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HYDROXYL RADICAL SCAVENGING ACTIVITY IN RAW AND COOKED BROWNISH YELLOW AND PURPLE POTATO TUBERS

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ABSTRACT

This study was to evaluate and compare the hydroxyl radical (OH) scavenging activity of extracts from common brownish yellow potato and purple potato cultivars during cooking methods. The potato (*Solanum tuberosum* L.) is the third most important food crop in the world after rice and wheat in terms of human consumption. The amount of malonaldehyde produced by disassembling deoxyribose from hydroxyl radiological by Fenton reaction was measured to check the hydroxyl radical scavenging ability. OH scavenging activity of raw extract (common brownish yellow potato) evaluated at 2.0 mg/ml was 52.6% and that of boiled potatoes was only 19.3% at same concentration. OH scavenging activity of the roasted potato evaluated only 17.0%. Raw and cooked potatoes at all concentrations showed significant differences in OH scavenging ability (p<0.05-0.001). Boiling and roasting treatments showed reducing effects on the OH scavenging activity of common brownish yellow potato and purple potato cultivars.

KEYWORDS: Hydroxyl radical (OH), yellow potato and purple potatoes.

INTRODUCTION

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.[1] ROS are mainly produced by mitochondria, during both physiological and pathological conditions, that is, $O_2^{\bullet-}$ can be formed by cellular respiration, by lipoxygenases (LOX) and cyclooxygenases (COX) during the arachidonic acid metabolism, and by endothelial and inflammatory cells.[2] Consumption of antioxidant-enriched fruits and vegetables is known to lower the risk of several diseases caused by free radicals.[3]

Hydroxyl radicals (OH) generated in the human body may play an important role in tissue injury at sites of inflammation in oxidative stress-originated diseases.

The potato (*Solanum tuberosum* L.) belongs to the *Solanaceae* family and is a globally important crop plant producing high yields of nutritionally valuable food in the form of tubers. Potatoes traces its origin to Andean and Chilean land races developed by pre-Colombian cultivators and are still a very popular food source. It has been the focus of substantial study because of its use both as a staple food crop and as a potentially significant source of compounds of interest.[4] Tubers, spherical to ovoid in shape, are swellings of the rhizome. The flesh of the tubers varies in colour from white to yellow to blue and the skin varies from white through yellow to tan and from red through blue. The colour of the flesh may or may not correspond to the colour of the skin. The skin of potatoes is generally brown, red or yellow, and may be smooth or rough, while the flesh is yellow or white.

The main source of concern when it comes to **raw potato** consumption is a **toxic** compound called solanine, which can cause headaches, nausea, diarrhea, and even death in extreme cases. **Cooking** or **cookery** is the art, science and craft of using heat to prepare food for consumption. Cooking techniques and ingredients vary widely across the world, from grilling food over an open fire to using electric stoves, to baking in various types of ovens, reflecting unique environmental, economic, and cultural traditions and trends. When raw potatoes are cooked, they undergo a process called the Maillard reaction, namely, a chemical reaction that occurs between amino acids and a reducing sugar in the presence of heat. Conversely, heat treatment can cause thermal degradation of these chemical compounds.[5] 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.[6] The antioxidant activity of vegetables is mainly based on their phytochemical compounds, like polyphenols. The flesh of potato varieties is often tinged with yellow to a greater or lesser extent and this is

mainly due to the presence of carotenoids.[7] 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 OH 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.

Determination of hydroxyl radical scavenging activity

The amount of malonaldehyde produced by disassembling deoxyribose from hydroxyl radiological by Fenton reaction was measured to check the hydroxyl radical scavenging ability of the samples.[8] The hydroxyl radical scavenging capacity was measured using modified method as described previously.[9] Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled de-ionized water. Reaction mixtures contained, in a final volume of 1 ml, $FeCl_3$ (20 µM), EDTA (100 µM), H_2O_2 (1.42 mM), deoxyribose (2.8 mM) and *S. sarmentosum* extracts (0.25, 0.5, 0.75, and 1.0 mg/ml). All components were dissolved in KH_2PO_4-KOH buffer 10 mM, pH 7.4. The mixture was then incubated at 37 ºC for 1 h. 1.0 ml portion of the incubated mixture was mixed with 1 ml of 2.8% trichloroacetic acid (w/v) and 1 ml of 1% thiobarbituric acid (TBA) (w/v) to develop the pink chromogen. The mixture was heated in a water bath at 100ºC for 15 min. Corresponding blank sample was prepared and H_2O_2 (1.0 µg/ml) was used as reference standard (positive control). The absorbance of the resulting solution was measured at 530 nm with Microplate Reader (VersaMax, Califonia, USA). This assay was also performed without ascorbic acid or EDTA, in order to check for pro-oxidant or metal chelation activities. The range of extract concentrations and measurement frequencies were established experimentally. Considering IC_{50} and T_{IC50}, affect the antiradical capacity, antiradical efficiency was defined: $AE = 1/ IC_{50} \cdot T_{IC50}$.[10] Antiradical efficiency parameter allowed dividing the extracts into different antiradical activity groups: AE = 1 10-3 low antiradical activity. 1· 10-3 <AE < 5· 10-3 : medium antiradical activity, 5· 10-3 <AE < 10 · 10-3 : high antiradical activity, AE > 10 · 10-3 : very high antiradical activity.

Inhibition types were then determined by Lineweaver–Burk plot (1/v versus 1/[S]) where [S] analysis of data is according to Michaelis–Menten kinetics.[11]

Statistical analysis

All experiments were performed thrice and the results averaged Data were expressed as mean±SD. 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. 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.

IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined. Regression analysis by a dose response curve was plotted to determine the IC_{50} values.

RESULTS

The hydroxyl radical scavenging abilities of water extracts of potato on several concentrations were shown Figures 1 and 2. A concentration dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in the deoxyribose assay. The OH scavenging activity of the raw potatoes was high, but not when boiled or baked. Both common brownish yellow and purple potatoes had radical scavenging effects at all concentration. However, their effects were not great levels. OH scavenging activity of raw extract (common brownish yellow potato) evaluated at 2.0 mg/ml was 52.6% and that of boiled potatoes was only 19.3% at same concentration. OH scavenging activity of the roasted potato evaluated only 17.0%. There was no significant difference between boiled and roasted potatoes (p>0.05). OH scavenging activity of the raw purple potato was evaluated at 2.0 mg/ml was 55.4%, that of boiled potato was 22.6% at same concentration. OH scavenging activity of roasted potatoes evaluated 20.8%. Although purple potatoes were slightly higher in OH activity than common potatoes, there was no significant difference (p>0.05). However, raw and

cooked potatoes at all concentrations showed significant differences in OH scavenging ability (p<0.05-0.001) (Table 1). In particular, as the concentration increased, the difference between raw potatoes and cooked potatoes was significant in OH scavenging ability.

Figure 3 was shown the rate of OH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for potatoes on 2.0 M. The values for raw, boiled, and roasted states of common yellow potatoes were 64.9%, 23.8%, and 21.0%, respectively. The values for raw, boiled, and roasted states of purple potatoes were 68.4%, 27.9%, and 25.7%, respectively. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value was inversely related to the antioxidant activity of crude extracts. The values of IC_{50} for raw, boiled, and roasted states of common yellow potatoes were 236.9 µg/ml, 425.2 µg/ml, and 473.4 µg/ml, respectively (Table 2). Those of IC_{50} for purple potatoes were 248.5 µg/ml, 476.3 µg/ml, and 502.1 µg/ml, respectively.

According to antiradical efficiency parameter, the results showed that the raw potatoes were medium antiradical activity and the cooked and roasted potatoes were low antiradical activity (Table 3).

The Lineweaver–Burk plot was used to determine important terms in enzyme kinetics, such as K_m and V_{max}. The mode of inhibition of the tea extracts against OH was confirmed by Lineweaver–Burk plots (Figure 4). Crude water extracts competitively inhibited OH.

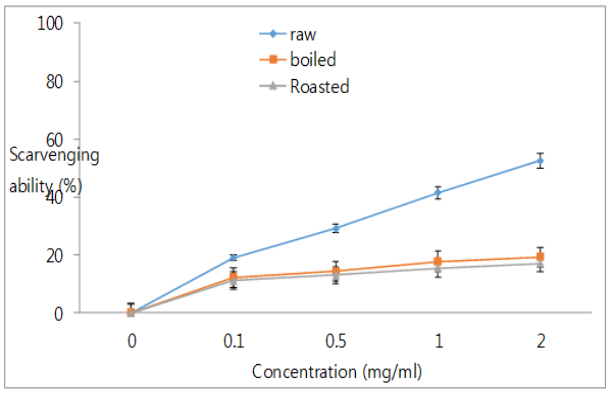

Figure 1. Scavenging activity (%) of OH by raw and cooked brownish yellow potatoes at different concentrations.

Data represented the mean ± SD from three replicates.

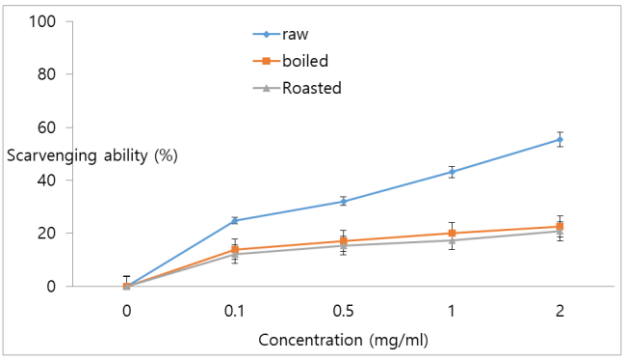

Figure 2. Scavenging activity (%) of OH by raw and cooked purple potatoes at different concentrations.

Table 1: Compare *t*-test of difference in means of three samples by raw and cooked brownish yellow and purple potato tubers at same concentration.

Concentration (mg/ml)	Color of potato	
	Brownish yellow	Purple
0.1	4.892*	19.007**
0.5	26.078**	25.236**
1.0	31.058**	18.804**
2.0	542.463***	257.973***

*, p<0.05; **, p<0.01; ***, p<0.001.

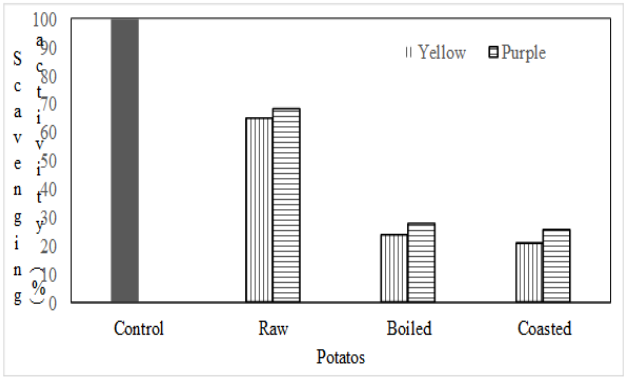

Figure 3. The rate of OH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for raw and cooked brownish yellow and purple potatoes on 2.0 mg/ml.

Table 2. The 50% inhibition (EC_{50}) of OH of raw and cooked brownish yellow and purple potato.

Sample	Brownish yellow potatoes	Purple potatoes
Raw tubers	236.9	221.9
Boiled tubers	425.2	413.6
Coasted tubers	473.4	488.4

Table 3. The 50% inhibition (EC_{50}) of OH for raw and cooked brownish yellow and purple potatoes

Sample	Color	EC_{50}	T_{IC50}	AE (10^{-3})	Antiradical efficiency classification
Raw tubers	Yellow	205.5	3.8	1.28	Medium antiradical activity
	Purple	198.6	3.7	1.36	Medium antiradical activity
Boiled tubers	Yellow	389.6	4.5	0.57	Low antiradical activity
	Purple	377.3	4.2	0.63	Low antiradical activity
Roasted tubers	Yellow	403.4	5.5	0.45	Low antiradical activity
	Purple	394.5	5.4	0.47	Low antiradical activity

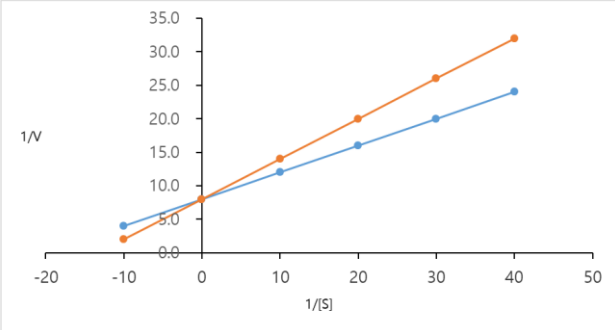

Fig. 4. Lineweaver-Burk plot for the activity of OH in the presence of concentration (1 ug/ml) of the raw brownish yellow potato and inhibitor.

DISCUSSION

Color-fleshed potatoes have higher antioxidant and stronger antiproliferative activities than white-fleshed potatoes.[12] The red colored potato had the high activity at all extract concentrations except at 500 μg·mL-1, and particularly showed about 55% OH scavenging activity at 2,000 μg·mL-1.[12] The activities of white fleshed potato, four colored potatoes, 'Red', 'Haryoung', 'Blue', 'Jaseo', and 'Jasim' were 26.5, 35.2, 32.3, 35.9, 35.9, and 42.3%, respectively, at 2,000 μg·mL-1; all the colored potato extracts showed significantly stronger scavenging activities than the white fleshed potato extract.[12] Chu et al.[13] found that flavonoid or flavone extracts of potato skins showed 94% scavenging activity towards hydroxyl radicals. Flavonoids showed a similar development such as phenolic compounds and their function is mainly combined with the role as a yellow pigment in the tuber.[15]

A significant negative correlation for phenolic content and EC50 values was observed in potato varieties, indicating that these phenolic compounds may contribute directly to the radical scavenging activity.[14]

Food contains many heat sensitive nutrients which include vitamins, minerals, and nutrients having functional properties such as pigments, antioxidant, bioactive compounds. Many processes during manufacturing of food cause detrimental effects on these nutrients.[16] The basic purpose for the thermal processing of foods is to reduce or destroy microbial activity, reduce or destroy enzyme activity and to produce physical or chemical changes to make the food meet a certain quality standard. When infrared treatment at high temperatures (60 and 70◦C) at a short period of time, the peel and leaf content of total phenols were higher because phenolic compounds resist thermal breakdown. However, boiling had the most detrimental effect on carrot polyphenols, resulting in a complete loss of each compound likely because of their diffusion in the boiling water. Steaming and frying had a less negative effect on total phenolics (-43 and -31%, respectively), exclusively because of the loss of chlorogenic acid (-95 and -93% for the two processes, respectively).[17]

In the present study, the effect of boiling and roasting on antioxidant properties (OH) radical scavenging ability and singlet oxygen scavenging ability) of potatoes were evaluated. Namely, antioxidant capacity of potatoes were significantly lost during common cooking practices such as boiling and roasting.

CONCLUSIONS

The hydroxyl radical 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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