Respiration Rate and Shelf Life of Radish (*Raphanus sativus* L.) as Influenced by Postharvest Application of Calcium Nitrate and Humic Acid Concentration

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Abstract

Postharvest application of calcium has a significant effect on postharvest quality and respiration rate of some horticultural crops. However, there is little literature citing the effect of calcium and humic acid on the respiration rate and shelf life of radish during storage. Hence, a study was conducted to assess the efficacy of calcium nitrate and humic acid on respiration rate and shelf life of radish. The experiment was laid out in a completely randomized design (CRD) with four treatments and three replications. The treatments tested were composed of calcium nitrate, and humic acid concentration applied alone and in combination and control. Data on visual quality rating, sprouting, shelf life, color changes, CO_2 concentration and respiration rate were subjected to the analysis of variance for CRD. Radishes applied with varying postharvest treatment were significantly different regarding sprouting and shelf life during storage but not significant regarding the visual quality rating. The color value of radish decreases as the storage days increases. The decrease in the value of color indicated a loss of whiteness of radish. Also, CO_2 concentration and respiration rate remarkably influenced by the application of calcium nitrate and humic acid. Thus, application of calcium nitrate and humic acid alone or in combination is beneficial in improving the respiration rate and shelf life of radish.

Keywords: calcium nitrate, humic acid, postharvest treatment, shelf life, respiration rate

1. Introduction

Radish is under *Brassicaceae* family. *Raphanus sativus* is a cool season annual but depending on when it is planted. Usually, commercial radishes are about 2 cm in diameter and are either red or white. They reach market size in 21 to 28 days (or extended in cold weather). It differs in color from white to black and a rapid growing cool season root vegetable. Also, radishes turn into stronger in flavor in hot weather. Furthermore, the roots stay in salable condition only a little period beforehand becoming pithy. On another hand, the growth must be continuous and rapid for better quality (Aruna and Nishadh, 2014).

Postharvest handling of these commodities raises some issues both regarding allowed procedures and of their effectiveness in maintaining the quality of the product. On the other hand, the postharvest performance of the product obtained from specific sustainable procedures may be somewhat affected by preharvest conditions (Amodio *et al.*, 2007).

Calcium as postharvest treatment has been utilized as firming agents to prolong postharvest shelf life in whole and fresh-cut fruits (Mahmud *et al.*, 2008). Postharvest calcium application has likewise been revealed to have a significant result on several storage parameters of many fruits and vegetables. For example, the usage of calcium chloride in regulating the rapid aging in collected fruits has been examined by several authors (Wills and Tirmazi, 1977 and Prakash *et al.*, 2007). Moreover, the postharvest calcium chloride application decreases respiration, reductions in ethylene production, and delays senescence in newly harvested produce such as tomatoes (Hussain *et al.*, 2012). Furthermore, postharvest calcium application preserves cell turgor, membrane integrity, tissue firmness and deferments of membrane lipid catabolism, in consequence, prolonging the storage life of fresh fruits (Chaplin and Scott, 1980; Picchion *et al.*, 1998).

Most of the study focused more on the preharvest of crop production particularly radish plant than the application of humic acid and calcium on postharvest aspect. Thus, a study was conducted to evaluate the effect of humic acid and calcium nitrate on the visual quality rating, sprouting, color, shelf life, CO_2 concentration, and respiration rate of radish.

2. Methodology

Radish was harvested at the mature stage or 45 days after planting from the FARMI, Vermicompost facility area, Visca, Baybay City, Leyte, (Latitude: 10° 44' 38.2884" N and Longitude: 124° 47' 57.6492" E). The radish tubers were packed with plastic, lined with banana leaves, and placed in the plastic crate. The packed radish tubers were transported directly to the Post Harvest Laboratory at the Department of Horticulture, College of Agriculture and Food Technology, Visca, Baybay City, Leyte (Latitude: 10° 44' 47.1696" N and Longitude:124° 47' 34.9692" E) at about 300 m from the field. Tubers were weighed and washed with running water upon arrival.

Selected radish tubers with uniform size, color, and physical integrity were sanitized in a 100 ppm sodium hypochlorite solution for 20 minutes and rinsed 3-4 times and let it dry. Then, the roots dipped for 10 minutes in the following concentrations: 1) control (distilled water); 2) 0.6% calcium nitrate solution; 3) 0.6% humic acid; and, 4) 0.6% calcium nitrate + 0.6% humic acid solution.

The experiment was done in a completely randomized design. Each treatment included 15 tubers and was replicated three times. Two tubers were set aside as destructive sampling. Five (5) tubers per replication were manually placed in plastic crates and stored at ambient temperature (23 ± 2ºC and 75% relative humidity (RH) for ten days. A randomly selected sample of 5 tubers at day one and two days intervals wherein three parameters such as visual quality rating, sprouting, and color were collected from each replication in treatment during the storage period. All experiments were carried out in triplicate. Data on the postharvest parameters were recorded.

2.1 Visual Quality Rating and Shelf Life

The shelf life of the radish tubers was calculated by counting the days required for them to attain the last stage of ripening and until the tubers are acceptable for marketing. The physical appearance of each tuber sample was assessed daily by the main researcher with the supervision of the secondary author using the following visual quality rating (VQR): 9-excellent, field fresh, no defect, 7-good, defect minor, 5-fair, defect moderate, limit of marketability, 3-limit of edibility and 1-non-edible (Bautista *et al.*, 2007) under usual condition. The number of days to VQR 5 was taken as the potential storage life of the tubers under ambient condition. The major defects responsible for tuber quality deterioration (e.g., senescence, browning, etc.) was noted.

2.2 Sprouting

The leaves of the radish were cut up to 10 mm from the top of radish tubers. Sprouting was measured at two days interval and recorded accordingly.

2.3 Color Changes

Color changes on lightness (L*), was determined using a colorimeter (Minolta CR-300, Osaka, Japan). A decrease in L* value indicated a loss of whiteness (brightness). Readings were conducted directly on the whole radish just after harvesting and at two days interval.

2.4 Carbon Dioxide (CO_2) Concentration

The CO_2 concentration of packaged radish tuber was determined using a CO_2 gas analyzer (Checkmate 3, PBI Dansensor, Ringstead, Denmark) as described by Banda *et al.*, (2015). Gas analysis was done by inserting a needle attached to the gas analyzer through a rubber septum on the 1.1 liters (L) glass jars. Gas sampling was done before opening the package to remove the radish roots. Three additional replications per treatment were used to monitor in-package CO_2 concentration during the entire storage period.

2.5 Respiration Rate

Respiration rate of radish roots was determined using the closed system method at 5^{o}C. On each sampling day, five radish roots from each of the treatments were separately weighed into 1.1 liters (L) glass jars. The glass jars were hermetically sealed by incorporating Vaseline petroleum jelly in the gap between the lid and the jar. Gas samples were drawn at hourly intervals over a period of 4 h through a rubber septum fitted on the jar, and the gas composition was monitored by the gas analyzer (Banda *et al.*, 2015). Measurements were repeated on each of the sampling days using fresh samples each time to determine the effect of the treatments. Respiration rate was calculated following the formula espoused by Banda *et al.* (2015):

$$\text{Respiration rate } (mg\ CO_2.kg^{-1}.h^{-1}) = \frac{C1 - C0}{100} \times V \times \frac{1}{(t)(w)} \times \frac{44\ mg\ CO_2}{24\ ml\ CO_2} \quad (1)$$

Where:
C_1 – % CO_2 after a time interval
C_0 – % CO_2 at zero time, 0.03%
V – headspace volume, ml = vol. of respiration jar - vol. of commodity
t – time interval, hour
w – weight of commodity, kg

2.6 Data Analysis

Analysis of variance (ANOVA) was used to identify the treatment effect using Statistical Tool for Agricultural Research (STAR) software of the International Rice Research Institute (STAR, 2014). Mean separation was done by using Tukey's test.

3. Results and Discussion

3.1Visual Quality Rating, Sprouting, and Shelf Life

Radishes applied with varying postharvest treatment were significantly (P<0.01) different regarding sprouting and shelf life during storage but not significant (P>0.05) concerning visual quality rating (Figure 1 to 3). Application of combined calcium nitrate and humic acid revealed the highest VQR ranges from 5.05-8.87 throughout the study (Figure 1).

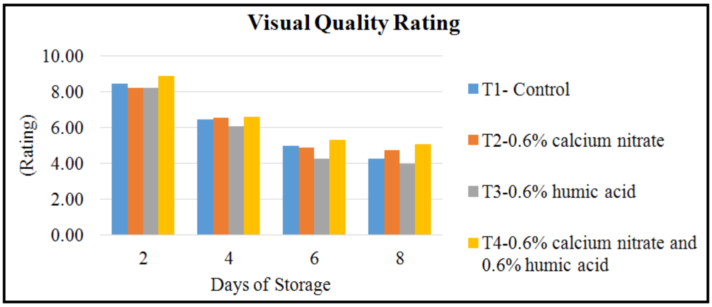

Figure 1. Visual quality rating as influenced by postharvest humic acid and calcium nitrate treatment {Coefficient of Variation (CV), 2 days= 6.98%, 4 days= 6.43%, 6 days=14.09%, 8 days=15.29%}.

On another hand, the lowest VQR was obtained by plant applied with 0.6 % humic acid with a VQR of 3.96-8.20. On the other hand, it can be observed on day 2 of storage the radish roots applied with calcium nitrate and humic acid alone and in combination got the highest value on sprout compared to the control treatment (Figure 2). However, in day 4 to 8, the pattern of effect was not any more similar as compared to day two but it is clear that there is a considerable increase in sprouts from day 2 to day 8. The increased in sprouting with the application of humic acid of this study was in agreement with results reported by Hassanpanah and Khodadadi (2009) for other crops such as potato where the application of humic acid resulted in increased sprouting percentage. According to Panneerselvam and Jaleel (2008) showed that there was a slight reduction in starch breakdown during the initial sprout emergence, but, as the sprout continued to grow, a sharp decrease in starch was found in yam tubers and turmeric rhizomes.

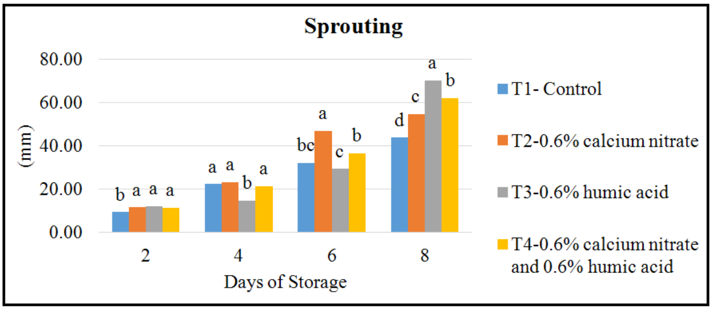

Figure 2. Sprouting as influenced by postharvest humic acid and calcium nitrate treatment {Coefficient of Variation (CV), 2 days=7.82%, 4 days=6.46%, 6 days= 8.24%, 8 days=3.13%}.

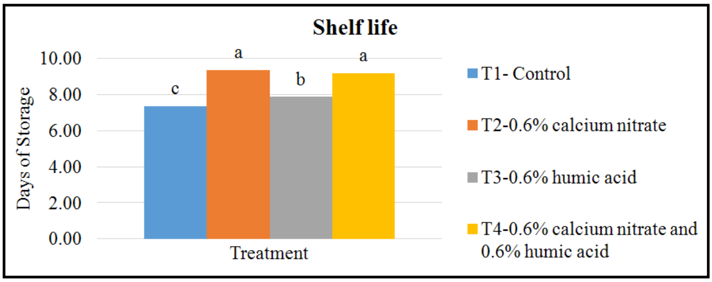

Figure 3. Shelflife as influenced by postharvest humic acid and calcium nitratetreatment (Coefficient of Variation (CV)=2.51%)

The increase in shelf life as applied with calcium was reported by Nirupama *et al*. (2010) who stated that calcium application helps preserve membrane integrity, tissue firmness, cell turgor also in delaying membrane lipid catabolism and prolonging the storage life of fruit. The defense of cell membrane integrity by Ca during senescence has been described by the capability to tie to membrane phospholipids and, in this manner, to alleviate the membrane and to regulate the membrane-related functions (Thompson, 1988 as cited by Cheour and Souiden, 2015). In this study also, the top portion of the roots was removed. According to Lutz *et al.* (1954) as cited by Wang (1998) total removal of the tops retards sprouting and prolongs the shelf life of radishes. Additionally, Bhattarai and Gautam (2006) reported that firmer fruits are known to be more resistant to physical damage during handling and transportation and thus contribute to extending storage life which has an economic benefit. Also, Gill *et al.* (2005) noted that pre and post-harvest application of chemicals such as calcium chloride and calcium nitrate are better known to enhance the quality and shelf life of fruits during storage.

3.2 Color Changes

The color of radish was significantly ($P<0.01$) affected by the treatment used in the study (Figure 4). The result shows that color value of radish substantially decreases as the storage days increases. At all treatment, there was a significant decrease in the value of L*, which indicated a loss of whiteness of radish just after 2 d of storage and throughout the storage period but with the exception for the radish root treated with humic acid. This suggests that color was lost in the control, calcium and the combination of calcium nitrate and humic acid samples following storage. L* values are an indication of the lightness of a color, wherein the scale of values range from 0 for pure black to 100 for pure white (Valida *et al.*, 2016). Also, when treatments were compared, it was observed that treatments having humic acid could preserve the color quality of radish as shown in days 2 to 8. The humic acid application shows higher value which indicated better results in color quality characteristics of radish. The change in total chlorophyll content in response to humic acid was primarily owed to the change in chlorophyll a content since there was no significant effect of humic acid on chlorophyll b content. On the other hand, humic acid treatments significantly enhanced the green color of cucumber fruit skin (Unlu *et al.*, 2011).

The application of calcium nitrate appeared to decrease the color value which could be a negative effect on the quality which leads to browning.

Bolin and Huxsoll (1991a) reported that development of white tissue was due to lignification, which apparently was not influenced by Ca treatment in their experiments. This could be due to inhibition of polyphenol oxidase (PPO) activity by the chloride ion, accompanied by reduced loss of subcellular compartmentalization and subsequent leakage of PPO and its substrates due to the firming action of calcium (Garcia and Barrett, 2002).

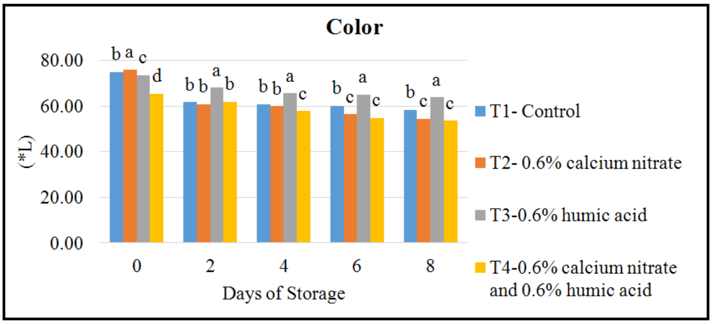

Figure 4. Color as influenced by postharvest humic acid and calcium nitrate treatment {Coefficient of Variation (CV), 0days=0.56%, 2 days=1.83%, 4 days=1.12%, 6 days=1.53%, 8 days=1.24%}.

3.3 CO_2 Concentration

CO_2 concentration and respiration rate of radish were significantly influenced by humic acid and calcium nitrate treatment as shown in Figure 5 to 6. CO_2 concentrations among samples changed accordingly as a response to the respiration process from day 0 until day 8 of storage (Figure 5). In day 0, the CO_2 concentration ranged from 0.33-0.70 part per million (ppm), the higher CO_2 was observed in the control treatment and humic acid treatment and lower at calcium nitrate treatment. Data on day 4 shows that the CO_2 concentration ranges from 0.10-0.16 ppm, humic acid treatment obtained the lowest CO_2 concentration and the three other treatments were not significantly different from each other. In day 8, it was calcium nitrate treatment that got the lowest CO_2 concentration and T3, T4 and control got the highest value and were not significant from each other. The CO_2 concentration during day 8 ranges from 0.60-1.11 ppm. An increase in CO_2 concentration with lower O_2 level is an indication of a lower in respiration rate of the plant. It is beneficial in delaying senescence and controlling fungal growth. However, complete removal of O_2 is not advisable as an anaerobic environment is harmful to the quality of the product as it results in fermentation, decay and development of off flavor, and change in color and texture (Rahman, 2007). According to Kubo *et al.* (1989) that CO_2 had a robust controlling effect on mitochondrial activity, including citrate and succinate oxidation and CO_2 could be suppressed ethylene (C_2H_4) production instead of having a direct effect on the respiration process.

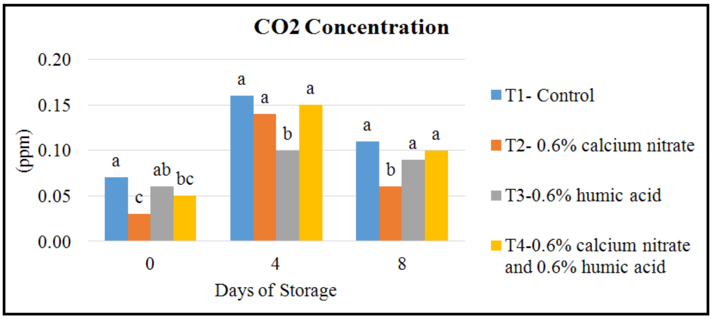

Figure 4. CO_2 concentration as influenced by postharvest humic acid and calcium nitrate treatment {Coefficient of Variation (CV), 0 days= 22.67%, 4 days=9.28%, 8 days=15.38%}.

3.4 Respiration Rate

The respiration rate of radish was significantly influenced by the application of different treatment (Figure 6). There was a significant increase as observed from initial reading to the final readings. The pattern of effects on the initial reading was similar to that of the final reading was control treatment got the highest rate in both initial and final readings. The respiration rate during initial reading ranges from 0.32-0.77 ml CO_2 .kg-1/hour whereas 1.65-2.63 ml CO_2 .kg^{-1}/hour at the final reading. The lowest respiration rate was observed in humic acid alone and calcium nitrate alone.
An increase in the respiratory rate of the study might be due to a dysfunction in the vegetal tissue or to its use as a respiratory substrate, considering that, besides soluble carbohydrates, the organic acids, lipids, and proteins could also be oxidized for energy production during aerobic respiration (Taiz and Zeiger, 2004).

In the present study, there is a reduction in respiration rate with the application of calcium nitrate. It is also possible that such decrease in the respiration rate happens because respiratory substrates stop reacting with the enzymes of the cells present in the cut surface. The subsequent reduction in the respiration rate after one h is probably due to the self-regulation of the respiratory tissue activity caused by the great production of ATP (Purvis, 1997). Faust and Klein (1974) and Bangerth *et al*. (1972) found reduced respiration rates from discs of apple fruit tissue incubated in solutions containing Ca^{2+}.

Some of the reductions in respiration rate may be explained by protective osmotic effects of high salt concentrations. Bangerth *et al*. (1972) suggested that a Ca^{2+} effect could arise from an influence of Ca^{2+} on substrate transport, perhaps indirectly due to Ca^{2+} -mediated alteration of membrane permeability, or from the screening of negative charges in the cell wall. At present, there is less than convincing evidence for Ca^{2+} directly inhibiting senescence by reducing respiration or preventing a respiratory rise, although this does occur in conjunction with other retarding effects of Ca^{2+} (Ferguson *et al*., 1983). Del Aguila *et al*. (2006) also reported that whole, sliced and shredded radish, after processing and storage at 5°C for 1 and 4 h, produced 4.5 and 2.4, 4.6 and 2.9, and 5.6 and 3.4 micrograms of carbon dioxide per kilograms per second (µg CO_2 /kg/s), respectively almost similar with the result of this study.

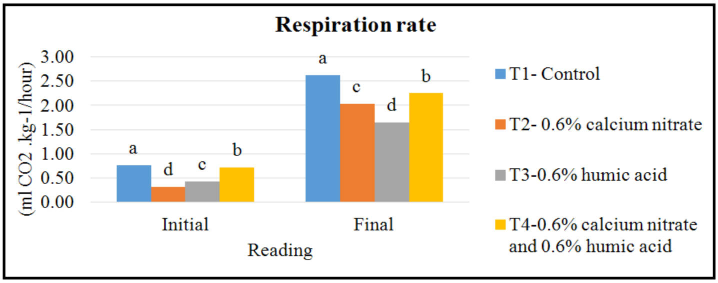

Figure 6. Respiration rate as influenced by postharvest humic acid and Calcium nitrate treatment {Coefficient of Variation (CV), initial=3.96%, final=2.51%}.

4. Conclusion

Humic acid and calcium nitrate significantly enhanced the sprouting and lengthen the shelf life of radish but does not influence the visual quality rating. It also delays the color development of radish which is a good indication for delay browning. On another hand, CO_2 concentration and respiration also affected by different treatment in which application of humic acid reduced the respiration rate of radish during storage. However, control treatment increases the respiration rate of radish indicating a negative effect on radish. Therefore, application of humic acid and calcium nitrate is helpful in reducing respiration rate and prolonging the shelf life of radish.

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