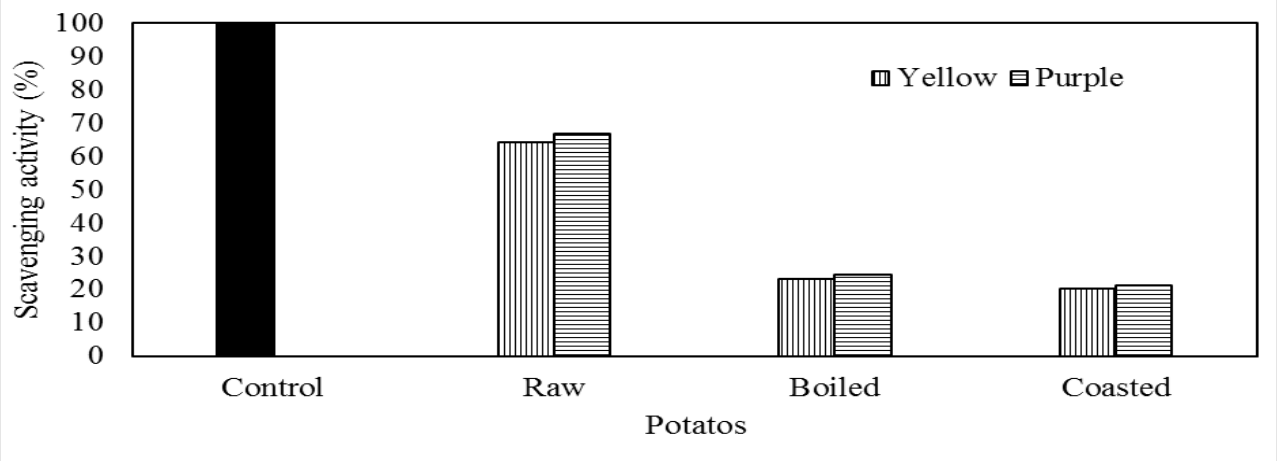

Figure 2. The rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for raw and cooked brownish yellow and purple potato on 1.0 M.

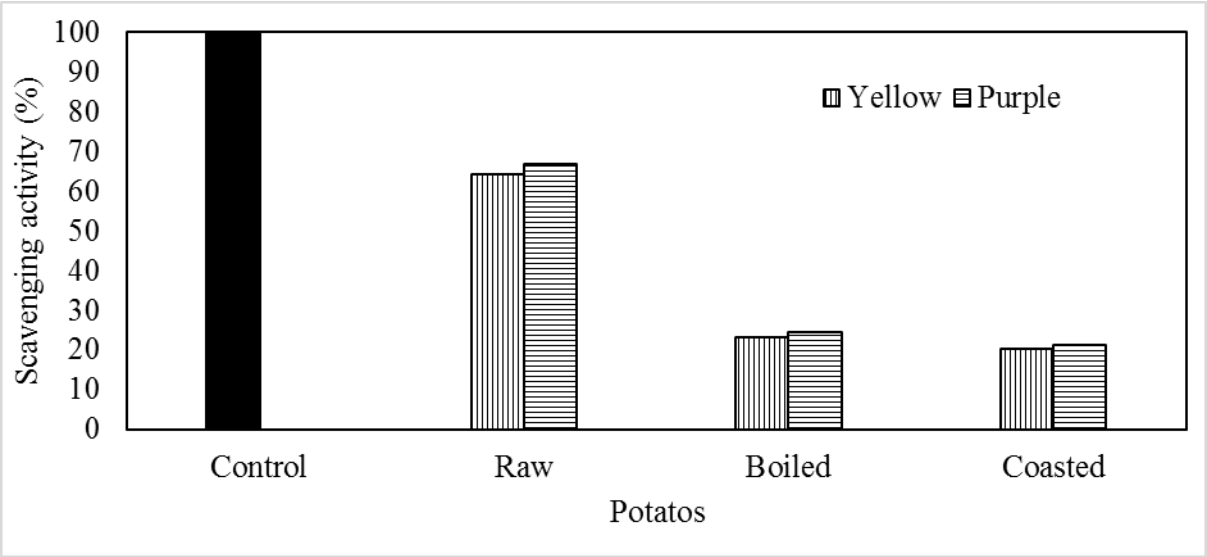

Figure 3. The rate of APX inhibitory of H_2O_2 (positive control) and relative inhibitory rate for raw and cooked brownish yellow and purple potato on 1.0 M.

Table 4. The 50% inhibition (EC_{50}) of DPPH, ABTS, and APX of raw and cooked brownish yellow and purple potato

Sample	Brownish yellow potatoes			Purple potatoes		
	DPPH	ABTS	APX	DPPH	ABTS	APX
Raw tubers	201.6	190.5	241.9	198.6	187.2	247.9
Boiled tubers	231.7	199.2	273.9	225.1	197.5	271.4
Coasted tubers	224.2	196.7	270.7	220.4	195.3	268.5

DISCUSSION

Heating or cooking treatments in food materials soften the cell walls and facilitate the extraction of bioactive compounds such as polyphenols[16] and carotenoids.[11] There are published investigations on the influence of cooking methods on the antioxidant activity of 20 vegetables.[17] According to the method of analysis chosen, griddling, microwave cooking, and baking alternately produce the lowest losses, while pressure- cooking and boiling lead to the greatest losses; frying occupies an intermediate position.[17] In the present study, the effect of boiling and roasting on antioxidant properties (DPPH, ABTS, and APX) radical scavenging ability and singlet oxygen scavenging ability) of potatoes were evaluated. DPPH, ABTS, and APX scavenging activities were shown a significant difference between raw and cooked (boiled and roasted) potatoes ($p < 0.05$). Namely, antioxidant capacity of potatoes were significantly lost during common cooking practices such as boiling and roasting. It is considered that the total polyphenols, carotenoids and antioxidant capacity of selected green leafy vegetables are significantly altered during common cooking practices such as boiling, steaming, and frying. After cooking in boiling water for 8 min, the broccoli tissue had a 51% relative peak force of the fresh tissue.[18] Likewise, Park et al. noticed that the DPPH radical scavenging activity and total phenolic content of raw garlic extract were higher than those of heated garlic extract.[19] Although roasting purple potatoes were slightly higher in DPPH activity than boiling common potatoes, the impact of cooking on the changes in bioactive concentrations and antioxidant capacities are dependent on the species and the method of cooking.[20] In addition, thermal degradation of antioxidants may occur in some vegetables, while the antioxidant activity can increase through the improved extractability and matrix softening effects of heat treatment.[9]

CONCLUSIONS

The DPPH, ABTS, and APX activity of the raw potatoes was high, but not when boiled or baked. Boiling and roasting treatments showed reducing effects on the antioxidant properties of common brownish yellow potato and purple potato cultivars.

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