POSTHARVEST ASCORBIC ACID TREATMENTS ON COLOR AND SUGAR CHANGES ON FRESH-CUT CARROT

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ABSTRACT

The purpose of the present study was to investigate the effects of ascorbic acid on white discoloration and microbial growth at fresh-cut carrots surface. Fresh-cut carrot cubes were treated with 250, 500, 1000 and 2000 ppm ascorbic acid solutions for 90 s. Carrots were stored in a cold room at 5±1°C temperature and 85-90 RH for 21 days. L*color values, whiteness index, hue angle values, saturation index, polyphenol oxidase activity, glucose, fructose, sucrose and total sugar content and weight loss were determined at the beginning and with 7-day intervals during the storage. The results showed that 500 and 1000 ppm ascorbic acid treatments were effective to reduce with ppo activity and assure the color stability of the fresh-cut carrot cubes for 21 days. Also, glucose level content of samples treated with 500 ppm were found to be high whereas the carrot cubes treated with 250 ppm AA had the highest sucrose and total sugar content during storage. Psychrotrophic microorganism growth was reduced significantly compared to control in all ascorbic acid treated samples, especially in the one treated by 2000 ppm dose of ascorbic acid.

Key Words: Carrot, Fresh-Cut, Color, Sugar, Microorganism

1. INTRODUCTION

Carrot (Daucus carota L.) is one of the important root crops cultivated throughout the world with a firm structure and is a good source of natural antioxidants such as carotenoids and phenolic compounds (Baranski et al. 2012). Fresh-cut carrots could be found on the market place as: whole peeled (baby), sticks and sliced, shredded, grated and diced. Carrot is considered one of the vegetables consumption of which, both fresh and processed, has increased over the past years due to its nutritional and health benefits and the introduction of new carrot-derived products (Augspole and Rakcejeva 2013).

Cut or grated carrot is one of the most widely used products in ready-to-eat salads; however, the main problems limiting its shelf-life are surface white blush discoloration and microbial spoilage (Emmambux and Minnaar 2003). “White blush” occurs as consequence of exposure of damaged cell wall materials on processed or cut edges to drying conditions. While the drying is a physical process, post-processing accumulation of lignified material can occur as a wound response, intensifying the incidence and severity of the “white blush” (Toivonen and Brummell 2008). Once carrots are exposed to air, they easily dehydrate, and dried cell debris acquires a whitish color, forming a white layer on the carrot surface. At this stage, the quality defect can be reversed by dipping carrots in water and allowing for rehydroxylation (Cisneros-Zevallos et al. 1995). As the lignification process is enzyme mediated, some dipping treatments directed to inactivate the responsible enzymes have been tested. A successful result was obtained with a treatment combining heat inactivation and an acidic environment. Carrots peeled with coarse sandpaper and dipped for 20-30 s. in a 2% of citric acid solution at 70°C did not develop the defect for at least five weeks in cold storage; product taste was not affected by the treatment (Bolin and Huxsoll 1991). Citric acid dips of 1 mM or higher concentration reduce respiration rate of shredded carrots by 50% or more (Kato-Noguchi and Watada 1997).

Ascorbic acid (Vitamin C) is a reducing agent often used to prevent oxidation reactions such as
browning; however, there may be effects on other physiological processes in the cut tissues (Toivonen and Deell 2002). Ascorbic acid dips reduced the respiration of "Fuji" apple slices stored in a 0% of O₂ atmosphere. In air atmosphere, the ascorbic acid dips reduced ethylene production and increased the respiration of apple slices (Gil et al. 1998). In our previous study (Kasim and Kasim 2014), ascorbic acid particularly in the dose of 4% was found to be more effective for preventing lignification of fresh-cut carrot shreds.

Fresh cut fruits and vegetables have been popular for the bioavailability of numerous vitamins, minerals and other phytochemicals. However, they may naturally contain a wide variety of bacteria, fungi and yeast species. Commercial or home-made fresh-cut fruits and vegetables are prepared by some simple treatments such as washing, cutting, grating, shredding and packaging. Among these steps, washing may be considered as the most critical step since it removes the soil particles and reduces the microbial load from the surface.

Antimicrobial effects of chlorine based sanitizers on fresh cut products have been previously reported, because they have been widely used as washing solution for fresh cut products to eliminate the microorganisms (Tornuka et al. 2012). However the effects of organic acids on microorganisms have not been studied eventhough organic acids (e.g. lactic acid, citric acid, acetid acid, tartraric acid) have been described as strong antimicrobial agents against psychrophilic and mesophilic microorganisms in fresh-cut fruits and vegetables. Citric acid and ascorbic acid were used to reduce microbial populations on salad vegetables. Ascorbic acid and its various neutral salts and other derivatives are the leading Generally Recognised as Safe (GRAS) antioxidants for use on fruit and vegetables. Commercial or home-made fresh-cut fruits and vegetables are prepared by washing solution for fresh cut products to eliminate excessive surface solution.

2.2. Sample Preparation

Processed carrots (250 g for each replicate) were dipped in 3 L solution of 250 ppm,500 ppm,1000 ppm, and 2000 ppm ascorbic acid (AA) or distilled water (control, K) for 90 s at room temperature. Treated carrot cubes were dried for 2 min at room temperature using a salad spinner to remove excessive surface solution.

2.3. Packaging and Storage Conditions

250 g of carrots sliced as cubes were placed in a plastic box with cover, and stored in a cold room at 5±1°C temperature and 85-90 % relative humidity for 21 days.

2.4. Color Measurements

Color measurements (L*, a* and b* values) were performed using a chromometer CR-400 (Konica Minolta Inc. Osaka, Japan) with illuminant D65 with 8 mm aperture. The instrument was calibrated with a white reference tile (L*=97.52, a*=-0.56, b*=3.57) prior to measurements. The L* (0=black, 100=white), a* (+red, -green) and b* (+yellow, -blue) color coordinates were determined according to the CIELAB coordinate color space system.

Whiteness index (WI, Eq. (1)), saturation index (SI, Eq. (2)), hue angle (H, Eq. (3)), total color difference (ΔE, Eq.(4)), and browning index (BI, Eq. (5)) were calculated using measured L*, a* and b* values as follows and these index values were used to determine the color changes as compared with control of fresh-cut green beans samples (Saricoban and Yilmaz 2010).

\[
WI = 100 - \sqrt{(100 - L*)^2 + a*^2 + b*^2} \\
SI = \sqrt{a*^2 + b*^2} \\
H = \arctan\left(\frac{b*}{a*}\right) \\
\Delta E = \sqrt{(L_0 - L*)^2 + (a_0 - a*)^2 + (b_0 - b*)^2} \\
BI = \frac{100(x - 0.31)}{0.17}
\]

where subscript “0” refers to the color reading of control sample used as the reference and a larger ΔE indicates greater color change from the reference fresh-cut green bean samples.

2.2. Plant Material

2.1. Material Methods

Carrots were obtained from Kocaeli Wholesale Distribution Center. After that, carrots were immediately brought to the laboratory, and screened for uniformity which is being free from any mechanical damage and diseases, and also same stage of maturity. Then, carrots peeled and simultaneously trimmed of tap root and stem plate prior to sample preparation. Afterwards, carrots were cut with a sharp knife to obtain carrot cubes of 1.0 cm x 1.0 cm x 1.0 cm, and divided into five batches.


\[ x = \frac{(a + 1.75L)}{(5.645L + a - 0.3012b)} \]  

(6)

2.5. Sugar Analysis

15 mL of water was added to 3g of fresh-cut carrot and the mixture was filtered through Whatman No. 1 filter paper. 20 µL of filtrate was injected to HPLC system (Agilent, HP 1210) with the following HPLC parameters: Column: Zorbax Carbohydrate Analysis, 4.6 mm ID x 150 mm (5 µm); Mobile phase: 75/25 acetonitrile/water; Flow rate: 1.4 mL/min; temperature: 30°C; Detector HP110 RID, 30°C. Sample volume: 20µL in 50/50 acetonitrile/water.

2.6. Polyphenol oxidase activity (PPO)

In order to measure polyphenol oxidase activity, 5 g of homogenized fresh-cut carrot was extracted with 0.1 M phosphate buffer, pH 7, containing 5 g of polyvinylpyrrolidone by magnetic stirrer for 15 min. The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate was collected as an enzyme extract. PPO activity was determined by a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva et al. 2000). Phosphate buffer pH 7 (0.1 M, 1.95 mL), 1 mL of 0.1 M catechol (substrate) and 50 µL of the enzyme extract were pipetted into a test tube and mixed thoroughly. The mixture was rapidly transferred to a cuvette of path length 1 cm. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible spectrophotometer (Arnnok et al. 2010).

2.7. Weight Loss Determination

The weight of each sample with three replication of each treatment group was recorded on the day of harvest and on the sampling dates. The cumulative weight loss was expressed as percentage loss of original weight.

2.8. Microbial Analysis of Psychrophilic Microorganisms

In order to evaluate the microbiological efficiency of the AA on the fresh-cut carrot cubes microbiota, microbiological analysis of psychrotrophs was carried out in the treated and untreated carrot samples at 0, 7, 14 and 21 storage days. The following medium and incubation conditions were used for microorganism enumeration: Plate Count Agar (PCA) (Oxoid) for total aerobic psychrotrophic count, spread plated and incubated at 22°C for 6 days. Microbiological counts were done in triplicate (3 packages/analysis day for both treated and untreated carrots) by taking 15 g of sample and mixing it 135 mL peptone saline solution (8.5 g/L NaCl and 1 g/L peptone) in a sterile breast milk storage bags and manual mixing for 60 s. Ten fold dilution series were made in peptone saline solution for plating (Gómez-Lopez et al. 2007).

2.9. Statistical Analysis

The experiments were conducted in a completely randomized design with a minimum of three replications per storage treatments per sampling date. Data were analyzed by ANOVA and differences among means were determined by the Duncan’s multiple range test with significance level at p<0.05.

3. RESULTS AND DISCUSSION

3.1. L* values and Whiteness Index (WI)

In Table 1, the results of L* (lightness) and whiteness index (WI) from all treated and untreated samples are shown. L* values in all treatment group were increased until the 14th day, then decreased. L* values of samples treated with 2000 ppm ascorbic acid (AA) were lower than the other AA treatments and control on the 7th day, but the differences among treatments were not statistically significant (p>0.05). After this time, it was seen that AA treatment did not affect L* values, and it was higher in samples treated with AA than control group at the day 14, however, at the end of the storage the lowest L* value was obtained in samples treated with 1000 ppm AA (61.587) and followed by 250 ppm AA (62.013), control (62.753), 200 ppm AA (62.423) and 500 ppm AA (62.817). Similarly L* values, whiteness index (WI) of all treatment groups was increased during storage, and the highest increase was found in samples treated with 500 ppm AA and followed by 2000 ppm, control, 1000 ppm and 250 ppm AA treatments on the day 14th. Also the differences between 250 and 1000 ppm AA treatments and the other treatments were statistically significant at p<0.05 level.

The bright orange color of fresh carrots can disappear in stored fresh-cut products, particularly when abrasion peeling is used. Carrots may develop “white blush”, also known as “white bloom”, a discoloration defect which results in the formation of a white layer of material on the surface of peeled carrots, giving a poor appearance to product (Garcia and Barret 2002). Kenny and O’Brien (2010) found that different cutting methods lead to an increase in the L values of fresh-cut carrots, but L*
values of manually peeled carrots were the lower compared with fine abrasion peeling method and coarse abrasion method. In our research, L* and WI values of carrots that manually peeled and cut as cubes form was increased in all treatment groups until the 7th day. After that they were remained constant except for samples treated with 250 and 2000 ppm AA, and also it was found that decreased in all AA treated samples compared to control at the end of the storage. Therefore it can be said that AA treatments applied to fresh-cut carrot cubes were effective in reducing "white blush". This study agrees with the results of Rico et al. (2007). They reported that increases in luminosity can be correlated with the development of whiteness in the samples when analysing the color using a colorimeter. In this study L* and whiteness index scores of fresh-cut carrots were correlated to each other (data not shown).

3.2. Hue angle (H°) and Saturation Index (SI)

Hue angle (H°) and saturation index (SI) values of samples were decreased in the first 7 days of storage in all treatment groups (Table 1). The H° and SI values of samples treated with 2000 ppm ascorbic acid, were the lowest among all treatments, at the day 7, but differences among the treatments were not found statistically significant (p>0.05). SI value of 250 ppm AA treated samples was the highest (116.91) among all treatments at the second week of storage, and also differences among 250 ppm AA and 1000 ppm AA and the other treatments, were determined as statistically significant at p<0.05 level. Although, SI values of the control group were decreased during storage they changed as increase/decrease of carrot cubes treated with AA. Hue angle values of samples in control, 250 ppm AA or 1000 ppm AA, were remained nearly constant compared to 500 ppm or 2000 ppm AA treated samples during storage.

Hue angle is expressed as a value indicating the actual color of the product. (Mcguire 1992). In the present study, AA applications was caused lightening of orange color compared to control in the first week of storage, it was determined that color of samples treated with 500 and 2000 ppm AA were maintained better than the other treatments in the second week. Cutting-induced injury affects immediately visual quality of the products, and also has long-term effects on metabolism with consequent quality changes that can be detected later (Hodges and Toivonen 2008), also, impact of application on metabolism of fresh-cut products may be occurred later. Therefore in this study it can be said that AA treatments affected the color of carrots for two weeks but after that the effect of treatments was lost. Saturation index results also confirms these findings.

3.3. Browning Index (BI) and Polyphenol Oxidase (PPO) Activity

Browning Index (BI) scores indicated that browning in fresh-cut carrots was inhibited by application of the AAs, and the samples treated with high concentration of AA showed a better result than the samples treated with low dose of AA at the day 7 (Table 2). Gil et al. (1998) reported that AA reduces browning incidence by reducing o-quinones back to phenolic compounds prior to polymerization and subsequent formation of colored pigments. In the present study, AA treatments decreased browning scores for the first 7 days of storage but after this, BI values of samples in 500 and 1000 ppm AA was a little bit increased compared to the other treatments, and differences between these two treatment and other treatments were found to be statistically significant at the day 14 (p<0.05).

However BI values of samples treated with AA at all doses were high in comparison to control samples by the end of 3-week storage. Unlike BI scores; while polyphenol activity of fresh-cut carrot cubes treated with AA increased, it decreased in control group, during the first 7 days of storage. Also the differences between control and 250 ppm AA treatment and the other AA treatments were found to be statistically significant (p<0.05). At the second week, PPO activity of the samples treated with AA continued to increase and this increase was the lowest in 2000 ppm AA treated carrots and the highest in control samples. PPO activity in all groups decreased at the end of storage. Enzymatic browning is one of the most limiting factors on the shelf-life of fresh-cut products, and it is the discoloration which results from the action of a group of enzymes called polyphenol oxidases (PPO), which have been reported to occur in all plants. The color variation in products of enzymatic oxidation is related to the phenolic compounds involved in the reaction, and both color intensity and hue of pigments formed vary widely (Garcia and Barrett 2002). AA is an antihrowning agent, and is probably the most widely used for prevent enzymatic browning. In addition to its reducing properties, it also slightly lowers pH, and it has a direct effect on PPO (Whitaker 1994). In the present study, it was not observed browning on the surface of fresh-cut carrots during the storage, although PPO activity was high compared to the initial level. This result was supported with low
browning index scores of carrots. When analysing the color using a colorimeter, a decrease of luminosity can be an indication of browning appearance was stated by Rico et al. (2007). In the present study, the increase in L* values of carrot samples was indication that there were not browning.

3.4. Glucose, fructose, sucrose and total sugar content of fresh-cut carrot cubes

Glucose, fructose and total sugar content of fresh-cut carrots in all treatment groups decreased during the 14 days (Table 3), then increased till at the end of storage. According to the sugar results; the highest glucose content was obtained by 500 ppm AA treatments, whereas the samples treated with 250 ppm AA had the highest sucrose and total sugar content during the storage and the differences between 500 ppm AA and 250 ppm AA treatment were statistically significant at level of p<0.05. Meanwhile, the fructose level of all treatments was decreased at the day 7; after that it increased in all treatments except 250 and 1000 ppm AA treatments during storage. Also, it was found that fructose content of samples treated with 500 ppm AA was higher compared to fructose content of control samples and the other AA treatments during storage. Furthermore, fructose content of 250 ppm AA treated carrots was greatly low in comparison with the other treatments (p<0.05) at the end of storage. One of the most important qualities of vegetables is their sweetness, closely related to the soluble sugar content (Ozaki et al. 2009). Carrot is mainly constituted by water (approximately 95%) and carbohydrates, which account of 5% of edible portion. It has been reported that carbohydrate is one of the most important sensory quality indicator for consumers. As it is known that the major sugars in carrots are fructose, glucose and sucrose; and many research has been published the effect of different processing and storage condition on their content in carrots (Soria et al. 2009). The present study revealed the positive influence of AA on total sugar content and fructose, glucose and sucrose content of fresh-cut carrots (Table 1).

Total sugar content of carrots in all AA treatments was found to be higher compared to total sugar content of control during storage. We not found any literature about the effect of ascorbic acid on sugar content of carrot. But, in a study; Augspole et al. (2014) reported that, the content of total sugars decreased by 30% in average, if carrot were treated with different hydrogen peroxide doses for treated time 30-90s. But in this study, any decrease in the sugar content of carrots was not observed, on the contrary it can be said that total sugar and also sweetness of fresh-cut carrots was increased with the AA treatments.

3.5. Weight Loss

According to Fig. 1, the weight loss of samples in all treatment groups was increased during storage. But, AA treatments reduced weight losses in comparison with control group. It was also determined that while weight loss of control group was the highest, it was the lowest in samples treated with 2000 ppm AA. Also the differences between 2000 ppm AA treatment and the other treatments were statistically significant at level of p<0.05. Fresh-cut products tend to be more vulnerable to water losses because they are no longer intact after peeling and cutting or shredding, slicing, etc. Peel or skin is a very important barrier to loss of turgor and desiccation; many commodities have a protective waxy coating, highly resistant to water loss. Evidently, peel removal renders commodities more perishable. The mechanical injury brought on by cutting and the method used, directly expose the internal tissues to the atmosphere, promoting desiccation (Garcia and Barrett 2002). Also, the type of peeling or cutting process can also influence the degree of physiological response by tissues. According to Barry-Ryan and O’Beirne (1998), fine abrasion peeling results in lower weight loss of packaged slices made from the peeled carrot (0.1 %) as compared with coarse abrasion peeling (0.2 %), which causes more tissue injury. In the present study, weight loss of samples treated with AA was lower than that of control, so AA treatments reduced weight loss of fresh-cut carrots.

![Image](https://example.com/weight_loss.png)

Figure 1. Weight loss of fresh-cut and AA treated carrots. It includes mean ± standard deviation of three replicatons.
3.6. Microbial growth analysis

The changes in psychrotrophic microorganism population during the study can be observed in Fig.2. Organic acids (e.g. lactic acid, citric acid, acetic acid, tartaric acid) have been described as strong antimicrobial agents against psychrophilic and mesophilic microorganisms in fresh-cut fruits and vegetables. Citric acid and AA were used to reduce microbial populations on salad vegetables (Rico et al. 2007). AA led to low growth of psychrotrophic microorganisms at all concentrations. The treatment had a satisfactory performance at 2000 ppm AA concentration; it inhibited the growth of psychrotrophic microorganisms up to 11 days. According to the Fig.5, fresh-cut carrots can be long lasting when they were treated by 2000 ppm AA. According to Debevere’s (1996) specification which is 8 log CFU/g for psychrotrophic microorganisms, the control samples of MP carrots almost reached the end of the shelf life from the microbiological point of view, however AA treated samples had lower levels.

Conclusions

During storage surface white discoloration on fresh-cut affects the product quality and limits of storage life. Also, fresh-cut carrots may be contaminated by pathogens in different ways after harvest. The present study investigated the potential of AA treatments for the preventing of whitening and reducing microorganism of fresh-cut carrots. The experimental findings showed that AA treatments at 500 and 1000 ppm were effective in improving color of fresh-cut carrot surface. Also AA treatments reduced both PPO activity and browning of samples. Fructose and glucose level of fresh-cut carrots treated with 500 ppm AA was found to be high. Furthermore, the microbial growth was inhibited by AA treatments.

References


Table 1. L* values, whiteness index (WI), hue angle (H') and saturation index (SI) of fresh-cut and AA treated carrots.

<table>
<thead>
<tr>
<th></th>
<th>L* values</th>
<th>WI</th>
<th>H'</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
</tr>
<tr>
<td>Control</td>
<td>58.1 61.8 62.4 62.8</td>
<td>31.1 39.9 41.8 a 42.4</td>
<td>56.2 54.1 53.7 b 53.9</td>
<td>54.7 46.5 44.3 b 44.0</td>
</tr>
<tr>
<td>250 ppm</td>
<td>58.1 62.4 62.8 62.0</td>
<td>31.1 40.2 40.0 a 40.3</td>
<td>56.2 52.9 53.1 b 52.6</td>
<td>54.7 46.5 47.1 b 46.0</td>
</tr>
<tr>
<td>500 ppm</td>
<td>58.1 61.8 64.2 62.8</td>
<td>31.1 40.9 43.0 a 40.7</td>
<td>56.2 53.5 54.3 a 53.5</td>
<td>54.7 45.1 44.4 a 46.1</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>58.1 62.1 62.6 61.6</td>
<td>31.1 41.5 41.0 a 41.8</td>
<td>56.2 53.1 53.1 a 53.2</td>
<td>54.7 44.6 45.6 ab 43.7</td>
</tr>
<tr>
<td>2000 ppm</td>
<td>58.1 61.0 63.1 62.4</td>
<td>31.1 42.3 42.3 ab 40.2</td>
<td>56.2 50.7 54.6 a 53.4</td>
<td>54.7 42.5 44.3 b 46.5</td>
</tr>
</tbody>
</table>

Values are the mean of three replicates. Means with different letters are significantly different at the 0.05 level.

Table 2. Browning index (BI) and polyphenol activity (PPO) of fresh-cut and AA treated carrots.

<table>
<thead>
<tr>
<th></th>
<th>BI</th>
<th>PPO activity</th>
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</thead>
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<tr>
<td></td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
</tr>
<tr>
<td>Control</td>
<td>168.4 117.2 107.3 b 105.3</td>
<td>0.45 0.29 b 1.78 a 0.44</td>
</tr>
<tr>
<td>250 ppm</td>
<td>168.4 115.7 116.9 a 114.4</td>
<td>0.45 0.46 b 1.54 ab 0.48</td>
</tr>
<tr>
<td>500 ppm</td>
<td>168.4 111.7 103.8 b 113.3</td>
<td>0.45 0.83 ab 1.60 a 0.56</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>168.4 108.7 111.8 ab 106.8</td>
<td>0.45 0.97 a 1.32 b 0.46</td>
</tr>
<tr>
<td>2000 ppm</td>
<td>168.4 103.4 105.9 b 115.5</td>
<td>0.45 1.11 a 1.35 b 0.51</td>
</tr>
</tbody>
</table>

Values are the mean of three replicates. Means with different letters are significantly different at the 0.05 level.

Table 3. Fructose (%), glucose (%), sucrose (%) and total sugar (%) contents in untreated control and AA treated carrot samples, at day 0, 7, 14 and 21 of storage at 5±1°C.

<table>
<thead>
<tr>
<th></th>
<th>Fructose (%)</th>
<th>Glucose (%)</th>
<th>Sucrose (%)</th>
<th>Total Sugars (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
<td>0  7 14 21</td>
</tr>
<tr>
<td>Control</td>
<td>0.50 0.59 1.27 1.35 a</td>
<td>1.46 1.00 0.65 1.56 ab</td>
<td>2.27 1.82 1.30 3.58 bc</td>
<td>4.22 3.41 3.22 6.49 ab</td>
</tr>
<tr>
<td>250 ppm</td>
<td>0.50 0.42 1.04 0.96 b</td>
<td>1.46 0.93 0.59 1.26 c</td>
<td>2.27 2.14 1.99 4.38 a</td>
<td>4.22 3.48 3.62 6.60 a</td>
</tr>
<tr>
<td>500 ppm</td>
<td>0.50 0.60 1.22 1.38 a</td>
<td>1.46 1.20 0.70 1.67 a</td>
<td>2.27 2.00 1.37 3.30 c</td>
<td>4.22 3.79 3.30 6.35 b</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>0.50 0.54 1.35 1.28 a</td>
<td>1.46 0.97 0.66 1.47 ab</td>
<td>2.27 1.95 1.59 3.58 bc</td>
<td>4.22 3.47 3.60 6.33 b</td>
</tr>
<tr>
<td>2000 ppm</td>
<td>0.50 0.59 1.00 1.25 a</td>
<td>1.46 0.80 0.60 1.53 ab</td>
<td>2.27 2.31 1.89 3.74 b</td>
<td>4.22 3.69 3.48 6.52 ab</td>
</tr>
</tbody>
</table>

Values are the mean of three replicates. Means with different letters are significantly different at the 0.05 level.