Influence of Sulfur Fertilization on the Antioxidant Activities of Onion Juices Prepared by Thermal Treatment

Eunmi Koh¹ and Jeonghee Surh²

¹Department of Food and Nutrition, Seoul Women’s University, Seoul 01797, Korea
²Department of Food and Nutrition, Kangwon National University, Gangwon 25949, Korea

ABSTRACT: Two onions (Sulfur-1 and Sulfur-4) cultivated with different sulfur applications were thermally processed to elucidate the effects of heat treatment on browning index and antioxidant activity. Sulfur-4 onion had higher sulfur content compared with the Sulfur-1 onion. After thermal processing, browning intensity was different between the two onions juices, with lower values observed for Sulfur-4 onion juice. This suggests that sulfur inhibits the Maillard browning reaction. The total reducing capacity of the juices increased at higher thermal processing temperatures; however, it was also lower in the Sulfur-4 onion juice. This suggests that the heat treatment of onions enhanced their antioxidant activity, but the effect was offset in the Sulfur-4 onion juice presumably due to higher sulfur content. This study indicates that sulfur, a core element for the functionality of onions, can decrease the antioxidant activity of thermally processed onions because of its potential as a Maillard reaction inhibitor.

Keywords: onion, sulfur, thermal processing, antioxidant activity, browning

INTRODUCTION

Onions (Allium cepa L.) contain both phenolic compounds and sulfur-containing compounds such as thiosulfinates and thiosulfonates. These compounds are responsible for the characteristic flavor and aroma of onions. In addition, they have anti-inflammatory, anti-cholesterol, anticancer, and antioxidant properties (1). Therefore, their contents are critical to the taste and pungency of onions as well as to their health-enhancing properties. However, a strong and pungent flavor of sulfur-containing compounds makes people hesitate to consume large quantities of onions. Alternatively, onions are thermally processed into onion juice to meet daily consumption as a health supplement.

Earlier studies demonstrated that the content of sulfur-containing compounds in onions increased significantly with sulfur fertilization of the soil (2,3). Bystrická et al. (4) found a positive correlation between the sulfur content in the soil and the level of total polyphenolic compounds in onions. Choi and Surh (5) reported that onions cultivated with sulfur applications had about 2-fold higher values of total reducing capacity compared with onions grown without sulfur treatment. Further, Yun and Surh (6) found higher anti-inflammatory activity of onion juice prepared from sulfur-fertilized onions compared with sulfur-untreated onion juice. These results indicate that sulfur fertilization during cultivation leads to an increase of desirable bioactive compounds in onions.

It is well known that natural antioxidants occurring in foods are significantly degraded during heat treatment due to enzymatic or chemical oxidation of polyphenols. Recently, thermal treatments were reported to induce the formation of novel compounds with antioxidant properties and/or an increase in the amount of free polyphenolic compounds from covalently bound polymers, thereby resulting in improvement of antioxidant capacity. Reviews of Maillard reactions and antioxidant capacity of processed foods found that Maillard reaction products (MRPs) exhibit chain breaking and oxygen scavenging activities (7). MRPs formed during heat treatment are a particularly complex mixture of various compounds. High antioxidant capacity of brown products has been associated with the formation of brown melanoidin. Indeed, a prolonged heat treatment facilitated browning reactions and enhanced the antioxidant activity of tomato and coffee (8). Woo et al. (9) also demonstrated that the antioxidant activity of onion extracts increased with increasing heating temperatures (110∼150°C) and times (1∼5 h). The formation of MRPs was strongly affected by other compounds present in foods as well as processing conditions.
conditions (10). For example, the sulfur-containing amino acid such as cysteine suppressed the color formation during the Maillard reaction (10).

A number of studies have demonstrated the antioxidant capacity of fresh onions and processed onions. However, to the best of our knowledge, no data are available in the literature regarding the effect of heat treatment on onion juice prepared with sulfur-fertilized onions. Therefore, the objective of this study was to evaluate the effects of sulfur fertilization on the antioxidant activities of onion juice produced under five different thermal conditions. Thereby, we wanted to weigh the benefits and losses caused by sulfur fertilization of onion, particularly in terms of the antioxidant activity of onion juices that are thermally processed.

MATERIALS AND METHODS

Chemicals
Folin-Ciocalteu’s reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), xanthine, xanthine oxidase (from bovine milk), nitroblue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), and quercetin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Distilled and deionized water were used for the preparation of all solutions.

Preparation of samples
Onions and onion juices were provided from Samcheok Eco-friendly Onion Agricultural Cooperatives (Samcheok, Korea) in July and September 2012, respectively. The cultivation method for the two types of onions used, Sulphur-1 and Sulphur-4, was described in detail in the previous study (5). Briefly, the onions were grown in soil fertilized with sulfur (in the form of methylsulfonylmethyl anhydride, 3 kg/740 m²) before being harvested in June 2012. The Sulfur-1 and Sulfur-4 onions were washed 5 times using tap water without any chemical treatments. The washed onion bulbs, covered with a layer of tunic, were transferred into a fruit crusher (D&J Medical, Gwangmyeong, Korea) and then to a juicer connected to an automatic packing machine (Korea Invention Patent 0582248, D&J Medical). The onions were squeezed in the juicer after thermal treatment at one of the following conditions: 105°C for 5 h, 110°C for 5 h 10 min, 115°C for 5 h 20 min, 120°C for 4 h 30 min, or 125°C for 5 h 30 min. The onion juices were packaged in 100 mL polyethylene pouches and then stored at −20°C until analysis.

Elemental analysis
Contents of elements in the onions were measured with an elemental analyzer (Flash EA 1112, Thermo Finnigan LLC, San Jose, CA, USA), according to the manufacturer’s instructions for CHNS determination. The carbon, nitrogen and sulfur content, each detected as peaks of CO₂, N₂, and SO₂, were determined using 2,5-bis(5-tert-butyl-benzoxazol-2-yl)thiophene as a standard.

Browning index
Ten-fold dilutions of the thermally treated onion juices were measured at 420 nm (A420) and 520 nm (A520) using a spectrophotometer (UV-1650, Shimadzu Corporation, Kyoto, Japan). The browning index was expressed as A520/A420.

Total reducing capacity
Total reducing capacity was determined using Folin-Ciocalteu’s reagent that reacts with any reducing substances. One mL of onion juice (1/50) was added to 1.0 mL of diluted Folin-Ciocalteu’s reagent and 1.0 mL of saturated Na₂CO₃. After placement at room temperature for 1 h, the absorbance was measured at 700 nm. Total reducing capacity was expressed as quercetin equivalents.

Superoxide anion radical scavenging activity
The scavenging activity for superoxide anion radicals was measured using a xanthine/xanthine oxidase system coupled with the reduction of NBT. An aliquot of 100 μL of onion juice was mixed to 2.8 mL of reducing solution (250 μM xanthine in 0.1 mM EDTA-phosphate buffer pH 7.4 and NBT). After vortexing, the reaction was initiated by the addition of 200 μL of xanthine oxidase (0.1 unit/mL), and was allowed to stand in a water bath at 37°C for 40 min. The resulting NBT end product was measured at 550 nm. The activity was calculated as the % inhibition of the reduced NBT relative to the blank (as 100%). A solution of 0.148% (w/v) quercetin was used for comparison. The quercetin concentration was selected based on the content of flavonoids typically present in onion juices (0.04 to 0.15%) that are thermally processed (11).

DPPH radical scavenging activity
The free radical scavenging activity of onion juice was evaluated with the DPPH method. Five hundred μL of DPPH solution (0.2 mM in ethanol) was added to 200 μL of the onion juice supernatant, and was shaken vigorously for 5 s. The absorbance was monitored at 525 nm every 5 min during the reaction at room temperature in the dark until no noticeable change in the absorbance was observed. The activity was calculated as the % decrease of the absorbance at 525 nm relative to the blank (as 100%). A solution of 0.148% (w/v) quercetin was used for comparison.
Statistical analysis
All experiments were conducted with more than three replicates, and results were expressed as the averages and standard deviations. Analysis of variance (ANOVA) and Duncan’s multiple range tests were performed using the SAS program for Windows (SAS 9.1, SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Chemical composition of Sulfur-1 and Sulfur-4 onions
The chemical composition of the two onion samples is presented in Table 1. Sulfur-4 onion had significantly higher (P<0.01) nitrogen content (that is an indicator of protein and nitrogenous compounds) compared with Sulfur-1 onion, while two onions were not significant different (P=0.05) in carbon content (an indicator of organic matter). Sulfur content was also higher in the Sulfur-4 onion than the Sulfur-1 onion (Table 1). This is in agreement with the results of Bloem et al. (2), demonstrating that the application of sulfur fertilizer significantly increased the sulfur content of onions. In comparison, the total reducing capacity was not significantly different between the two onions (P>0.05). This is opposite to the results of Bystrická et al. (4) who reported that higher sulfur content in soil increased the level of total phenols in onions. These results indicate that the effects of sulfur fertilization on sulfur content and antioxidant activity of onion vary, depending on other factors such as sulfur type, dose, and timing.

Table 1. Comparison of chemical compositions and total reducing capacity of Sulfur-1 and Sulfur-4 onions

| Onions     | Sulfur-1 | Sulfur-4 | P-value |
|------------|----------|----------|---------|
| Carbon (% of dry matter) | 38.03±0.15 | 40.37±5.64 | 0.332   |
| Nitrogen (% of dry matter) | 1.47±0.14  | 1.88±0.21  | 0.003   |
| Sulfur (% of dry matter) | 0.27±0.12  | 0.43±0.13  | 0.053   |
| Total reducing capacity (PQ µg/g)a | 57.07±6.30 | 63.23±9.99 | 0.419   |

Values are expressed as mean±standard deviation of six replicates.
aValue was cited from Choi and Surh (5). Value for the control onion which was neither fertilized nor sprayed with sulfur was 30.50±4.87 µg/g.
aQuercetin equivalents.

Browning index of onion juices
The browning index of the onion juices showed that colored compounds were formed to a greater extent in the Sulfur-1 onion juices than in the Sulfur-4 juices (Table 2). Huang et al. (10) demonstrated that the color formation was markedly suppressed by the sulfur-containing amino acids such as cysteine during the Maillard reaction. In this study, the content of nitrogen, a core element in the reactants involved in non-enzymatic browning reactions, was higher in the Sulfur-4 onions (Table 1). Our previous study (5) found that the proportion of cysteine among the total sulfur-containing amino acids was higher in the Sulfur-4 onion compared with the Sulfur-1 onion, which were the same onion samples processed in the present study. The brownish color indicates the formation of colored compounds through the Maillard reaction and/or caramelization. The lower browning index of the Sulfur-4 onion juices (Table 2) indicates the inhibition of color formation, possibly mediated by sulfur compounds. At initial stage of Maillard reaction, the carbonyl group of sugar reacts with the amino group of amino acid, so that produces N-substituted glycosylamine. It is likely that sulfur combines either with the aldehyde group of reducing sugars or with the decomposition products of a glycosylamine, thereby resulting in the inhibition of melanoidin formation in its final stage. This type of sulfur effect is frequently observed in the Maillard reaction, but not much in caramelization (10). For these reasons, the lack of difference in the two onion juices processed at 120°C (Table 2) can be attrib-

Table 2. Effect of thermal processing on browning index, total reducing capacity, and superoxide anion radical scavenging activity of onion juices

| Heating condition | Browning index (A_{520}/A_{420}) | Total reducing capacity (PQ µg/mL)a | Superoxide anion radical scavenging activity (%)a |
|-------------------|----------------------------------|------------------------------------|-----------------------------------------------|
|                   | Sulfur-1 | Sulfur-4 | P-value | Sulfur-1 | Sulfur-4 | P-value | Sulfur-1 | Sulfur-4 | P-value |
| 105°C, 5 h        | 0.35±0.06 | 0.11±0.05 | <0.01   | 556±52a | 396±32a | <0.05   | 49.8±2.7a | 21.8±5.5 | <0.01   |
| 110°C, 5 h 10 min| 0.34±0.00 | 0.20±0.01 | <0.001  | 899±126a | 464±30a | <0.01   | 30.2±0.4a | 21.2±1.6 | <0.001  |
| 115°C, 5 h 20 min| 0.32±0.00 | 0.24±0.04 | <0.05   | 804±77a | 506±46a | <0.05   | 32.9±4.7b | 17.6±2.3 | <0.01   |
| 120°C, 4 h 30 min| 0.33±0.00 | 0.23±0.07 NS |        | 704±15a | 46±15b | <0.001  | 15.6±3.0a | 11.0±4.9 NS |
| 120°C, 5 h 30 min| 0.31±0.01 | 0.29±0.14 NS |        | 745±12a | 498±44a | <0.01   | 26.3±6.6a | 17.1±6.5 NS |<0.001 NS |
| P-value           | NS      | NS       |         | <0.05   | <0.05   |         | <0.01   | NS      |         |

Values are expressed as mean±standard deviation of triplicate experiments.
Values with different letters (a-c) in the same column are significantly different.
NS: not significant.
aQuercetin equivalents.
aThe activity of 0.148% quercetin used for comparison was 16.52%.
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Fig. 1. Effect of thermal processing on the kinetics of DPPH radical scavenging activity of onion juices prepared with Sulfur-1 (A) or Sulfur-4 (B) onions. Onions were heated at 105°C for 5 h, 115°C for 5 h 20 min, or 120°C for 5 h 30 min. Quercetin (0.148%) scavenged 91% of the DPPH radical within 10 min, reaching a plateau under the identical experimental condition.

Antioxidant activities of onion juices
Superoxide anion radical scavenging activity was the highest in the onion juice processed at 105°C, which indicates that superoxide scavengers such as phenolic compounds were higher than those in the other onion juices (Table 2). On the contrary, total reducing capacity, which measures reducing substances including phenolics and non-phenolics, was the lowest at 105°C and appreciably increased at 110°C or higher (Table 2). This indicates that considerable amounts of non-phenolics rather than phenolics might have been generated from a browning reaction (e.g., dicarbonyl compounds, reductones, and melanoidin, etc.), which contributed to the significant increase in the total reducing capacity, but not much to the superoxide scavenging activity. MRPs have been known to have strong antioxidant properties (7). Furthermore, they have effective synergy in combination with phenolic antioxidants. Antioxidant activity of the onion juices was also measured by DPPH radical-scavenging activity assay as a function of incubation time between the onion juice and DPPH radicals (Fig. 1). The onion juices quickly reached the maximum DPPH radical scavenging activity with an increasing incubation time. The maximum DPPH radical scavenging activity was faster at a higher temperature. As the temperature increased from 105°C to 120°C, the time to reach the maximum activity (i.e., plateau) decreased from 195 min to 160 min for Sulfur-1 onion juice (Fig. 1A) and 70 min to 60 min for Sulfur-4 juice (Fig. 1B). In particular, the Sulfur-1 onion juice showed a proportional increase in the maximum DPPH radical scavenging activity from 78% to 85% as the heating temperature increased from 105°C to 120°C (Fig. 1A), while the Sulfur-4 onion juice showed an opposite trend (93% at 105°C decreased to 78% at 120°C; Fig. 1B). At the relatively lower temperature of 105°C, the Sulfur-4 onion juice showed much higher maximum activity (93%) than the Sulfur-1 juice (78%). However, this difference reduced with the increasing temperature, eventually being

Total reducing capacity of onion juices
The total reducing capacity was the lowest in the onion juices processed at 105°C, the mildest condition among the thermal processing procedures employed in this study. Thermal processing at higher temperatures of 110～120°C increased the total reducing capacity (Table 2). It is well established that heat treatment increases the extractability and bioavailability of bioactive compounds via the disruption of cell wall and the hydrolysis of glycosidic bonds (9). Furthermore, heat treatment of vegetables induces the formation of antioxidants through the Maillard reaction (7,9). Nevertheless, there was no further increase in the total reducing capacity by a temperature increase from 110°C to 120°C. It could be linked to the fact that there was no significant difference in the browning index of the onion juices at the temperature ranges (Table 2), indicating that the contents of MRPs at the temperatures were not sufficiently different to induce a significant increase in the total reducing capacity. Meanwhile, it was remarkable that the total reducing capacity was significantly higher in the Sulfur-1 onion juices than the Sulfur-4 juices (Table 2), although there was no difference in the values of total reducing capacity between two onions before thermal processing (Table 1). This is presumably related to the lower content of sulfur in the Sulfur-1 onion than its counterpart (Table 1), thus resulting in a lesser inhibition of browning reaction by sulfur during the thermal processing and subsequently higher content of MRPs in the Sulfur-1 juices.
reversed at 120°C with the activity being 85% and 78% for the Sulfur-1 and Sulfur-4 onion juices, respectively. This indicates that the heat treatment of onions enhanced their antioxidant activities and the effect of thermal processing was offset in the Sulfur-4 onion juice.

This study showed that the heat treatment of onion induced an increase in its antioxidant activities, which was presumably due to the formation of MRPs that have antioxidant activity, and/or the enhanced activity of other antioxidants. Most notably, sulfur that inhibits the Maillard browning reaction appeared to have offset the antioxidant activity of thermally processed onion juices. This study indicates that sulfur enhances the functionality of onions and simultaneously decreases the antioxidant activity of thermally processed onions because of its potential as a Maillard reaction inhibitor.

**AUTHOR DISCLOSURE STATEMENT**

The authors declare no conflict of interest.

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