JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Impact of Food Disinfection on Beneficial Biothiol Contents in Strawberry

Omca Demirkol,*,† Arzu Cagri-Mehmetoglu,† Zhimin Qiang,‡ Nuran Ercal,§ and Craig Adams||

Muhendislik Fakultesi, Gıda Muhendisligi Bolumu, Sakarya Universitesi, Esentepe, Sakarya, Turkey, Research Center for Eco-Environmental Sciences (RCEES), Chinese Academy of Sciences, Beijing 100085, China, Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri 65409, and CEAE Department, University of Kansas, Lawrence, Kansas 66045

In this study, the impact of four food disinfectants including hydrogen peroxide, free chlorine, and gaseous- and aqueous-phase ozone with industrial doses on the concentration of biothiol compounds $\gamma$-glutamylcysteinylglycine (GSH) and cystein (CYS) in strawberry was investigated for 1, 5, 15, 30, and 60 or 120 min. Additionally, the amount of oxidized glutathione (GSSG) was analyzed for calculation of the GSH/GSSG ratio as an indicator of oxidative stress. After this treatment, thiol contents of strawberry samples were examined using high-performance liquid chromatography (HPLC) technique. According to the results of measurements, free chlorine treatment for only 60 min significantly decreased CYS content in strawberry ($p < 0.05$). A significant decline in the GSH/GSSG ratio was also observed when $H_2O_2$ was applied for all time intervals except for 1 min ($p < 0.05$). However, aqueous-phase ozone treatment did not significantly affect the thiol levels ($p < 0.05$). In conclusion, this study may provide optimum disinfection methods for strawberry to minimize loss of beneficial biothiols.

**KEYWORDS: Biothiol; antioxidant; chlorine; hydrogen peroxide; ozone; disinfection; strawberry**

## INTRODUCTION

The increasing number of food-borne disease outbreaks associated with fruits and vegetables in recent years has promoted disinfection usage in processing and preserving various food products (*1*−*4*). Disinfectants are antimicrobial agents that are applied to eliminate pathogenic organisms (i.e., viruses, bacteria, and protozoa) to prevent food-borne diseases as well as used to control the growth of fungi to increase shelf life. Ozone (either gaseous- or aqueous-phase), free chlorine ($HOCl/OCl^-$), and hydrogen peroxide ($H_2O_2$) are the disinfectants most commonly studied and/or used in the food industry. The U.S. government has recently approved ozone as an antimicrobial agent on food (21 CFR Part 173.368) (*5*). Dissolved ozone, ozone gas stream, or its products including hydroxyl radicals has a great disinfection effect on microorganisms by direct physical contact. Ozone with desired properties such as high reactivity, good penetrability, and spontaneous decomposition to nontoxic oxygen gas was stated as a very demandable food disinfectant (*6*−*8*). Either gaseous- or aqueous-phase ozone has been tested against the growth of some pathogenic bacteria (e.g., *Salmonella* species, *Escherichia coli* O157:H7, and *Listeria monocytogenes*) in lettuce (*9*), whole black peppercorn and ground black pepper (*10*), apple cider and orange juice (*11*), apples, strawberries, and cantaloupe (*12, 13*), cucumbers (*14*), cabbage (*15*), and beef (*16, 17*), as well as to stop the growth of mold and yeasts to increase the shelf life of some fruits (*18*).

Free chlorine has been applied in three forms including pressurized gas, as hypochlorous acid, or as hypochlorite. Sodium or calcium hypochlorite is the most commonly used, since it is safe and easy to use. The dissociation of hypochlorous acid (HOCl) to hypochlorite ($OCl^-$) ($pK_a = 7.6$ at 20 °C) reduces disinfection effectiveness because the relative bacterial inactivation efficiency of HOCl is about 40−80 times that of $OCl^-$ (*19*). Chlorine has been widely applied in fresh fruits and vegetables during harvest or postharvest for a long time due to its many advantages including its effectiveness and being chemically steady, readily accessible, comparatively cheap, and simply applied. For example, chlorine has been routinely used for disinfection of vegetables such as lettuce, carrots, and spinach (*12, 20, 21*), fruits such as strawberries, apples, cantaloupe, honeydew melons, and tomatoes (*22*−*26*), chicken (*27*), and fish (*28*). A recent development in chlorine disinfection is the production of chlorine water through electrolysis (*29, 30*).

Hydrogen peroxide, the third popular disinfectant, kills bacteria directly or indirectly through other cytotoxic oxidizing species such as hydroxyl radicals. Hydrogen peroxide may also

* To whom correspondence should be addressed. Telephone: 90 264 229 3009. Fax: 90 264 295 5601. E-mail: omcad@sakarya.edu.tr.
† Sakarya Universitesi.
‡ Chinese Academy of Sciences.
§ Missouri University of Science and Technology.
|| University of Kansas.

10.1021/jf802209t CCC: $40.75 
Published on Web 10/18/2008

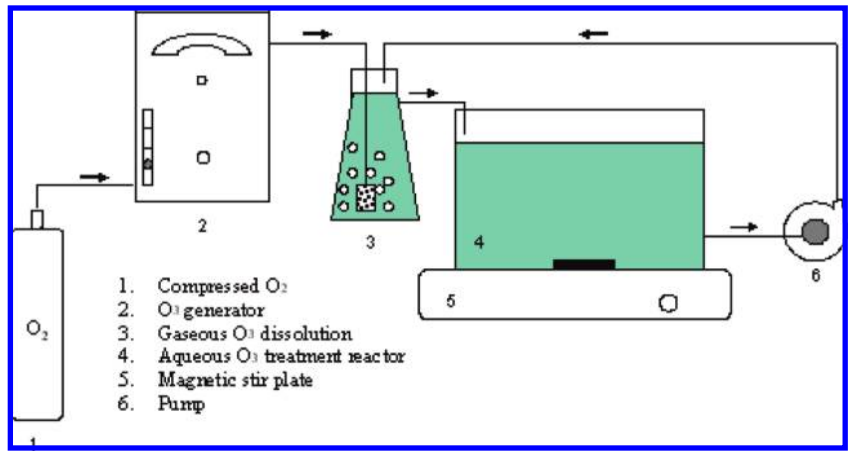


**Figure 1.** Schematic diagram of the aqueous-phase treatment system.

be produced in bacteriological media by exposure to light or oxygen, thus becoming an important toxic agent for bacteria (*31*). The sporicidal activity of hydrogen peroxide coupled with fast breakdown to water and oxygen makes it an attractive disinfectant for use on some food contact surfaces (*32*). Hydrogen peroxide has several applications in disinfecting vegetables, fruits (*33*−*35*), and poultry (*36*).

However, application of ozone, free chlorine, or hydrogen peroxide to the surface of foods may also diminish the level of some nutrients (e.g., antioxidants). Hydrogen peroxide could damage plant cells isolated from tomato, cucumber, and soybean through oxidation of cell wall polysaccharides (*37*). When γ-glutamylcysteinylglycine (GSH) in aqueous solution reacted with hydrogen peroxide, most of the cysteine contained in the GSH was oxidized to the monoxide or dioxide, and some cysteine was even directly oxidized to sulfinic acid (*38*). Similarly, ozone treatment could also result in a 40% decreased emission of volatile esters in postharvest strawberries (*13*) and a 16−25% decrease of ascorbic acid content in potatoes, carrots, and cabbage (*39*). It is reasonably supposed, therefore, that some biologically important antioxidants (e.g., biothiols) contained in vegetables or fruits may decompose upon exposure to disinfectants.

Strawberries contain varied concentrations of important biothiols with strong antioxidant properties. Biothiols are a type of mercaptan having a sulfhydryl functional group and are among the most important antioxidants that protect human cells against oxidative damage which leads potentially to cancers, Alzheimer's disease, and other maladies (*40, 41*). These important biothiols include GSH and cysteine (CYS). Our previous work showed that the common disinfection technologies applying hydrogen peroxide, free chlorine, and gaseous- or aqueous-phase ozone may result in significant loss of beneficial biothiols including GSH, *N*-acetyl-L-cysteine (NAC), L-glutamyl-L-cysteine (GGC), and CYS in spinach, red pepper, and cucumber (*42*). The GSH and CYS contents were ranged from 39 to 59 nmol/g (wet weight), respectively, in a variety of strawberry (*43*). The purpose of this study was to examine the impact of ozone (gaseous- and aqueous-phase) and other disinfectants (including free chlorine and hydrogen peroxide) with treatment times (1, 5, 30, 60, or 120 min) on the bulk concentrations of GSH or CYS in strawberry samples. The level of oxidized glutathione (GSSG) was also examined prior to and after disinfection as an indicator of oxidative stress in terms of the ratio of GSH to GSSG. Strawberry was selected for this study because it has relatively high levels of biothiols as determined in our previous work (*43*)

## MATERIALS AND METHODS

**Reagents and Chemicals.** HPLC grade acetonitrile, acetic acid, and *o*-phosphoric acid and certified ACS grade boric acid, hydrochloric acid, sodium hydroxide, sodium phosphate (99.9%), and hydrogen peroxide (ca. 30 wt %) were purchased from Fisher Scientific (Fairlawn, NJ). GSH, CYS, GSSG, *N*-(1-pyrenylmaleimide) (NPM), L-serine, trizma hydrochloride, diethylenetriaminepentaacetic acid (DETAPAC), potassium indigo trisulfonate, potassium permanganate (99.3%), and sodium hypochlorite (>4 wt %) were purchased from either Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). Sodium oxalate was obtained from Acros Organics (Fairlawn, NJ) as a primary standard to titrate the concentration of potassium permanganate. Milli-Q (MQ) water with a resistivity > 18.2 M·cm was produced by a Simplicity 185 water purification system (Millipore Co., Bedford, MA) to prepare HPLC mobile phases and biothiol standard solutions.

Strawberries were obtained from a local grocery store in Rolla, MO. All samples from this supplier were simply rinsed three times with tap water for cleaning purposes, and none were treated by any disinfectant. In our reaction systems, strawberries were treated whole and then cut up into about 0.5 g pieces for biothiol analysis.

**Reaction Systems.** Batch reactions were conducted for free chlorine and hydrogen peroxide in a large glass jar reactor containing 10 L of oxidant solution as a reaction reservoir. The oxidant concentration was maintained approximately constant (i.e., 515−485 mg/L free chlorine, 5.10−4.90% $H_2O_2$) throughout the course of reaction. Strawberries were soaked in the oxidant solution with a porous ceramic plate (nonreactive toward the oxidants) sitting on top to maintain submersion of the strawberries. The working solutions of free chlorine and hydrogen peroxide were prepared by diluting the purchased sodium hypochlorite (>4 wt %) and hydrogen peroxide (ca. 30 wt %) solutions with distilled water to reach concentrations of 500 ± 15 mg/L and 5.0 ± 0.10% (by weight), respectively, with the pH of both solutions adjusted to 7.6.

Semibatch reactions were conducted for aqueous-phase ozone, whose setup is illustrated in **Figure 1**. An ozone gas stream was produced from compressed pure oxygen by a GLS-1 ozone generator (PCI-WEDECO Environmental Technologies, West Caldwell, NJ). The ozone stream was bubbled through a stone diffuser into a glass bottle containing 4 L of buffered MQ water (5 mM sodium phosphate, adjusted to pH 7.6). The dissolved ozone solution then circulated into the 10 L reactor through an overflow outlet on one side of the glass bottle. A pump was utilized to continuously circulate the working solution between the 4 L bottle and the 10 L reactor. The system was allowed to equilibrate and stabilize for 20 min to reach a steady-state concentration of dissolved ozone before the strawberry samples were placed into the reactor.

Continuous reactions were carried out for gaseous-phase ozone, whose setup is illustrated in **Figure 2**. A small ozone generator (AquaZone 50 mg/h, Red Sea Fish Pharm Ltd., Houston, TX) was used to generate a low-concentration ozone gas stream (i.e., 40 ppm) from compressed air. The flow rate of the compressed air was maintained at 5.0 standard liters per minute (SLPM) with a mass flow controller (2900

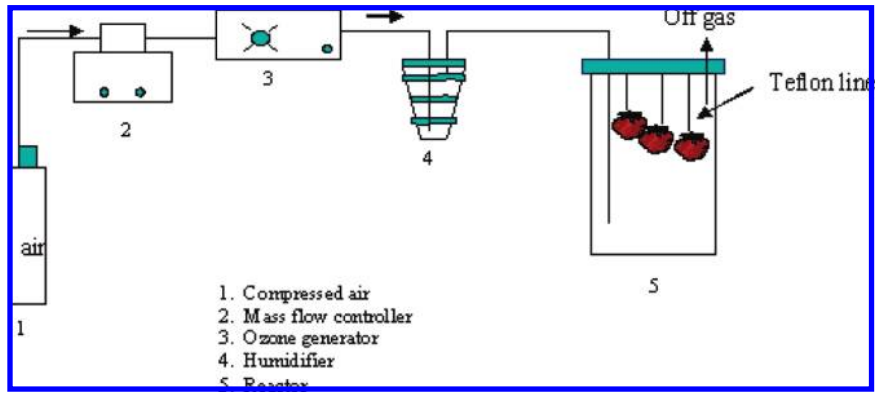


**Figure 2.** Schematic diagram of the gaseous-phase treatment system.

Series, Tylan General, Inc., Austin, TX). The ozone gas stream then passed through a humidifier containing about 50 mL of MQ water to get saturated with water. Saturated humidity not only minimizes the gas-stripping loss of water originally contained in the strawberries but also enhances the efficiency of bacterial inactivation. It was reported that the inactivation rates of *Bacillus* spores by gaseous-phase ozone increased with increasing exposure humidity (*44*). Strawberries were hung in a 5 L glass reactor with Teflon lines (nonreactive with ozone) to ensure sufficient contact of the strawberry surface with ozone gas.

Common oxidant dosages and contact times were employed in this work. Specifically, the dosages of free chlorine, hydrogen peroxide, and aqueous-phase ozone were 500 ± 15 mg/L, 5.0 ± 0.10% (by weight), and 8.0 ± 2.0 mg/L, respectively, with a contact time of 1, 5, 30, 60, or 120 min at pH 7.6 for each disinfectant. The working solution was gently mixed with a magnetic stir plate to eliminate an oxidant concentration gradient toward the strawberry surface. For gaseous-phase ozone, a concentration of 40 ppm and a contact time of 1, 5, 30, 60, or 120 min were utilized. Chemical analysis confirmed that the oxidant concentration remained nearly constant throughout the course of reaction.

**Oxidant Analysis.** The total chlorine concentration was determined using Hach DPD Method 8167 with a DR/2010 portable spectrophotometer (Hach Co., Loveland, CO) after appropriate dilution. The hydrogen peroxide concentration was titrated with potassium permanganate, whose concentration was first standardized by a primary standard, sodium oxalate. The aqueous ozone concentration was measured by an indigo colorimetric method (*45*) with a Cary 50 Conc spectrophotometer (Varian Australia Pty Ltd., Australia). The small ozone generator was standardized by a modified indigo colorimetric method which is suitable for determination of the ozone concentration in the gas phase by utilizing a humidifier. Specifically, 200 mL of an indigo solution (0.12 mM) was added to the humidifier. An ozone gas stream was allowed to pass through the humidifier for 2 min, where ozone reacted rapidly with the indigo solution. The molar absorptivity of indigo decreased by 20 000 $M^{-1}\cdot cm^{-1}$ at 600 nm with respect to each mole of ozone consumed (*45*). The gaseous ozone concentration was then determined on the basis of the decrease of the indigo absorbance.

**HPLC System.** A Finnigan high-performance liquid chromatography (HPLC) system (Thermo Electron Co., San Jose, CA), which consisted of a vacuum membrane degasser, an injection valve with a 5 μL injection filling loop, two gradient pumps, an autosampler, and a fluorescence detector, was used to determine the concentrations of biothiols and GSSG. The fluorescence detector was operated at an excitation wavelength of 330 nm and an emission wavelength of 376 nm. A reversed-phase Reliasil ODS-1 $C_{18}$ column (5 μm, 250 × 4.6 mm) (Column Engineering, Ontario, CA) was utilized for biothiol separation. The mobile phase consisted of 70% acetonitrile and 30% MQ water and was adjusted to approximately pH 2.5 by addition of 1 mL of acetic acid and 1 mL of *o*-phosphoric acid per liter of mobile phase. Prior to use, the mobile phase was vacuumed under sonication for 30 min to drive out dissolved gas bubbles.

**Biothiol Analysis.** Because our preliminary work showed that the biothiol contents for a batch of strawberry may vary significantly from one sample to another, 15 strawberries were analyzed in replicates for both control (untreated) and disinfectant-treated samples. Specifically, samples were prepared in five replicates, with each replicate containing a mix of three cutlets (about 0.5 g per piece) cut from individual whole treated strawberries. A cutlet represented a piece of strawberry cut from the central part of a strawberry body. A cutlet was cut across the whole central body. As a result, the analyzed biothiol level represented the level over the whole body of strawberry. The three-cutlet mix was placed in a serine borate buffer (SBB) to prevent potential oxidation of biothiols by atmospheric oxygen. The SSB buffer comprised 100 mM Tris-HCl, 10 mM borate, 5 mM serine, and 1 mM DETAPAC with the final pH adjusted to 7.0 with concentrated NaOH solution. The samples were homogenized in the SBB buffer with a Tissue-Tearor instrument (Biospec Products, Inc., Bartlesville, OK) on ice for 2 min and centrifuged at 10 000*g* for 15 min at a controlled temperature of 4 °C to extract biothiols out of the vegetables. Thereafter, 40 μL of supernatant was withdrawn and derivatized with *N*-(1-pyrenylmaleimide) (NPM), which reacts with free sulfhydryl groups of the biothiols to form fluorescent derivatives. Each sample was diluted with 210 μL of MQ water and derivatized with 750 μL of NPM (1 mM in acetonitrile). The resulting solution was vigorously mixed and allowed to react at ambient temperature for 5 min. HCl solution (10 μL, 2 M) was then added inside to stop the reaction. After being filtered through a 0.20 μm nylon filter (Advantec MFS, Inc., Dublin, CA) using a 3 mL syringe, the derivatized samples were injected onto the HPLC system.

The biothiol-NPM derivatives were separately eluted from the HPLC column with the mobile phase flowing isocratically at a rate of 1 mL/min. GSH and CYS could be determined concurrently, since all these biothiols form fluorescent derivatives with NPM. The biothiol-NPM peaks were quantified with Thermo LC software (Thermo Electron Co., San Jose, CA). The linearity of standard calibration curves was confirmed over a concentration range of 0−10 μM for each biothiol in a mixture.

**GSSG Analysis.** GSSG, the primary oxidation product of GSH, was determined by reacting 84 μL of strawberry supernatant with 16 μL of 2-vinylpyridine (6.25% in ethanol) for 1 h to block any pre-existing GSH. After the reaction, 95 μL of an NADPH solution (2 mg/mL) and 5 μL of a glutathione reductase solution (2 units/mL) were added sequentially. An aliquot of 100 μL of the resulting solution was quickly withdrawn and mixed with 150 μL of MQ water and 750 μL of NPM (1 mM in acetonitrile) for derivatization. After 5 min, the reaction was stopped by adding 5 μL of HCl solution (2 M). The samples were then filtered through a 0.20 μm nylon filter and injected onto the HPLC system.

**Statistical Analysis.** The values represent mean ± standard deviation (SD) of five replicated samples. The one-way analysis of variance (ANOVA) test was used to analyze the data from the experimental and control groups, with $p$ values of $<0.05$ considered to be significant.

## RESULTS

**Gaseous-Phase Ozone.** The results of the study indicated that gaseous-phase ozone treatment did not significantly affect

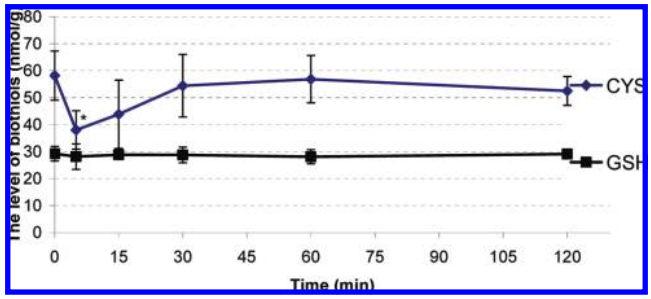


**Figure 3.** CYS and GSH levels after gaseous-phase ozone treatment. Values are mean ± SD. (*) Significantly different when compared with control ($p < 0.05$). ($n = 5$/group.)

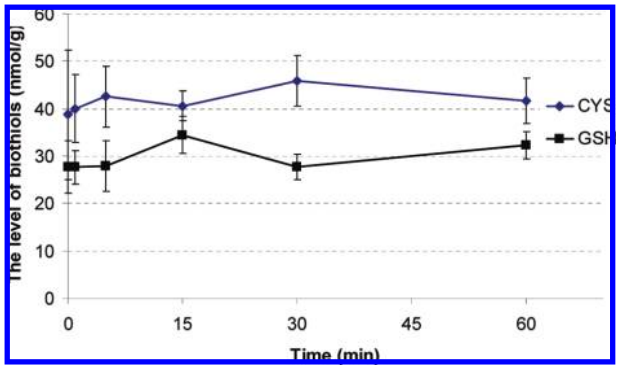


**Figure 4.** CYS and GSH levels after aqueous-phase ozone treatment. Values are mean ± SD. (*) Significantly different when compared with control ($p < 0.05$). ($n = 5$/group.)

the level of CYS in strawberry for 15, 30, 60, and 120 min, except for 5 min (**Figure 3**) ($p < 0.05$). The response attribute of CYS toward gaseous-phase ozone application for different time durations drew an unusual pattern. In fact, although the CYS level dropped significantly by 35% after 5 min treatment, it started to elevate again with increasing exposure time. The amount of CYS in strawberry decreased approximately by 24% by 15 min, 6% by 30 min, 2% by 60 min, and 9% by 120 min of exposure of gaseous-phase ozone compared to the control (no ozone treatment). On the other hand, unlike CYS, the GSH level was not statistically influenced by gaseous-phase ozone treatment for all tested time intervals (**Figure 3**) ($p < 0.05$). Similarly, the ratio of GSH to GSSG (reduced glutathione to oxidized glutathione), an oxidative stress parameter, was not statistically affected in strawberry samples (**Figure 7**).

**Aqueous-Phase Ozone.** The treatment of liquid ozone could not significantly reduce the concentrations of GSH and CYS in strawberry samples at any time intervals (**Figure 4**) ($p < 0.05$). However, 15 and 60 min exposure to liquid ozone insignificantly increased the GSH level in strawberries from 27.7 (the control) to 34.4 and 32.3 nmol/g ($p = 0.057$ and 0.13), respectively. Similarly, the concentration of CYS in strawberry samples was insignificantly raised with all tested treatment times. A maximum increase was observed from 37.5 (the control) to 45.84 nmol/g at 30 min ($p = 0.38$). The results also showed that 15 min liquid ozone treatment increased the ratio of GSH to GSSG by approximately 7.6% (statistically insignificant, $p = 0.12$) (**Figure 7**).

**$H_2O_2$.** The CYS level in strawberry samples was not statistically influenced by $H_2O_2$ treatment at any of the applied time durations (**Figure 5**) ($p < 0.05$). However, the GSH level statistically declined from 28.6 (the control) to 22.8 nmol/g (20%) only with 5 min exposure of $H_2O_2$ ($p < 0.05$). The amount of GSH was insignificantly reduced by approximately 12, 11, and 2% with the application of $H_2O_2$ to strawberry

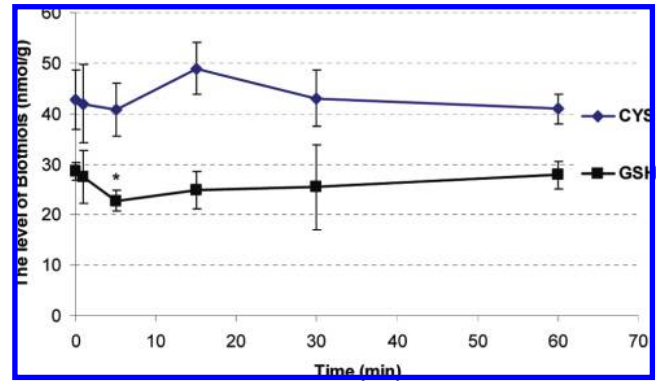


**Figure 5.** CYS and GSH levels after $H_2O_2$ treatment. Values are mean ± SD. (*) Significantly different when compared with control ($p < 0.05$). ($n = 5$/group.)

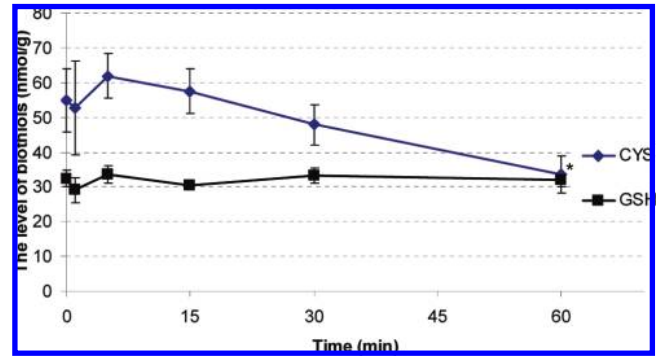


**Figure 6.** CYS and GSH levels after free chlorine treatment. Values are mean ± SD. (*) Significantly different when compared with control ($p < 0.05$). ($n = 5$/group.)

samples for 15, 30, and 60 min, respectively, compared to the control. Results further showed that the mean GSH/GSSG ratio decreased significantly from 11.9 (the control) to 7.3, 7.2, 6.8, and 7.4 with application of hydrogen peroxide for 5, 15, 30, and 60 min, respectively; however, 1 min treatment had no significant effect on the ratio (**Figure 7**) ($p < 0.05$).

**Free Chlorine.** The results also showed that the amount of CYS in strawberries insignificantly decreased with the application of free chlorine by 4% at 1 min and 12% at 30 min (**Figure 6**). At the 5 min exposure of chlorine, an insignificant slight increase (12%) was observed ($p < 0.05$). Nevertheless, only 60 min free chlorine treatment could result in a statistically significant decline in the level of CYS in strawberry ($p < 0.05$).

Unlike the case of CYS, the concentration of GSH or the ratio of GSH to GSSG in strawberries was not significantly affected by chlorine treatment at any examined time period (**Figures 6** and **7**) ($p < 0.05$). The GSH level was reduced only by 10, 6, and 1.7% with 1, 15, and 60 min free chlorine treatment, respectively, compared to the control ($p < 0.05$), which was statistically insignificant. In contrast, although also insignificant statistically, the GSH level increased by 3 and 2% with 5 and 30 min treatment, respectively ($p < 0.05$).

## DISCUSSION

Glutathione is an essential antioxidant for the detoxification of reactive oxygen substances (*46*). Cystein, one of the biothiols, is used for synthesis of GSH in organisms. The synthesis of GSH and the protective effect of GSH against diseases (protein malnutrition, adult respiratory distress syndrome, and AIDS) were particularly promoted under various nutrition and pathologic conditions with increasing

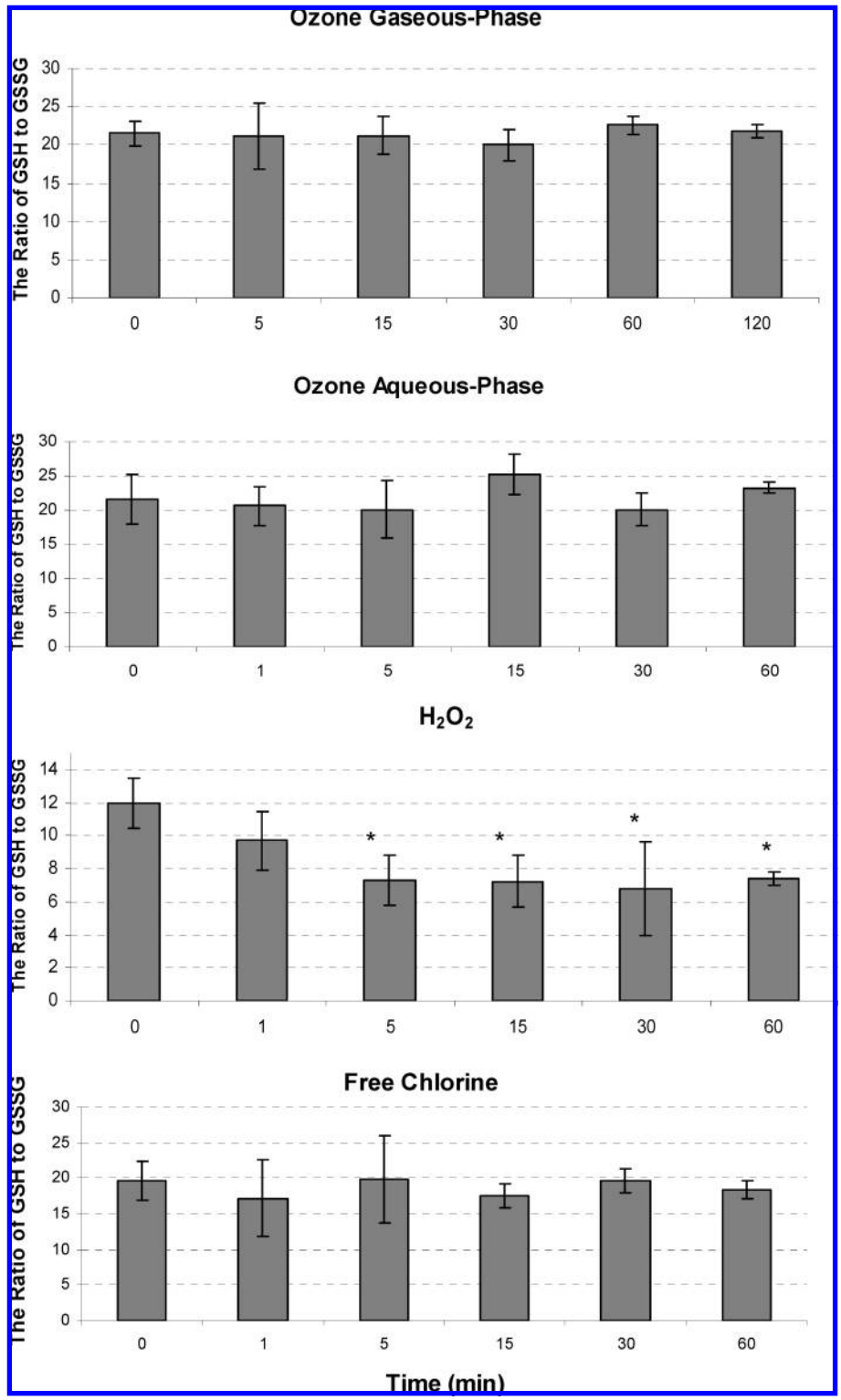


**Figure 7.** Ratio of GSH to GSSG after gaseous-phase ozone (GPO), aqueous-phase ozone (LPO), $H_2O_2$, and free chlorine treatments. Values are mean ± SD. (∗) Significantly different when compared with control ($p < 0.05$). ($n = 5$/group.)

intake levels of cystein or its precursors (e.g., cystine, *N*-acetyl-cysteine, and L-2-oxothiazolidine-*a*-carboxylate) in the cell (*47*). In the present study, among tested disinfectants, only $H_2O_2$ treatment for 5 min decreased the GSH level significantly, by 20.3%. Similarly, the CYS level was significantly reduced by 38.9% and 34.6% by application of free chlorine for 60 min and by application of gaseous-phase ozone for 5 min, respectively. Moreover, like in our previous study, the CYS level was found to be higher than the GSH level in strawberry samples, also in this study (*43*).

A significant decrease in the concentration of CYS only with 5 min gaseous-phase ozone treatment might result from increasing biosynthesis of GSH as part of the defense mechanisms. In fact, CYS is needed to synthesize GSH, which is one of the most powerful and active antioxidants in the plant (*47*). In the case of more than 5 min gas ozone treatment, in which no

significant decrease was observed in the CYS level, the strawberry cells might find enough time to produce more CYS to supply the CYS used for GSH synthesis.

Oxidative stress results in the development of neurodegrative and age-related diseases and damages proteins, DNA, and lipids (*48*). Oxidative damage is believed to be one of the major causes involved in chronic diseases such as cancer and heart disease (*49*). In living organisms, there is a balance between oxidants and antioxidants. When the balance is broken in favor of oxidants, the state called oxidative stress will develop and the concentration of reactive oxygen substances (ROS) will increase (*50*). In general, this fact can explain the slight increase in the level of GSH or CYS in strawberry under disinfectant treatment as a source of oxidative stress in this study.

The terms reactive oxygen substance (ROS) or oxygen derivative substance are used for both oxygen radicals ($O_2^{\bullet-}$, $OH^{\bullet}$, $LOO^{\bullet}$) and reactive agents carrying oxygen (HOCl, $H_2O_2$, $O_2$, $O_3$) (*51*). In fact, the disinfectants tested in this study have important roles in the oxidation process. In the antioxidant dependent defense system of a living organism, superoxide dismutase enzyme (SOD) catalyzes the conversion reaction of $O_2^{\bullet}$ into $H_2O_2$ and glutathione peroxidases (GSHPX) utilize $H_2O_2$ to convert reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase enzyme (GR) catalyzes the regeneration of GSH from GSSG; thereby, the damage of organism by free radicals is prevented. Besides the antioxidant defense enzymes, there are also some other antioxidants such as GSH, uric acid, tocopherol, and ascorbic acid scavenging free radicals in the living organism. In a study, Wang et al. (*52*) determined the activation of GSHPX, SOD, and GR as 27.5, 11.7, and 7.9 nmol/mg of protein·min, respectively, in strawberry. Apart from the presence of high activity antioxidant enzymes in strawberry, the strawberry fruit has excellent capability for inhibiting active oxygen substances ($O_2^{\bullet-}$, $H_2O_2$, $OH^{\bullet}$, and $O_2$) (*53*), which explains why the concentration of thiols in strawberry treated with disinfectants did not statistically decrease in the current study.

Similar to the findings in this study, Perez et al. (*13*) reported that the concentration of vitamin C significantly increased in strawberry stored in an ozone atmosphere compared to control. They suggested that the defense system was activated by exposure to ozone, which might raise the concentration of vitamin C in strawberry. This result and other studies agreed with our findings, reporting that ozone, by having high oxidative capacity and producing toxic molecules, acts as a phototoxic agent activating defense mechanisms in plants (*54*).

In the aqueous-phase, the steady-state concentration of dissolved ozone was measured to be 8.0 (2.0 mg/L) at pH 7.6 in our reaction system. In the gaseous-phase, the ozone concentration was maintained at 40 ppm. It should be noted that 1 ppm ($\mu$L/L) ozone in air contains only about 1/500 as many molecules of ozone as 1 ppm (mg/L) in water. The oxidation potential of aqueous-phase ozone on glutathione may be reduced by generating hydroxyl radicals at high pH. According to Wang and Jiao (*53*), glutathione was more effective in inhibiting superoxide radicals and $H_2O_2$ free radical activities (20.1 and 19.9%, respectively) than OH radicals and $O_2$ (5.19 and 3.61%, respectively). Thus, in agreement with the study above, the aqueous-phase ozone treatment possessing hydroxyl radicals could not affect the GSH level in our study.

Another possible explanation for the low effectiveness of the aqueous-phase ozone against biothiols in strawberries is that a hydroxyl radical, possessing an oxidation potential higher than that of ozone, generally reacts with organic materials at a diffusion rate. It is thus hypothesized that the majority of hydroxyl radicals will be consumed on the fruit surface for bacterial inactivation, whereas the oxidation of interior biothiols is minimized.

The ratio of GSH/GSSG in strawberry samples was also significantly influenced by $H_2O_2$ exposure in this study. This ratio is accepted as one of the most important indicators for oxidative stress (*42*). Thiols produce disulfide forms when reacting with $H_2O_2$. For instance, GSH produces GSSG when reacting with $H_2O_2$ (*55*), so during oxidative stress a decreasing level of GSH must increase the GSSG level. A significant decrease in the ratio of GSH/GSSG in strawberry samples treated with $H_2O_2$ demonstrated that oxidative stress in strawberry was significantly induced by $H_2O_2$. In our previous study, 30 min treatment (60 min for ozone gaseous-phase) of the same disinfectants at the same concentrations as those in the present study was tested to observe their effect on biothiols in some vegetables (*42*). $H_2O_2$ treatment was found to reduce the GSH level by 61, 11, 15, 4, and 71% in spinach, green beans, asparagus, cucumber, and red pepper, respectively. $H_2O_2$ also decreased the concentration of CYS in spinach by 80% and in asparagus by 7%. Similar to the present study, the most significant decrease in the ratio of GSH/GSSG was also observed in all vegetables treated with $H_2O_2$.

Moreover, in the present study, only 60 min free chlorine treatment could induce a statistically significant decline in the level of CYS in strawberry ($p < 0.05$). Similarly, based on our previous study, free chlorine treatment for 30 min did not reduce the CYS level in green beans, red pepper, asparagus, or spinach. At the same time, 30 min free chlorine application was proved to inactivate bacteria by about 100% on the surfaces of vegetables in the same study (*42*).

The major goal of vegetable and fruit disinfection is to inactivate pathogenic microorganisms. The efficiency of pathogen inactivation usually depends on the type of disinfectant, contacting conditions (e.g., exposure time, disinfectant concentration, and pH), and characteristics of vegetable and fruit surfaces. Typically, the microbial population on vegetable or fruit surfaces is reduced by 90−99% (*56*). In our previous study, among the four disinfection technologies (aqueous- or liquid-phase ozone, free chlorine, $H_2O_2$) investigated, free chlorine was determined as the most effective disinfectant. Hydrogen peroxide was found as the second most effective disinfectant, but a concentration as high as 5% (by weight) was required in comparison with much lower concentrations of other oxidants studied. Hydrogen peroxide is more expensive and also decomposes more quickly than free chlorine once used for vegetable processing (*42*).

In this study, the disinfectants, used in common dosages, generally did not significantly affect the concentration of CYS and GSH in strawberry samples. Only 60 min free chlorine and 5 min gaseous-phase ozone treatment decreased the CYS level significantly, and only 5 min $H_2O_2$ treatment decreased the GSH level significantly in strawberries. In conclusion, when applying these dosages, gaseous-phase ozone, aqueous-phase ozone, free chlorine, or $H_2O_2$ for 30 min was found to be optimal for disinfecting purposes and, at the same time, maintaining beneficial levels of biothiols.

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**Received for review July 18, 2008. Revised manuscript received September 10, 2008. Accepted September 10, 2008.**

JF802209T