Journal of Agricultural Science; Vol. 4, No. 10; 2012
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Antioxidant Content and Quality of Fruits as Affected by Nigari, an Effluent of Salt Industries, and Fruit Ages of Sweet Pepper (*Capsicum annuum* L.)

M. J. Rahman[1] & H. Inden[1]

[1] Laboratory of Advanced Horticulture, Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan

Correspondence: H. Inden, Laboratory of Advanced Horticulture, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan. Tel: 81-985-58-7164. E-mail: inden@cc-miyazaki.u.ac.jp

Received: June 13, 2012 Accepted: July 3, 2012 Online Published: August 24, 2012

doi:10.5539/jas.v4n10p105 URL: http://dx.doi.org/10.5539/jas.v4n10p105

Abstract

Nigari, an effluent of salt industries, is less expensive fertilizer. Without testing its effect on bioactive substances, it may not be suitable for fertilizer. Greenhouse trials were conducted over two years to evaluate antioxidant content, fruit quality, yield, and mineral contents in fruits and leaves of sweet pepper 'Papri new-E-red' by application of three nigari concentrations at 0, 2 and 4 mL L^{-1} and additional N P K to equal the standard. Capsaicin, β-carotene, ascorbic acid, fruit quality attributes, and fruit mineral compositions were evaluated at five different ages of fruits at 25 Days after fruit set (DFS), 35 DFS, 45 DFS, 55 DFS, and 65 DFS. Results revealed that capsaicin, β-carotene, ascorbic acid, fruit quality, and mineral contents in fruits and leaves of sweet pepper increased with increasing rate of nigari compared to the control. Furthermore, capsaicin, and β-carotene increased linearly with the advancement of fruit ages, but not ascorbic acid. Exceptionally, ascorbic acid increased until the turning of fruit maturity at 45 DFS and after that decreased drastically. Total soluble solid (^{o}Brix) and titratable acidity (TA) were higher at 45 DFS, although an increasing trend was found for most of the mineral contents with maturing fruits. We concluded that a higher amount of antioxidant and improved fruit quality with higher yield and moderate mineral contents in fruits could be achieved by application of 2 mL L^{-1} nigari to sweet pepper in soilless culture. We can also suggest harvesting sweet pepper fruits between 45 to 55 DFS.

Keywords: capsaicin, β-carotene, ascorbic acid, mineral contents in leaves and fruits

1. Introduction

There is increasing attention being given towards reducing the production cost of agricultural crops. Nigari application in soilless culture can contribute to the agricultural production at low cost as it contains many macro and micro nutrients and can be an alternative fertilizer source. Nigari is an effluent of salt industries, and cheaper than the commercial fertilizers. Nigari contains high amounts of calcium (Ca^{2+}), magnesium (Mg^{2+}) and other micronutrients that may have an effect on quality, and antioxidant content of crops. It also contains some extent of sodium (Na^{+}) that may impose mild salinity, but it contains some amount of silicon (Si) that may minimize the negative impact of salinity (Bradbury & Ahmad, 1990; Liang et al., 1996). Significant economic losses of horticultural crops have been linked to inadequate Ca^{2+} nutrition (Grattan & Grieve, 1999). Nigari can supply adequate Ca^{2+} with micronutrients to sweet peppers. Thus, it can reduce fertilizer inputs and make agricultural practices more sustainable. However, there has been no research on nigari application and its impact on bioactive compounds, even though it is used as alternative fertilizer in Japan. Therefore, it is desirable to investigate the effect of nigari on antioxidant contents, yield, and fruit quality on crops like sweet pepper.

Sweet pepper (*Capsicum annuum* L.) can be considered a functional food, because it contains many health-promoting phytochemicals such as vitamins A, B, C, E, phenolic compounds, carotenoids, and capsaicin (Bloch & Thomson, 1995). These compounds are reported to have antioxidant, anticarcinogenic, antimutagenic, anti-aging, and antibacterial properties (Chu et al., 2002; Surh, 2002). Antioxidants are of particular interest because they reduce free radicals and reactive oxygen species. In addition to their role in defense against human diseases, antioxidants have an important role in plant defense and are produced in response to both biotic and abiotic stresses (Sakihama et al., 2002; Slater et al., 2003).

Capsaicin, a phytochemical, produced only in *Capsicum* spp. is widely using in food, medicine and pharmaceutical industries (Prasad et al., 2005). It also shows a high antioxidant activity (Lee et al., 1995) and its content in sweet pepper is very low as compared to hot chilies. Sweet pepper is usually consumed as fresh vegetable and if we can improve capsaicin content in sweet peppers in commercial cultivars, we can take more capsaicin in fresh vegetables.

Fresh sweet peppers are a significant source of provitamin A and have a noticeable level of flavonoids and phenolic acids (Mar´in et al., 2004). These phytochemicals and vitamins are responsible for high antioxidant activity that has been strongly correlated with several human health disorders (Chu et al., 2002). Paprika carotenoids are also used in the food industry as natural colorants.

Fresh sweet peppers have been assessed as one of the main food sources of ascorbic acid (vitamin C), as a serving of 100 g of fresh peppers supplies more than 100% of the recommended dietary allowance (RDA of 60 mg day^{-1}) (Simonne et al., 1997).

The antioxidant content of fruits and vegetables is becoming increasingly important for growers who want to satisfy the demand of consumers for products with a high content of health-promoting constituents. An increase in antioxidant content in fresh peppers can be attained by improving crop production practices, e.g. selection of varieties rich in phytochemicals and optimization of plant nutrition and water supply (Lee et al., 1995). Furthermore, nigari, as cheaper fertilizer alternative, maybe used for improving the antioxidant content in fruits of sweet pepper as well as reducing production cost.

Composition and concentration of antioxidant can vary in owing to maturation of fruits, and synthesis and degradation also differed for individual antioxidant. It is important to find out a suitable fruit age, which could supply a much amount of different antioxidant combinations, for example capsaicin, β-carotene, and ascorbic acid. Therefore, the present work was aimed to study the effect of nigari on antioxidant content, fruit quality, plant tissue mineral compositions, and yield of sweet pepper. Furthermore, the aim was to evaluate the effect of fruit ages on antioxidant content, fruit quality, and fruit mineral compositions.

2. Materials and Methods

2.1 Experimental Site, Plant Materials and Growing Conditions

Two repeated trials were conducted in the greenhouses at University of Miyazaki, Japan. The transplanting and final harvesting dates of the first trial were 25 November 2010 and 10 June 2011, and of the second trial were 18 February 2011 and 24 August 2011, respectively. Seeds of sweet pepper cv. 'Papri new-E-red' (Marutane Seed Co., Kyoto, Japan) was selected on the basis of yield performance under high temperature conditions (Rahman & Inden, 2012). Two 8-week-old seedlings were transplanted 20 cm apart into 40 L containers containing a 50:45:5 (v/v) mixture of bora (volcanic soil), perlite and shodo (burned loam soil), respectively. Each row consisted of 15 containers and treated as a replication. The nutrient solution was continuously applied to the crop by ultra-drip irrigation tube with pH and electrical conductivity (EC) controlled nutrient solution (Table 2). The average temperatures during cultivation were 22±2 ^{o}C (day) and 18±2^{o}C (night), and 29±2 ^{o}C (day) and 21±2 ^{o}C (night), respectively for trial 1 and trial 2.

2.2 Experimental Design and Treatments

The 3 × 5 factorial experiment in a randomized completely block design consisted of three levels of nigari concentrations and five levels of fruit ages, resulting in 15 treatment combinations replicated three times. Two plants served as an experimental unit. Three levels of nigari concentrations were: standard nutrient solution as control, 2 mL L^{-1} nigari + additional N P K to equal the standard, and 4 mL L^{-1} nigari + additional N P K to equal the standard; and five levels of fruit ages were: 25 days after fruit set (DFS), 35 DFS, 45 DFS, 55 DFS, and 65 DFS. The standard nutrient solution composition was selected according to Rahman & Inden (2012). Standard nutrient solution was continuously applied to the plants for all treatments until one month after transplanting. After that time, nigari treatments were started and applied every day until harvest. Nigari was collected from Miyazaki Sun Salt Co., Limited, Miyazaki, Japan and was analyzed by inductively coupled plasma emission spectroscopy (ICPS-8100, Shimadzu Corp., Kyoto, Japan) for its nutritional composition (Table 1). We did not measure chloride (Cl^{-}), but we have a plan to analyze Cl^{-} in our future works.

Table 1. Composition of nigari analyzed by inductively coupled plasma emission spectroscopy.

Components	P	K	Ca	Mg	S	Fe	Mn	Zn	B	Mo	Na	Si
(mg L^{-1})	1860	63081	94658	139669	96854	4133	1471	53	941	454	32525	642

Table 2. Macro and micro nutrients, electrical conductivity (EC), and pH of nigari treatments

Nigari concentration	Macro nutrients (meq L^{-1})						pH	EC ($dS\ m^{-1}$)
	NO_3^-	P	K	Ca	Mg	S		
0 (control)	17.05	7.86	8.94	9.95	6.00	6.00	≈6.0	2.8
2 mL L^{-1}	17.05	7.86	8.94	9.47	23.28	12.11	≈6.0	3.5
4 mL L^{-1}	17.05	7.86	8.94	18.93	46.56	24.21	≈6.0	3.9
	Micro nutrients (μg L^{-1})							
	Fe	Mn	Zn	B	Mo	Cu	Na	Si
0 (control)	3000	1000	100	500	25	30	-	-
2 mL L^{-1}	8266	2942	106	1882	908	-	6505(	1284
4 mL L^{-1}	16532	5884	212	3764	1816	-	13010(	2568

2.3 Antioxidants Analysis

Capsaicin, β-carotene and ascorbic acid were measured by HPLC (LC-2010A HT, Shimadzu Corp., Kyoto, Japan) and individual analyzing procedure was described separately.

2.3.1 Measurement of Capsaicin

Capsaicin was measured according to the protocol of Collins et al. (1995) with little modifications. The absorbance detector was set at 280 nm and a Cadenza CD-C_{18} column of 75×4.8 mm size was used. In brief, the sliced fruits were dried at 70 ^oC for five days and ground with a 0.25 mm screen. A 1:10 (g: mL) ratio of dried sweet pepper powder to acetonitrile was placed into the bottles with lids. The bottles were placed in an 80 ^oC water bath for 4 h. The supernatant was filtered by 0.20 μm filter unit for using HPLC injection. The mobile phase was low pressure gradient with 70% solvent B (100% methanol) and 30% solvent A [10% methanol (by volume) in water], and flow rates of 1 mL min^{-1} and run duration of seven minutes were used. Capsaicin standard (8-methyl-n-vanillyl-6-nonenamide) (Sigma-Aldrich Co., MO, US.) was used for retention time verification and preparation of standard curve by using 1, 5, 10, 50, 100 and 500 ppm capsaicin in ethanol.

2.3.2 Measurement of β-carotene

β-carotene was measured according to Hussein et al. (2000) with some modifications. The absorbance detector was set at 450 nm and an YMC-Trait C18/S-3μm column of 75×4.8 mm size was used. In brief, 10 g in duplicate of the homogenized samples were extracted with 1 g $MgCO_3$, 10 g anhydrous Na_2SO_4 and 75 mL tetra-hydrofuron (THF) (stabilized with 0.01% butylated hydroxyl toluene). The extraction was repeated using 75 mL THF. The filtrates were combined and evaporated at <40 ^oC using rotary evaporator until almost dry and then slight N_2 stream was applied to the point of dryness. The residue was made up to 25 mL with mobile phase. The extracts were stored at -20 ^oC before injecting into the HPLC. A 25 μL extract was injected into HPLC for analysis. The mobile phase was a low pressure gradient with 100% solvent A, which was composed of acetonitrile: dichloromethane: methanol at the ratio of 7:2:1 (v/v/v) and flow rates of 1 mL min^{-1} and run duration of eight minutes were used. Standard β-carotene (Nacalai Tesque Inc., Kyoto, Japan) was used for retention time verification and standard curve preparation using 0.2, 0.5 and 1.0 ppm standard β-carotene.

2.3.3 Measurement of Ascorbic Acid

The extraction of ascorbic acid was performed following the method of Wimalasiri & Wills (1983) with some modifications. The samples were prepared immediately after harvest under reduced light to avoid loss of ascorbic acid. One gram of fresh sweet pepper fruit was dried into liquid nitrogen and extracted with 10 mL of 3% metaphosphoric acid (HPO_3). The extract of 5μL was immediately injected into HPLC. The absorbance detector was set at 255 nm and a Cadenza CD-C_{18} column of 75×4.8 mm size was used. The mobile phase was low pressure gradient with 10 mM ammonium dihydrogen phosphate ($NH_4H_2PO_4$), pH 4.0. Flow rates of 1 mL min^{-1} and run duration of eight minutes were used. Stand curve was prepared by using standard L-ascorbic acid of 10, 50, 100, 250 and 500 ppm in methanol.

2.4 Fruit Quality Measurement

Fruit quality attributes namely fruit shape index (FSI), pH, total soluble solids (^{o}Brix), titratable acidity (TA) and maturity index (MI) were measured. FSI was defined by the equatorial (diameter) to longitudinal length ratio. Total soluble solid was determined by digital refractometer and expressed as °Brix at 20 °C. TA was determined by potentiometric titration with 0.1 M NaOH to pH 8.1, using 10 mL of fruit extract and results were expressed as a percentage of citric acid in the juice, and MI was calculated by the ratio of ^{o}Brix to TA.

2.5 Leaves and Fruits Tissue Analysis

Leaf tissues were analyzed at the end of the experiment according to the nigari treatments only, but fruit tissues were analyzed according to fruit ages and nigari treatments. Fruits and leaves were dried at 105 °C for 96 h. The dried leaves and fruit samples were ground and digestion was done according to Rodushkin et al. (1999). NO_3-N, PO_4^{3-}, Ca^{2+}, K^+, Mg^{2+}, SO_4^{2-}, Na^+ and Cl^- were analyzed using HPLC ion analyzer (IA 300, DKK-TOA Corporation, Tokyo, Japan). Manganese (Mn) and iron (Fe) were analyzed using RQflex® 10 (Merck chemicals, Germany). Zinc (Zn) was analyzed using a spectrophotometer (DR 2800, HACH Co., USA) at 620nm.

2.6 Yield Parameters

Average fruit fresh weight, number of fruit per plant, percent of blossom-end rot (%BER) and yield per plant of each of the nigari treatments were recorded during the experiment.

2.7 Statistical Analysis

Data of two trials were combined and analyzed by two-way analysis of variance (ANOVA) using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) and the differences among means were determined using Tukey's test. But leaves mineral compositions and yield parameters were analyzed by one-way analysis of variance (ANOVA) for nigari treatments and the differences among the means were determined using Tukey's test.

3. Results and Discussion

3.1 Capsaicin Content

Capsaicin concentration in fruits was significantly affected by nigari, fruit age and their interactions (Table 3). Results revealed that capsaicin concentration increased with increasing rate of nigari. The highest capsaicin was found at 4 mL L^{-1} nigari, the highest nigari concentration of this experiment. A 2.5-fold higher capsaicin concentration was found at 4 mL L^{-1} nigari compared to the control. This result indicated that nigari had an effect on biosynthesis of capsaicin in fruits of sweet pepper. Jurenitsch et al. (1979) stated that capsaicinoids content ranged from 0.07% to 1.5% DW of fruit in some cultivars of *Capsicum annuum* L. Estrada et al. (1997) found 0.6% (fruit), 0.7% (seed), and 0.03% (pericarp) capsaicin content in *Capsicum annuum* L. var. Padron. Our study showed that the fruits without seeds of sweet pepper cv. 'Papri new-E-red' contained 0.74 mg 100 g^{-1} FW capsaicin, which was lower than the previous reports, but it was higher compared to the control. Pungency of fruits of genus *Capsicum* depends on the interaction between phenotype and environment (Contreras-Padilla & Yahia, 1998; Estrada et al., 1997). Ruiz-Lau et al. (2011) stated that water stress enhanced the activity of capsaicin synthase and decreased in the activity of peroxidase. In fact, nigari contains Na^+ that might have imposed mild salinity stress on pepper and EC level was also higher than the control. As a result, it might have enhanced the activity of capsaicin synthase, which is a very positive outcome for improving capsaicin content in sweet pepper fruit.

Table 3. Effect of nigari concentrations and fruit ages on antioxidant content in fruit of sweet pepper

Treatments	Capsaicin	β-carotene	Ascorbic acid
	(mg100 g^{-1} FW)		
Nigari (N)			
0 (control)	0.34 c[z]	1.60 c	142.55 c
2 mL L^{-1}	0.59 b	2.15 b	158.60 b
4 mL L^{-1}	0.74 a	2.81 a	167.05 a
Fruit ages (M)			
25 DFS	0.12 e	0.63 e	132.80 c
35 DFS	0.40 d	0.87 d	183.52 b
45 DFS	0.62 c	1.84 c	221.74 a
55 DFS	0.80 b	3.24 b	124.26 d
65 DFS	0.84 a	4.35 a	118.02 d
Significance			
N	***	***	***
M	***	***	***
N × M	***	***	***

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. Significance represents for ANOVA of nigari, fruit age, and interaction of N× M. *** significant at $P \leq 0.001$. DFS = Days after fruit set.

Regarding fruit ages, capsaicin content in fruit significantly increased with the advancement of fruit age from 25 DFS until 65 DFS. Our result revealed that the increment of capsaicin concentration was faster until 55 DFS and after that the increment was comparatively slower. But the capsaicin concentration was almost 7-folds higher at 65 DFS compared to 25 DFS. This might be due to the activities of capsaicin synthase and peroxidase. Contreras-Padilla & Yahia (1998) found a peak of capsaicinoid between 45 and 50 days after anthesis (DAA), and the maximum peroxidase activity found at 60 DAA in *C. chinense* fruits. But our study revealed that the capsaicin concentration was increased until 65 DFS without showing any peak.

Regarding interaction, the highest capsaicin content was found at 65 DFS under 4 mL L^{-1} nigari, meanwhile the lowest at 25 DFS under the control treatment (Data not shown).

3.2 β-carotene Content

Significant variability existed for β-carotene accumulation among the nigari treatments, fruit ages and their interaction (Table 3). The highest β-carotene was found at 4 mL L^{-1} nigari treatment, meanwhile the lowest at the control. It indicated that β-carotene accumulation increased with increasing rate of nigari, and almost double concentration was found at 4 mL L^{-1} nigari. Mar´in et al. (2004) stated that β-carotene content in sweet pepper cv. Vergasa ranged from 1.7 to 4.3 mg 100 g^{-1} FW. Our result agreed with this finding, but comparatively higher β-carotene was found in sweet pepper cv. 'Papri new-E-red' due to application of nigari. Oh et al. (2010) stated that mild water stress was better for enhancing phytochemical concentration in lettuce. Nigari also contained Si that might have effect on improvement of β-carotene content in sweet pepper. Stamatakis et al. (2003) observed that Si enhanced β-carotene synthesis in tomato that agreed our results in sweet pepper.

There was sharp increase in β-carotene accumulation with advancement of fruit age, which was found from 25 DFS to 55 DFS and after that increment was comparatively slower. However, with the advancing fruit ages about 7-folds variation was observed at 65 DFS.

In case of interactions between nigari and fruit age, the highest β-carotene content in sweet pepper fruits cv. 'Papri new-E-red' was found at 65 DFS under 4 mL L^{-1} nigari, meanwhile the lowest at 25 DFS under the control (Data not shown).

3.3 Ascorbic Acid Content

Results revealed that ascorbic acid content increased with increasing rate of nigari (Table 3). The highest ascorbic acid content was found at 4 mL L^{-1} nigari. Frary et al. (2008) showed that ascorbic acid content varied from 522 to 1631 mg kg^{-1} depending on pepper types. But a notable increment of ascorbic acid was found in our experiment. This might be due to nigari might have imposed mild salinity to the sweet pepper plant causing higher ascorbic acid. Environmental stresses, like mild water stress/drought stress, have been showed to activate the genes involved in the biosynthesis of some antioxidants and phytochemicals in many plants like lettuce (Oh et al., 2009).

Ascorbic acid content was found to vary significantly among different ages of fruit (Table 3). The highest ascorbic acid was found at 45 DFS and the lowest at 65 DFS. Ascorbic acid content showed increasing trend from 25 DFS until 45 DFS and after that a declining trend was found with advancing fruit maturity. A clear peak for ascorbic acid accumulation at 45 DFS was found and after that it was drastically reduced. Josefa et al. (2006) reported that the ascorbic acid content in sweet pepper was dependent on the fruit maturity state. Some authors have found divergences in ascorbic acid content of 30% between red and green peppers (Howard et al., 1994). Our results agreed with these findings, and 45 DFS contained more ascorbic acid. Furthermore, other authors have found that ascorbic acid increased or remained constant as pepper fruits matured (Howard et al., 2000), and declined with further ripening (Gnayfeed et al., 2001). Our results also supported these findings.

Regarding the interactions between nigari and fruit ages, the highest ascorbic acid was found at 45 DFS at 4 mL L^{-1} nigari, meanwhile the lowest at 65 DFS under the control (Data not shown).

3.4 Fruit Quality Characteristics

The fruit quality characteristics of sweet pepper cv. 'Papri new-E-red' so as FSI, pH and MI decreased with increasing rate of nigari, but increasing trend was found for TA and ^{o}Brix (Table 4). The highest FSI, pH and MI were found in the control, meanwhile the highest ^{o}Brix and TA were found at 4 mL L^{-1} nigari. TA and ^{o}Brix were two important traits for improvement of fruit quality, which could be improved by application of nigari. Ehret & Ho (1986) stated that plant under salinity accumulated less water and have a reduced water uptake that improved fruit quality in tomato. Nigari contained some amount of Na^{+} that might have created mild-salinity and it decreased water uptake of the plants. As a result, ^{o}Brix and TA increased with increasing rate of nigari. Our results also agreed with previous findings that showed a positive influence of salinity and water stress on quality

of tomato (Del Amor et al. 2001).

Table 4. Effect of nigari concentrations and fruit ages on fruit quality attributes of sweet pepper

Treatment	FSI	pH	TA (% citric acid)	°Brix (%)	MI
Nigari (N)					
0 (control)	0.80 a[z]	5.34 a	0.17 c	7.32 c	46.59 a
2 mL L^{-1}	0.79 b	5.13 b	0.20 b	7.66 b	40.01 a
4 mL L^{-1}	0.76 c	5.03 c	0.21 a	8.06 a	39.15 b
Fruit ages (M)					
25 DFS	0.70 d	5.47 a	0.11 e	5.14 e	47.61 a
35 DFS	0.78 c	5.33 b	0.14 d	6.57 d	48.17 a
45 DFS	0.81 b	5.16 c	0.21 c	8.98 b	42.90 b
55 DFS	0.82 a	4.96 d	0.27 a	9.58 a	35.83 c
65 DFS	0.82 a	4.93 e	0.23 b	8.11 c	35.07 c
Significance					
N	***	***	***	***	***
M	***	***	***	***	***
N × M	NS	***	***	***	***

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. Significance represents for ANOVA of nigari, fruit age and interaction of N × M. NS nonsignificant at $P \geq 0.05$, and *** significant at $P \leq 0.001$. FSI = fruit shape index, TA = titratable acidity, Total soluble solid = °Brix (%), MI = maturity index, DFS = Days after fruit set.

Regarding fruit ages, FSI, °Brix and TA of sweet pepper fruits increased with advancing fruit ages, meanwhile pH and MI decreased. FSI increased until 45 DFS and after that, FSI was statistically similar to that of 55 DFS and 65 DFS, although it was increasing. The pH and °Brix increased until 55 DFS and after that decreased. An increase in °Brix during ripening process in sweet pepper fruit may probably be due to accumulation of more sugars in the fruit due to hydrolysis of starch and slight decline at over ripe stage is owing to utilization of sugars during respiration process. Similar trend of °Brix in sweet pepper cv. Domino was observed by Tadesse et al. (2002).

In case of interactions between nigari and fruit age, the TA and °Brix in sweet pepper fruits cv 'Papri new-E-red' was found at 55 DFS under 4 mL L^{-1} nigari, meanwhile the lowest at 25 DFS under the control (Data not shown).

3.5 Mineral Compositions in Fruit

Mineral compositions in fruits were significantly affected by nigari treatments, fruit ages and interactions (Table 5). Among the minerals, NO_3-N, K^+, Mg^{2+}, Ca^{2+} and SO_4^{2-} concentrations appeared to increase with increasing rate of nigari. All these minerals sharply increased at 2 mL L^{-1} nigari, except for NO_3-N. The content of NO_3-N in fruits increased until 4 mL L^{-1} nigari. On the contrary, PO_4^{3-} accumulation in fruits decreased with increasing rate of nigari. Furthermore, micronutrients, including Na^+ and Cl^- showed a continuous increment until 4 mL L^{-1} nigari. Bar-Tal et al. (2001a) stated that Ca^{2+} deficiency in tomato fruit may be caused by inadequate supply of Ca^{2+} in the solution. We found the highest Ca^{2+} concentration in fruits at 2 mL L^{-1} nigari, which indicated a sufficient supply of Ca^{2+} in the solution. Because lower %BER, which was related to the concentration of Ca^{2+} in fruits, found in the same treatment. High K^+ level increased fruit acidity and decreased maturity index (Usherwood, 1985). We found high level of K^+ in fruits at 2 mL L^{-1} nigari that could improve fruit quality.

Table 5. Effect of nigari concentrations and fruit ages on mineral concentrations in fruits of sweet pepper

Treatment	NO_3-N	PO_4^{3-}	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Na^+	Cl^-	Mn	Fe	Zn
	g Kg^{-1} DW								ppm		
Nigari (N)											
0 (control)	0.29 c[z]	2.09 a	0.65 c	0.79 b	0.04 c	0.09 c	0.03 c	0.13 c	9.12 c	6.47 c	7.5 c
2 mL L^{-1}	0.32 b	1.88 b	0.94 a	1.94 a	0.98 a	0.15 a	0.05 b	0.19 b	12.54 b	8.82 b	8.65 b
4 mL L^{-1}	0.34 a	0.90 c	0.71 b	0.78 b	0.75 b	0.11 b	0.09 a	0.23 a	15.33 a	12.20 a	12.25 a
Fruit age (M)											
25 DFS	0.21 d	1.33 e	0.31 e	0.57 e	0.05 d	0.05 d	0.02 d	0.11 e	5.17 e	5.05 e	5.03 e
35 DFS	0.30 c	1.46 d	0.70 d	1.26 d	0.57 c	0.10 c	0.03 c	0.14 d	10.14 d	8.07 d	8.22 d
45 DFS	0.34 b	1.68 c	0.83 c	1.38 c	0.76 b	0.13 b	0.06 b	0.20 c	13.74 c	9.49 c	10.67 c
55 DFS	0.35 ab	1.77 b	0.93 b	1.34 b	0.78 ab	0.14 a	0.07 b	0.22 b	15.64 b	11.20 b	11.46 b
65 DFS	0.37 a	1.87 a	1.07 a	1.31 a	0.79 a	0.16 a	0.09 a	0.24 a	16.96 a	12.01 a	11.96 a
Significance											
N	***	***	***	***	***	***	***	***	***	***	***
M	***	***	***	***	***	***	***	***	***	***	***
N × M	NS	***	***	***	***	NS	***	***	***	***	***

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. Significance represents for ANOVA of nigari, fruit age and interaction of N × M. NS nonsignificant at $P \geq 0.05$ and *** significant at $P \leq 0.001$. DFS = Days after fruit set.

The various mineral elements entered at the different rates during aging of the fruits. The content of NO_3-N was increasing with the advancement of fruit ages, but the increasing rate was faster until 45 DFS and after that it became slower. Similarly, PO_4^{3+}, K^+, Mg^{2+}, and SO_4^{2-} increased with the maturing fruits, but the increasing rates were slower after 45 DFS. On the contrary, Ca^{2+} entered the fruit until 45 DFS treatment and afterthat it was decreasing. Transportation of Ca^{2+} in the plant is dominated by transport via xylem and new developing organs contain low Ca^{2+} concentrations (Ho et al., 1993). Bar-Tal et al. (2001b) also stated that Ca^{2+} concentration in the sweet pepper fruit decreased during fruit growth and ripening. Our result agreed with these findings, but the concentration of Ca^{2+} in fruit throughout the developmental stages was relatively higher than the previous reports. Furthermore, all micro nutrients, including Na^+ and Cl^- increased steadily until 65 DFS.

In case of interaction between nigari and fruit age, all macro nutrients studied in this experiment were the highest at 65 DFS under 2 mL L^{-1} nigari, except for NO_3-N which was the highest at 65 DFS under 4 mL L^{-1} nigari. On the other hand, all micro nutrients were the highest at 65 DFS under nigari at 4 mL L^{-1} (Data not shown).

3.6 Mineral Compositions in Leaves

Mineral compositions in leaves were significantly affected by nigari concentration (Table 6). Results revealed that NO_3-N and PO_4^{3-} concentrations decreased with increasing rate of nigari. Meanwhile, Mg^{2+}, Ca^{2+}, and SO_4^{2-} increased at 2 mL L^{-1} nigari, but decreased at 4 mL L^{-1} nigari. Furthermore, micronutrients including Na^+ and Cl^- increased with increasing rate of nigari. O'Sullivan (1979) stated that the critical level of plant tissue NO_3-N at least 4000 mg kg^{-1} is necessary to maintain fruit yield of sweet pepper. Our results showed that all the treatments had a higher level of tissue NO_3-N than the critical levels, although it was decreasing with increasing rate of nigari. The concentration of K^+ increased at 2 mL L^{-1} nigari and it was decreased at 4 mL L^{-1} nigari. Chartzoulakis and Klapaki (2000) reported that K^+ concentration in leaves of sweet pepper was not affected by salinity. Our result partially agreed with this result because K^+ concentration was higher at 2 mL L^{-1} nigari, but lower at 4 mL L^{-1} nigari. This suggests that sweet pepper plants are able to maintain high K^+ level in leaf lamina under mild external salinity due to application of 2 mL L^{-1} nigari. Fe and phosphorus are antagonistic to each other (Gutschick, 1987) and our result agreed with this finding. Leaf Fe increased with the increase of Fe in nutrient solutions, but leaf PO_4^{3-} concentration decreased with the increase of leaf Fe. The classical antagonism between leaf Fe and Mn was not evident in this study.

Table 6. Effect of nigari concentration on mineral contents in leaves of sweet pepper

Nigari treatment	NO_3-N	PO_4^{3-}	K^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Na^+	Cl^-	Mn	Fe	Zn
	g Kg^{-1} DW								ppm		
0 (control)	6.72 a[z]	3.11 a	42.6 b	20.4 c	9.01 c	1.49 c	1.18 c	2.32 c	57.3 c	26.3 c	20.8 b
2 mL L^{-1}	6.49 b	3.09 b	45.0 a	32.8 a	15.96 a	3.07 a	2.17 b	3.22 b	66.5 b	41.0 b	20.0 b
4 mL L^{-1}	4.87 c	2.66 c	33.9 c	22.9 b	11.50 b	1.83 b	4.08 a	5.05 a	76.3 a	49.8 a	27.3 a
Significance	***	***	***	***	***	***	***	***	***	***	***

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. Significance represents of one-way ANOVA for three levels of nigari concentrations (0, 2, and 4 mL L^{-1}). *** significant at $P \leq 0.001$.

3.7 Yield and Yield Components

Average fruit fresh weight, number of fruit, %BER, and yield were varied significantly by the application of nigari (Table 7). Average fruit fresh weight decreased with increasing rate of nigari and the highest fruit fresh weight was found from the control. On the contrary, number of fruit per plant was the highest at 2 mL L^{-1} nigari and the lowest at 4 mL L^{-1} nigari. As expected, %BER was the lowest at 2 mL L^{-1} nigari and it was the highest at 4 mL L^{-1} nigari. Per cent BER is one of the crucial problems in sweet pepper that reduce total marketable yield. Our result revealed that some degree of %BER might be reduced by application of nigari. It contains higher amount of Ca^{2+} and it might have contributed to the decrease of %BER. On the contrary, 4 mL L^{-1} nigari might have imposed more osmotic stress, causing higher %BER. Water stress and osmotic stress reduce Ca^{2+} transport particularly to the distal end region of sweet pepper fruit, where BER develops (Silber et al., 2005). The highest yield per plant was found at 2 mL L^{-1} nigari compared to the control. This might be due to the lower %BER and higher number of fruit per plant. Furthermore, nigari contains Si that might have a positive effect on fruit yield in sweet pepper. Stamatakis et al. (2003) found a positive effect of Si addition to the nutrient solution under saline condition in tomato fruit yield. Alexander & Clough (1998) also observed that marketable yield of pepper increased due to increased supply of Ca^{2+}, mainly because of decrease in BER-affected fruits. We observed similar results at 2 mL L^{-1} nigari applied in sweet pepper.

Table 7. Effect of nigari concentrations on average fruit fresh weight, number of fruit per plant, blossom-end rot and yield of sweet pepper

Nigari treatment	Average fruit fresh wt. (g)	No. of fruit per plant	% BER (by no.)	Yield/plant (g)
0 (control)	170.51 a[z]	18.32 b	16.36 b	2391.67 b
2 mL L^{-1}	162.87 b	21.92 a	13.55 c	2622.47 a
4 mL L^{-1}	146.45 c	16.09 c	21.49 a	2039.93 c
Significance	***	***	***	***

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. Significance represents of one-way ANOVA for three levels of nigari concentrations (0, 2, and 4 mL L^{-1}). *** significant at $P \leq 0.001$.

4. Conclusions

Nigari, effluent of salt industries, can significantly improve antioxidant content, fruit quality, and yield of sweet pepper cv. 'Papri new-E-red' under soilless culture. Capsaicin, β-carotene and ascorbic acid increased with increasing rate of nigari as compared to the control. Nigari at 2 mL L^{-1} improved yield and decreased %BER. Furthermore, mineral compositions in leaves and fruits also improved. Regarding fruit ages, accumulation of antioxidant, fruit quality, and mineral compositions in leaves and fruits of sweet pepper differed significantly with advancing fruit age. Capsaicin and β-carotene increased with the advancement of fruit age, whereas ascorbic acid decreased after 45 DFS. Furthermore, TA and ^{o}Brix were the highest at 45 DFS. Therefore, moderate amount of capsaicin, β-carotene, ascorbic acid, and fruit mineral contents with higher TA and ^{o}Brix contained fruits will be obtained at 45 to 55 DFS. Therefore, we can conclude that nigari at 2 mL L^{-1} can be applied in sweet pepper cv. 'Papri new-E-red' for producing a higher yield of high quality fruits and moderately amount of antioxidant compounds. Furthermore, we suggest harvesting sweet pepper fruit between 45 to 55 DFS.

References

Alexander, S. E., & Clough, G. H. (1998). Spun bonded row cover and calcium fertilization improve quality and yield in bell pepper. *HortScience, 33*, 1150-1152.

Bar-Tal, A., Aloni, B., Karmi, L., Oserovitz, J., Hasan, A., Itach, M., Rosenberg, R. (2001b). Nitrogen nutrition of greenhouse pepper. I. Effect of nitrogen concentration and NO_3:NH_4 on yield, fruit shape and the incidence of blossom-end rot in relation to mineral composition. *HortScience, 36*, 1244-1251.

Bar-Tal, A., Aloni, B., Karni, L., & Rosenberg, R. (2001a). Nitrogen nutrition of greenhouse pepper. II. Effects of nitrogen concentration and NO_3:NH_4 ratio on growth, transpiration, and nutrient uptake. *HortScience, 36*, 1252-1259.

Bloch, A., & Thomson, C. A. (1995). Position of the American Dietetic Association: phytochemicals and functional foods. *J. Am. Diet. Assoc., 95*, 493-496. http://dx.doi.org/10.1016/S0002-8223(95)001130-1

Bradbury, M., & Ahmad, R. (1990). The effect of silicon on the growth of *Prosopis juliflora* growing in saline soil. *Plant Soil*, *125*, 71-78. http://dx.doi.org/10.1007/BF00010745

Chartzoulakis, K., & Klapaki, G. (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. *Sci. Hortic.*, *86*, 247-260. http://dx.doi.org/10.1016/S0304-4238(00)00151-5

Chu, Y. F., Sun, J., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *J. Agric. Food Chem.*, *50*, 6910-6916. http://dx.doi.org/10.1021/jf020665f

Collins, M. D., Mayer-Wasmund, L., & Bosland, P. W. (1995). Improved method for quantifying capsaicinoids in *Capsicum* using high-performance liquid chromatography. *HortScience*, *30*, 137-139.

Contreras-Padilla, M., & Yahia, E. M. (1998). Changes in capsaicinoids during development, maturation and senescence of chile peppers and relation with peroxidase activity. *J. Agric. Food. Chem.*, *46,* 2075-2079. http://dx.doi.org/10.1021/jf970972z

Del Amor, F. M., Mart´ınez, V., & Cerda, A. (2001). Salt tolerance of tomato plants as affected by stage of plant development. *HortScience*, *36*, 1260-1263.

Ehret, D. L., & Ho, L. (1986). The effects of salinity on dry matter partitioning and fruit growth in tomatoes grown in nutrient film culture. *HortScience, 61*, 361-367.

Estrada, A. B., Pomar, F., Díaz, J., Merino, F., & Bernal, A. (1997). Evolution of capsaicinoids in *Capsicum annuum* L. var. *annuum* cv. Pardrón fruit at different growth stages. *Capsicum and Eggplant Newsletter*, *16*, 60-63.

Frary, A., Keçeli, M. A., Ökmen, B., Şiğva, H. Ö., Yemenicioğlu, A., & Doğanlar, S. (2008). Water-soluble antioxidant potential of turkish pepper cultivars. *HortScience*, *43*, 631-636.

Gnayfeed, M. H., Daood, H. G., Biacs, P. A., & Alcaraz, C. F. (2001). Content of bioactive compounds in pungent spice red pepper (paprika) as affected by ripening and genotype. *J. Sci. Food Agric.*, *81*, 1580-1585. http://dx.doi.org/10.1002/jsfa.982

Grattan, S. R., & Grieve, C. M. (1999). Salinity mineral nutrient relations in horticultural crops. *Sci. Hortic., 78*, 127-157. http://dx.doi.org/10.1016/S0304-4238(98)00192-7

Gutschick, V. P. (1987). *A functional biology of crop plants*. Timber Press, Portland, Ore.

Ho, L. C., Belda, R., Brown, M., Andrews, J., & Adams, P. (1993). Uptake and transport of calcium and the possible causes of blossom-end rot in tomato. *J. Exp. Bot.*, *44*, 509-518. http://dx.doi.org/10.1093/jxb/44.2.509

Howard, L. R., Smith, R. T., Wagner, A. B., Villalon, B., & Burns, E. E. (1994). Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapenos. *J. Food Sci.*, *59*, 362-36. http://dx.doi.org/10.1111/j.1365-2621.1994.tb06967.x

Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *J. Agric. Food Chem., 48*, 1713-1720. http://dx.doi.org/10.1021/jf990916t

Hussein, A., Odumeru, J. A., Ayanbadejo, T., Faulkner, H., McNab, W. B., Hager, H., & Szijarto, L. (2000). Effect of processing and packaging on vitamin C and β-carotene content of ready-to-use (RTU) vegetable. *Food Res. Int., 33*, 131-136. http://dx.doi.org/10.1016/S0963-9969(00)00027-2

Josefa, M. N., Pilar, F., Consuelo, G., & Vicente, M. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chem.*, *96*, 66-73. http://dx.doi.org/10.1016/j.foodchem.2005.01.057

Jurenitsch, J., David, M., Herescht, F., & Kubelka, W. (1979). Detection and identification of new pungent compounds in fruits of Capsicum. *Planta Med., 36*, 61-67. http://dx.doi.org/10.1055/s-0028-1097241

Lee, Y., Howard, R. L., & Villalon, B. (1995). Flavonoids and antioxidant activity of fresh pepper (*Capsicum annuum*) cultivars. *J. Food Sci.*, *60*, 473-476. http://dx.doi.org/10.1111/j.1365-2621.1995.tb09806.x

Liang, Y. C., Shen, Q. R., Shen, Z. G., & Ma, T. S. (1996). Effects of silicon on salinity tolerance of two barley cultivars. *J. Plant Nutr.*, *19*, 173-183. http://dx.doi.org/10.1080/01904169609365115

Mar´in, A., Ferreres, F., Tom´as-Barber´an, F. A., & Gil, M. I. (2004). Characterization and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum* L.). *J. Agric. Food Chem.*, *52,* 3861-3869. http://dx.doi.org/10.1021/jf0497915

O'Sullivan, J. (1979). Response of peppers to irrigation and nitrogen. *Can. J. Plant Sci.*, *59*, 1085-1091. http://dx.doi.org/10.4141/cjps79-16

Oh, M. M, Carey, E. E., & Rajashekar, C. B. (2009). Environmental stresses induce health-promoting phytochemicals in lettuce. *Plant Physiol. Biochem.*, *47*, 578-583. http://dx.doi.org/10.1016/j.plaphy.2009.02.008

Oh, M. M., Carey, E. E., & Rajashekar, C. B. (2010). Regulated water deficits improve phytochemical concentration in lettuce. *J. Am. Soc. Hort. Sci.*, *135*, 223-229.

Prasad, N. B. C., Shrivastava, R., & Ravishankar, G. A. (2005). *Evidence Based Integrative Med., 2*, 147-166.

Rahman, M. J., & Inden, H. (2012). Effect of nutrient solution and temperature on capsaicin content and yield contributing characteristics in six sweet pepper (*Capsicum annuum* L.) cultivars. *J. Food Agric. Environ., 10*, 524-529.

Rodushkin, I., Ruth, T., & Huhtasaari, A. (1999). Comparison of two digestion methods for elemental determinations in plant material by ICP techniques. *Anal. Chim. Acta*, *378*, 191-20. http://dx.doi.org/10.1016/S0003-2670(98)00635-7

Ruiz-Lau, N., Medina-Lara, F., Minero-Gracía, Y., Zamudio-Moreno, E., Guzmán-Antonio, A., Echvarría-Machado, I., & Martínez-Estévez, M. (2011). Water deficit affects the accumulation of capsaicinoids in fruits of *Capsicum chinense* Jacq. *HortScience*, *46*, 487-492.

Sakihama, Y., Cohen, M. F., Grace, S. C., & Yamasaki, H. (2002). Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, *177*, 67-80. http://dx.doi.org/10.1016/S0300-483X(02)00196-8

Silber, A., Bruner, M., Kenig, E., Reshef, G., Zohar, H., Posalski, I., & Assouline, S. (2005). High fertigation frequency and phosphorous level: Effects on summer-grown bell pepper growth and blossom-end rot incidence. *Plant Soil*, *270*, 135-146. http://dx.doi.org/10.1007/s11104-004-1311-3

Simonne, A. H., Simonne, E. H., Eitenmiller, R. R., Mills, H. A., & Green, N. R. (1997). Ascorbic acid and provitamin A contents in unusually colored bell peppers (*Capsicum annuum* L.). *J. Food Comp. Anal., 10*, 299-311. http://dx.doi.org/10.1006/jfca.1997.0544

Slater, A., Scott, N. W., & Fowler, M. (2003). *Plant biotechnology*. The genetic manipulation of plants. Oxford Univ. Press, Oxford, UK.

Stamatakis, A., Papadantonakis, N., Lydakis-Simantiris, N., Kefalas, P., & Savvas, D. (2003). Effects of silicon, and salinity on fruit yield and quality of tomato grown hydroponically. *Acta Hortic.*, *609*, 141-147.

Surh, Y. J. (2002). Anti-tumor promoting potential of selected spice ingredients with oxidative and anti-inflammatory activities: a short review. *Food Chem. Toxicol.*, *40*, 1091-1097. http://dx.doi.org/10.1016/S0278-6915(02)00037-6

Tadesse, T., Hewett, E. W., Nichlos, M. A., & Fisher, K. J. (2002). Changes in physicochemical attributes of sweet pepper cv. Domino during fruit growth and development. *Sci. Hortic.*, *93*, 91-103. http://dx.doi.org/10.1016/S0304-4238(01)00317-X

Usherwood, N. R. (1985). The role of potassium in crop quality. In: Potassium in agriculture (Munson R.S., ed). ASA-CSSA-SSSA, Madison, WI, USA. pp. 489-513.

Wimalasiri, P., & Wills, R. B. H. (1983). Simultaneous analysis of ascorbic acid and dehydroascorbic acid in fruits and vegetables by high-performance liquid chromatography. *J. Chromatography A, 256,* 368-371. http://dx.doi.org/10.1016/S0021-9673(01)88253-4