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Antioxidative Activities of the Ethyl Acetate Fraction from Heated Onion (*Allium cepa*)

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Abstract Heated onion juice was partitioned using the solvents hexane, chloroform, ethyl acetate, and butanol. The ethyl acetate fraction showed the strongest scavenging effect on the ABTS radical. The antioxidant activities of the ethyl acetate fraction from raw and heated onion (120, 130, and 140°C) were evaluated using radical scavenging assays. Radical and nitrite scavenging activities were higher in heated onion than raw onion, and the higher the temperature of heat treatment, the greater the radical and nitrite scavenging activities. Heated onion (140°C, 2 hr) was more effective than raw onion, having higher DPPH radical scavenging (5.7-fold), hydroxyl radical scavenging (6.4-fold), superoxide radical scavenging (2.3-fold), hydrogen peroxide scavenging (11.8-fold), and nitrite scavenging (4.3-fold) activities. Onion increased its physiologically active materials after heating, and in this regard, heated onion can be used as biological material for the manufacture of health foods and supplements.

Keywords: heated onion juice, hydroxyl radical, superoxide radical, hydrogen peroxide, nitrite scavenging activity

Introduction

Reactive free radicals have been postulated to contribute to the causes of chronic inflammatory proliferative diseases (CIPD), especially arteriosclerosis and cancer, through oxidative damage of essential enzymes, cells, and tissues. There is therefore widespread interest in defining the possible role of diet in preventing and reversing reactive oxygen species (ROS)-induced chronic diseases (1).

Onion (*Allium cepa* L.) has been reported to have anti-microbial, antispasmodic, anticholesterolaemic, hypotensive, hypoglycaemic, antiasthmatic, anticancer, and antioxidant properties (2-4). The fresh weight of onions is 60-70% water, and the most significant of the other components are organosulfur compounds. Protective effects of organosulfur compounds (e.g., S-allyl cysteine and diallyl disulfide) have been found in the form of scavenging ROS, inhibiting low-density lipoprotein oxidation and suppressing the formation of atherogenic lesions (5-7). Recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to various chemical changes during heat treatment (8). Woo *et al.* (9) reported that the total polyphenol content of heated onions increased significantly with increased heating temperature and time. The polyphenol and flavonoid contents and antioxidant activity increase with heat treatment in plants such as tomato (8), ginseng (10), licorice (11), garlic (12), pear (13), *shiitake* mushroom (14), sweet corn (15), and citrus peel (16).

The browning of onion due to thermal treatment is the result of several reactions. The Maillard reaction, which occurs between the free amino groups of a protein or an amino acid with reducing sugars during food processing and heating, may lead to the formation of a complex mixture of reaction products (17). The antioxidant activities of Maillard reaction products (MRP) have been studied extensively (18). MRP have been shown to serve as natural antioxidants to be added to food materials susceptible to oxidative deterioration. Many studies of onion have been conducted, but most have been limited to physiological examinations.

In this study, to examine the possibility of using heated onion as a health-functional food, and to evaluate the effects of heating, its electron-donating ability to the DPPH radical, and capacities to scavenge hydroxyl, superoxide, hydrogen peroxide, and nitrite were evaluated in raw vs. heated onion.

Materials and Methods

Reagents 2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), xanthine, xanthine oxidase (XO) grade I from buttermilk (EC 1.1.3.22), nitro blue tetrazolium (NBT), hydrogen peroxide (H_2O_2), 2-deoxyribose, and ferrous sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sample preparation Onion (*Allium cepa*), purchased from Hamyang, Gyeongnam, Korea, was harvested on June 2006 and stored at -20°C. The onion was put into a sample bottle and sealed tightly. The sample bottle was then placed into the thermal processing instrument and heat treatment was performed using high-pressure steam (Jisico, Seoul, Korea) (9). The samples were heated to temperatures of 120, 130, and 140°C for 2 hr. The heated samples were juiced and centrifuged at 1,800×g for 10 min, and then the supernatant was filtered through a 0.45 μm syringe filter (Millipore, Billerica, MA, USA).

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Raw onions were put through the same process as heated onions, except for heating. The onion juice was kept at -20°C until analysis.

Solvent fractionation Heated onion juice was partitioned consecutively in a separating funnel using solvents of increasing polarity: *n*-hexane, chloroform, ethyl acetate, and butanol. The solvents were concentrated using a rotary evaporator (N-1000; Eyela, Tokyo, Japan) and then lyophilized. The dried residues of the 5 extracts were used for antioxidant activity analysis.

ABTS radical scavenging activity The ABTS radical cation scavenging activities of the extracts and ascorbic acid as a control were determined according to Roberta *et al.* (19), with some modifications. The ABTS radical cation scavenging activity was expressed as the AEAC in mg of ascorbic acid equivalents.

DPPH radical scavenging activity The scavenging activity for the DPPH radical was evaluated using the method of Yen and Chen (20), at a wavelength of 517 nm with a UV-visible spectrophotometer (DU-650; Beckman Coulter, Fullerton, CA, USA). The radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \{1-(A_{sample}/A_{blank})\} \times 100$$

Hydroxyl radical scavenging activity The scavenging activity for the hydroxyl radical was evaluated using the method of Halliwell *et al.* (21) at a wavelength of 520 nm with a UV-visible spectrophotometer. The radical scavenging activity was calculated using the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = \{1-(A_{bs}-A_{bo})/(A_{bc}-A_{bo})\} \times 100$$

where A_{bo} is the absorbance at 520 nm with no treatment, A_{bc} is the absorbance of the treated control at 520 nm, and A_{bs} is the absorbance of the treated sample at 520 nm.

Superoxide radical scavenging activity The scavenging activity for the superoxide radical was evaluated using the following equation at a wavelength of 560 nm according to the xanthine-xanthine oxidase method (22):

$$\text{Superoxide radical scavenging activity (\%)} = \{1-(A_{sample}/A_{blank})\} \times 100$$

Hydrogen peroxide scavenging activity The scavenging activity of the hydrogen peroxide (H_2O_2) radical from heated onion juice was evaluated using the method of Marklun and Marklun (23) at a wavelength of 405 nm with an enzyme-linked immunosorbent assay (ELISA; Sunrise Tecan Co. Ltd., Vienna, Austria). The radical activity was calculated using the following equation:

$$\text{Hydrogen peroxide scavenging activity (\%)} = \{1-(A_{sample}/A_{blank})\} \times 100$$

Nitrite scavenging activity The nitrite scavenging effect was evaluated using a UV-visible Spectrophotometer (DU 650; Beckman Coulter) at a wavelength of 520 nm, according to the method reported by Gray and Dugan (24). A blank was prepared by adding 0.4 mL distilled water instead of the Griess reagent. The nitrite scavenging effect was determined from the following equation:

$$\text{Nitrite scavenging activity (\%)} = \{1-(S_{Abs}-B_{Abs})/C_{Abs}\} \times 100$$

where S_{Abs} is the absorbance of the reaction mixture containing sample extracts, B_{Abs} is the absorbance of the sample blank without Griess Reagent, and C_{Abs} is the absorbance of the reaction mixture containing distilled water.

Statistical analysis The data were subjected to analysis of variance and expressed as mean ± standard deviation. Analysis of variance (ANOVA) and difference among samples were determined by Duncan's multiple range tests using the SPSS (version 11.5; SPSS Inc., Chicago, IL, USA). A level of $p<0.05$ was used as the criterion for statistical significance.

Results and Discussion

ABTS radical scavenging activity of various solvent fractions The antioxidant activities of the 5 solvent fractions from raw and heated onion juice are shown in Fig. 1. The ABTS radical scavenging activity of the ethyl acetate fraction was higher than that of the hexane, chloroform, butanol, and water fractions. Kim *et al.* (25) reported that among the various solvent fractions, the ethyl acetate fraction showed the strongest scavenging effect on the ABTS radical. Mokbel and Hashinaga (26) reported that antioxidative activities in various extracts have been observed, in particular with the ethyl acetate fraction which exhibits 93% inhibition of the peroxidation of linoleic acid.

It has been assumed that the compounds with antioxidant activities would be present in ethyl acetate layers, and the reason for the increased ABTS radical scavenging activity is the increase in temperature due to heat treatment which increases the polyphenol content. Based on these results, we further investigated the radical and nitrite scavenging effects in the ethyl acetate fraction.

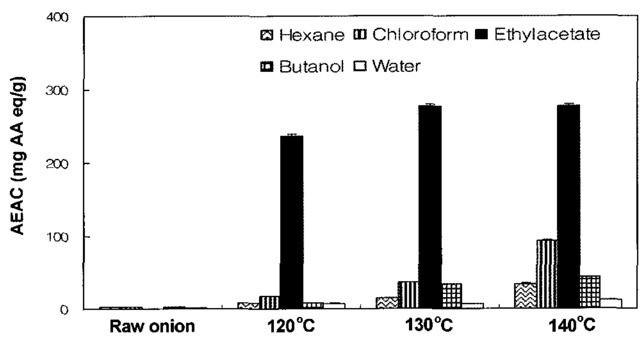

Fig. 1. ABTS radical scavenging activity of the solvent fractions from raw and heated onion juices. Results are expressed as the average of triplicate samples with mean±SD.

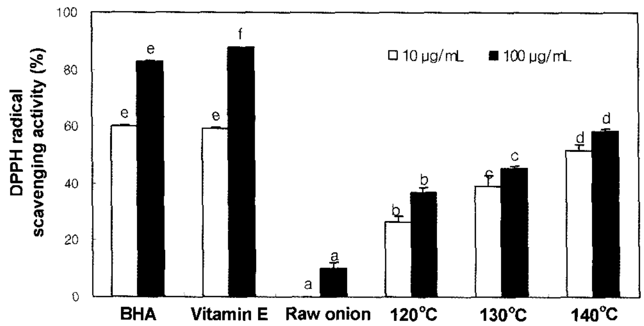

Fig. 2. DPPH radical scavenging activity of the ethyl acetate fraction from raw and heated onion juices. Results are expressed as the average of triplicate samples with mean±SD.

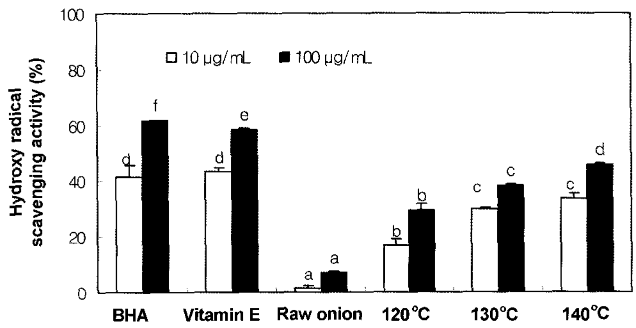

Fig. 3. Hydroxyl radical scavenging activity of the ethyl acetate fraction from raw and heated onion juices. Results are expressed as the average of triplicate samples with mean±SD.

DPPH radical scavenging activity The decrease in the absorbance of the DPPH radical caused by antioxidants is due to the scavenging of the radicals by hydrogen donation, causing a visible change from purple to colorless. Figure 2 shows the DPPH radical scavenging activities of raw and heated onion juice (120, 130, and 140°C for 2 hr) and compares them with the commercial antioxidants BHA and vitamin E. Raw onion showed no radical scavenging activity at a 10 g/mL, while 10% scavenging activity was seen at 100 g/mL. Heated onion juices at 100 g/mL showed high scavenging activities of 36% at 120°C, 45% at 130°C, and 58% at 140°C. It has been found that heated onion has higher DPPH radical scavenging activities than raw onion, and the activity increased with temperature. In a previous study (9), the polyphenol and flavonoid contents were found to increase during heating as the temperature increased, which is considered to be due to high molecular phenolic compounds and those phenolic compounds combined with proteins transformed into low molecular phenolic compounds by heat treatment. Velioglu *et al.* (27) reported strong relationships between antioxidant activity and total phenolic content in several fruits, vegetables, and grain products. Other research indicates that heating causes enhanced antioxidant activity in fruits and vegetables because of the enhancement of the antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products that have antioxidant activity (28, 29)

Hydroxyl radical scavenging activity The hydroxyl radical is an extremely reactive free radical formed in biological systems, and has been implicated as a highly damaging species in free radical pathology, capable of damaging the biomolecules of living cells (21). Figure 3 shows the hydroxyl radical scavenging activities of raw and heated onion juice (120, 130, and 140°C for 2 hr) and compares them with the commercial antioxidants BHA and vitamin E. Raw onion had no radical scavenging activity. Heated onion juices at 100 g/mL had high scavenging activities of 29% at 120°C, 38% at 130°C, and 45% at 140°C. It has been found that heated onion has higher hydroxyl radical scavenging activities than raw onion, and the activity increased with temperature. Yen and Hsieh (30) reported that xylose and lysine Maillard reaction products had scavenging activity toward the hydroxyl radical in a dose dependent manner, which might be attributed to the combined effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen. Borrellie *et al.* (31), Francisco and Salvio (32), and Jing and Kitts (33) reported that there exists a proportional relationship between the browning degree of melanodin, radical scavenging effect and antioxidation.

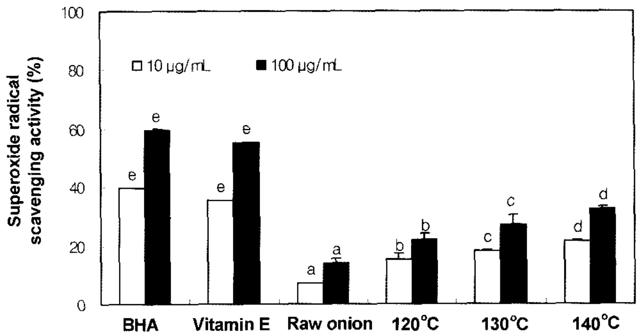

Fig. 4. Superoxide radical scavenging activity of the ethyl acetate fraction from raw and heated onion juices. Results are expressed as the average of triplicate samples with mean±SD.

Superoxide radical scavenging activity Xanthine/xanthine oxidase generates superoxide radicals that reduce tetrazolium blue into formazan blue (λ_{max}560 nm), but in presence of radical scavengers the formation of formazan blue is inhibited and therefore absorption at 560 nm decreases (23). Figure 4 shows the superoxide radical scavenging activities of raw and heated onion juice (120, 130, and 140°C for 2 hr) and compares them with the commercial antioxidants BHA and vitamin E. Raw onion had no radical scavenging activity at 10 g/mL, while 14% scavenging activity was seen at 100 g/mL. Heated onion juices at 100 g/mL had high scavenging activities of 21% at 120°C, 27% at 130°C, and 32% at 140°C. It has been found that heated onion had higher superoxide radical scavenging activities than raw onion, and the activity increased with temperature. Siddhuraju (34) reported that methanol and aqueous acetone extracts of dry heated seed coats showed the highest superoxide radical scavenging activities of 56.6 and 45.7%, respectively. Hence, the superoxide radical scavenging ability of the heated onion shown in this study suggests that heated onion has beneficial effects for decreasing the toxicity of superoxide radicals.

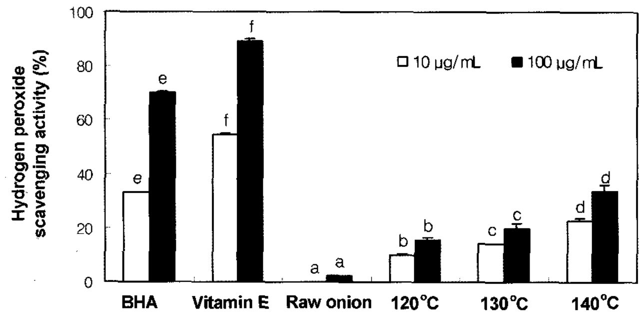

Fig. 5. Hydrogen peroxide scavenging activity of the ethyl acetate fraction from raw and heated onion juices.

Hydrogen peroxide scavenging activity Hydrogen peroxide (H_2O_2) can be formed *in vivo* by many oxidizing enzymes, particularly superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. H_2O_2 itself is not very reactive, however, it can be toxic to cells because it gives rise to hydroxyl radicals (35). Figure 5 shows the H_2O_2 radical scavenging activities of raw and heated onion juices (120, 130, and 140°C for 2 hr), and compares them with the commercial antioxidants BHA and vitamin E. Raw onion had no radical scavenging activity. Heated onion juices at 100 g/mL had high scavenging activities of 15% at 120°C, 19% at 130°C, and 28% at 140°C. It has been found that heated onion had higher H_2O_2 radical scavenging activity than raw onion, and the activity increased with temperature.

Nitrite scavenging activity RNS reacts with amines in protein-rich foods, medicines, and residual pesticides. They are also present in large quantities in pigments in meat and in both leaf and root vegetables (36). Nitrosamine converts to diazoalkane, protein, and intracellur components, which can increase the risk for cancer (36). Figure 6 shows the RNS scavenging activities of raw and heated onion juices (120, 130, and 140°C for 2 hr) and compares them with the commercial antioxidants BHA and vitamin E. Raw onion had no radical scavenging activity at 10 g/mL, while 8% scavenging activity was seen at 100 g/mL. Heated onion juices at 100 g/mL had high scavenging activities of 24% at 120°C, 28% at 130°C, and 34% at 140°C. It has been found that heated onion had higher

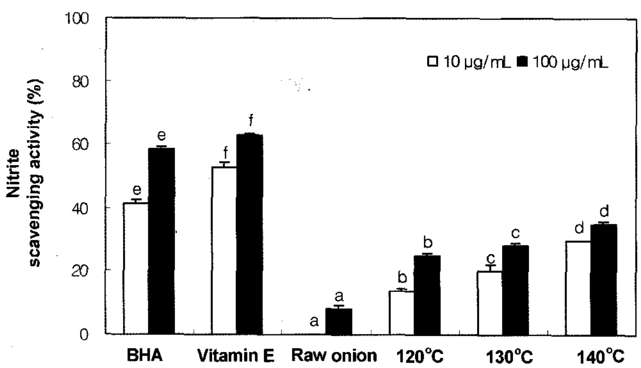

Fig. 6. Nitrite scavenging activity of the ethyl acetate fraction from raw and heated onion juices.

RNS scavenging activities than raw onion, and the activity increased with temperature. Woo *et al.* (9) report that the total polyphenol content of heated onions increased significantly with increased heating temperature and time. The highest total polyphenol content occurred after heating for 2 hr at 140°C and was 8 times greater than that in raw onion. Kang *et al.* (37) report that the total phenolic contents and MRP levels of heated ginseng were about 2.5 and 9.3 times increased. The DPPH-, •NO–, O_2–, ONOO–, •OH–, and AAPH-induced peroxyl radical scavenging activities of ginseng were significantly increased by heat processing. In summary, it is possible that the release of phenolic compounds and the browning degree of melanodin from onion after heat treatment could increase its antioxidant activity. Onion increases its physiologically activated materials after heating, and in this regard heated onion could be used as biological material for the manufacture of health foods and supplements. The present study suggests that heated onions are useful nutritional antioxidants for the nutraceutical industry. Further study is required to isolate and identify the antioxidant components within heated onion, and further determine their antioxidant activity *in vivo*.

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