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Anti-oxidative capacity and quality of strawberry fruit (*Fragaria* × *ananassa* Duch 'Selva') under the balance of minerals nutrition

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ABSTRACT: Anti-oxidative compounds play a protective role in biological systems via scavenging free oxygen radicals. Adjusting the balance between minerals in nutrient solutions, is one of the most important factors in minerals uptake, yield and quality improvement that affects the human health. In order to evaluate the effect of different ratios of K/Ca (120:100, 140:100, 160:100, 100:100, 85:140, 85:100 mg/l) on physiological and biochemical parameters of strawberries (*Fragaria* × *ananassa* Duch cv. Selva), an experiment was conducted in completely randomized design with three replications in hydroponic conditions. Based on the results, antioxidant compounds and fruit taste were affected by application of different K/Ca ratios ($P<0.01$). Accordingly, ascending trend revealed in total phenol, vitamin C content, anthocyanin content and fruit taste with increasing the K ratio in nutrient solution. Maximum antioxidant capacity and fruit taste index were in 140:100 comparing 80:100 ratio, which were 21% and 40% higher, respectively. Total yield and K ratio changes in nutrient solution showed a linear correlation. According to the results, K/Ca balance in nutrient solution could have a significant effects on improving quality and yield.

Key words: Antioxidant capacity, Fruit taste, Fruit quality, Mineral nutrition management,

INTRODUCTION

One of the most common causes of morbidity and mortality related to chronic diseases (Yach *et al*., 2004). Oxidative stresses are the cause of these diseases often happen by an imbalance between the production of free radicals within the body and antioxidative defense mechanisms (Bergamini *et al*., 2004). Phenol ring based antioxidants, containing OH groups, are compounds, which can scavenge free oxygen radicals and inhibit inflammatory processes and oxidation of macromolecules, lipids, nucleic acids and proteins (Noguchi *et al*., and Niki, 2000). Recently, adverse effects of using such synthetic antioxidant Butyl-hydroxy anisol (BHA (, butyl hydroxyl toluene (BHT) and beta-hydroxy quinone have been reported (Gao *et al*., 1999; Ebrahimzadeh *et al*., 2008). Accordingly, plant antioxidant compounds that have a protective role in biological systems (Girard-Lalancette *et al*., 2009), are among the scientific and practical approaches to nutrition and healthy food. The most important antioxidative sources of dietary are tocopherols, glutathione, ascorbic acid, ascorbate salts, carotenoids and phenolic compounds (Young and Woodside, 2001). Strawberries are from Rosacea family (Potter *et al*, 2007), which have no saturated lipid and are rich in antioxidant compounds including a variety of enzymes, phenolic compounds, vitamin C (ascorbic acid), vitamin E (tocopherol) and anthocyanin for nutritional health (Mandave *et al*., 2014).

There is a strong relationship between antioxidants found in stawberry and preventing cellular oxidative shocks (Tulipani *et al*., 2014), so the strawberry poly-phenols nutritional quality are very important in human health (Giampieri *et al*., 2012). Biosynthesis of mentioned compounds in fruits and vegetables, depend on various factors like genotype (Gündüz and Özdemir, 2014), Pre-harvest condition (Ayala-Zavala *et al*., 2004) especially mineral nutrition (Phuong *et al*., 2010) during growth and development. In plant nutrition, every mineral must be sufficiently supplied and properly balanced in solution, because nutritional imbalance reduces quality and productivity of crops (Ashraf and Foolad, 2007). Applying proper nutrient ratios and different formulations in various stages of growth and development in hydroponic systems, will led to the best quality and productivity (Jones, 2014). Among the minerals, potassium as an essential element, plays a critical role in plant metabolic processes (Marschner, 2005), including anti-oxidation capacity (Nguyen *et al*., 2010), quality, flavor and taste (Lynette, 2005). Calcium is also commonly used as an element of stability due to wall and cell membrane, creating a balance of cations and anions (Marschner, 1995), and increases mechanical strength and firmness of fruits (Kirby and Pilbeam, 1984). In addition to the optimal concentration of potassium and calcium in nutrient solutions, the balance between elements (Zamaniyan *et al*., 2012), is also one of the most important factors in growth, antagonistic and synergistic relationships and quality of products.

The aim of this experiment is to evaluate the balance of minerals and K/Ca on some antioxidant compounds, quality and taste of strawberry regarding limited information in this field.

MATERIALS AND METHODS

This research was conducted during the summer of 2014 in a hydroponic greenhouse whit day and night temperature 24±2°C and 18±2°C respectively and relative humidity 60%±5 was implemented. Strawberry Transplants (*Fragaria* × *ananassa* Duch cv. Selva) after disinfection with Benomyl was transformed into pots (3.5 liters / volume) containing perlite and peat (30:70). After the establishment of strawberry seedlings (one week), treatments included K/Ca ratios (120:100, 140:100, 160:100) K/Ca equal ratio (100: 100) and Ca/K (100:85, 140:85 mgL^{-1}) was supplied according to nutrient solution and pH of solution was 6.5 (Table 1). For yield evaluation, ripe fruits harvested, counted and weighted during experiment and considered as gram per plant.

A. Anti-Oxidative Capacity

One gr of fruit was dissolved in 4 ml of methanol. The prepared extract of methanol was centrifuged (Hermle Z216 MK) in 4°C for 15 minutes at 8000 rpm. In this protocol, 0.1mm DPPH was used as indicator. The absorption of samples was read in 517 nm using a spectrophotometer (UV / VIS Lambda 25 Perkin Elmer). Radical scavenging activity was calculated by the following formula (Eqn. 1).

The percentage of free radical trapping

DSE (%)-(As-Ac) / (Ac) ×100 …(1)

Where: As: Sample absorption, Ac: Control absorption, (Miliauskas *et al*., 2004).

Table 1: Fertilizer used for different K/Ca ratios.

Mineral sources	K^+/Ca^{++} Ratio [Amounts of salt in water (g 100 L^{-1})]					
	160:100 (1.6)	**140:100 (1.4)**	**120:100 (1.2)**	**100:100 (1)**	**85:100 (0.8)**	**85:140 (0.6)**
$Ca(NO_3)_2.4H_2O$	58.928	58.928	58.928	58.928	58.928	61.204
$MgSO_4.7H_2O$	45.639	45.639	45.639	45.639	45.639	45.639
KH_2PO_4	4.204	5.205	6.206	7.207	7.958	13.182
K_2HPO_4	11.491	10.21	8.929	7.647	6.687	0.0
KNO_3	7.298	7.298	7.298	7.298	7.298	5.349
K^+ EDTA	15.779	11.812	7.844	3.877	0.902	6.125
Ca^{++} EDTA	0.0	0.0	0.0	0.0	0.0	36.136

B. Total Phenol

Total phenol content was performed based on the Velioglu *et al*., (1998) method. Briefly, 0.1 g of fresh fruit tissue (ripped stage) with 5 ml of 80% methanol containing hydrochloric acid was grind and kept at room temperature for 2 hours and shacked (51 rpm, steplessly adjustable) and then centrifuged at 3000 rpm for 10 minutes. To measure total phenol, 100 µl of the supernatant mixed with 750 ml of Folin reagent and mixture was kept at room temperature for 5 minutes. Then 750 µl of sodium carbonate (6%) was added and after 90 minutes the absorption was read at 725 nm. Total phenol content was calculated using Gallic acid standard curve (mg Galic acid/grFW).

C. Ascorbic Acid

Determination of ascorbic acid (vitamin C) in ripped fruits was done using HPLC (Unicam-cristal-200, UK) (Nisperos-Carriedo, Buslig, and Shaw, 1992). Briefly, one gr of fresh fruit in 40 ml of sodium acetate buffer containing oxalic acid grinded, homogenized and centrifuged. The supernatant was removed, and then potassium phosphate 2% was added. One replication of every sample were injected in HPLC (4.6 and 25 mm internal diameter and column length, respectively). A Supelcosil LC-18 HPLC with KH2PO4 as column washing solvent at rate of 0.5 ml per minute and 260 nm) and vitamin C content was determined based on retention time of output peak and under curve surface comparing standard samples.

D. Anthocyanins

The total anthocyanin content of the extracts was measured using a pH difference (Wrolstad, 1976).

Extracting buffer (pH- 4.5) 1 M sodium acetate and 100 cc, 210 cc of normal hydrochloric acid 1, 310 ml of distilled water

How to prepare buffer (pH-1) 0.2 normal potassium chloride and hydrochloric acid, 125 ml, 385 cc normal 0.2

After the preparation of buffers, 1 gr of powdered extract of strawberries with 1 ml of every buffer separately was extracted in the mortar and centrifuged in 4000 rpm for 20 minutes. Extract absorption was done in 510 and 700 nm at two different pH (1 and 4.5). Buffers were applied as calibration extracts for spectrophotometer (UV / VIS Lambda25 Perkin Elmer. The (Equation 2) was used to calculate the amount of anthocyanin basis on Pelargonidin triglycoside.

A = (A510nm- A700nm) pH=1 - (A510nm- A700nm) pH = 4.

To determine the amount of total anthocyanin (mg of anthocyanin Cyanidin- 3 - glucoside per gr equation 2 was used) (Lako, 1974).

TAC = ((A*MW*100))/MA …(2)

(A - Absorption of the first formula, MW = 499.2 molecular weight, DF = dilution factor = 10, MA - 26900)

E. Total Soluble Solids

To calculate the amount of TSS ((Total Soluble Solids) (amount of fruit sugar)), a few drops of the supernatant were dropped on the refractometer (ATAGO) glass (temperature 20°C and dilution factor applied) and the concentration of dissolved solids was read as brix (Brix) degree (AOAC, 1980).

F. Titrable Acidity

2ml of fruit juice was reached to 20 ml solution using deionized water and titrated using a standard solution of sodium hydroxide (titrant) up to pH 8.1. Titratable acidity was calculated with equation 3. (Nadem *et al.*, 2010).

A = (N.V. Meq)/M × 100…(3)

A: The amount of organic acids in extract (mg / 100mL),

Meq: dominant acid in strawberry fruit (citric acid - 0.064),:

N: Applied NAOH normality (0.1 normal),:

M: Volume of fruit juice (mL),

V: Volume of applied NAOH

G. Taste Index

TSS/TA ratio was considered as taste index. This ratio has a positive correlation with the quality of strawberry fruit (Rutkowski, 2006).

H. Statistical Analysis

This experiment was performed in completely randomized design with three replications. Data analyzes and means comparison were done with SAS software version 9.2 and Duncan's multiple range test at 5% level, respectively.

RESULTS

Total phenol and ascorbic acid content were affected by different K/Ca ratios (P<0.01). (Table 2). K/CA (140:100) led to the maximum total phenol and ascorbic acid content whit 3.135 mg/grFw and 33.7 mg/100grFw respectively. Comparing with K/Ca (85:140 and 85:100) ratios and K/Ca (100:100), total phenol increased by 16% and ascorbic acid comparing the least K content in nutrient solution (K/Ca =85:100) showed a 71.5% increase. (Fig. 1 and 2).

Analysis of variance showed that the K/CA different ratios had a significant effect on total anthocyanin and antioxidant capacity (P<0.01) (Table 2). Accordingly the maximum amount of total anthocyanin and anti-oxidant capacity were showed K/Ca (140:100 mg/L) ratio with 73.3 mg/100gr plargonidine and 75.3 % respectively which were about 55% and 21% higher than K/Ca (100:100) ratio, (Figure 3 and 4).With increasing K level in nutrient solution, anthocyanin changes and antioxidant capacity showed a downward trend whit a gentle slope (Fig. 1, 2, 3 and 4).

According to the experiment results, ripening index ((fruit taste) (TSS/TA)) and titratable acidity were affected by different K/Ca ratios at 1% level and TSS at 5%, (Table 2). The maximum amount of dissolved solids obtained in the K/Ca (120:100 mg/L) ratio and the lowest was observed in (85:140 and 100: 100).

Table 2: Analysis of the variance of different K^+/Ca^{++} ratios on physiological and biochemical characteristics of strawberry 'Selva'.

S.O.V	df	Mean Square			
		Total Phenol	**vitamin C**	**Total Anthocyanin**	**DPPH**
K^+/Ca^{++} Ratios	5	223.4**	230.2**	721.65**	87.7**
Error	12	28.21	2.68	18.13	12.1
Coeff Var (%)	-	4.4	8.4	7.8	5.2
S.O.V	df	Mean Square			
		Total Soluble Solids	Titratable acidity	Taste index	Yield
K^+/Ca^{++} Ratios	5	1.03*	0.14**	7.32**	3826.47**
Error	12	0.22	0.014	0.37	237.36
Coeff Var (%)	-	6.17	9.65	9.3	16.5

*, ** indicate significant difference at 0.05, 0.01 respectively.

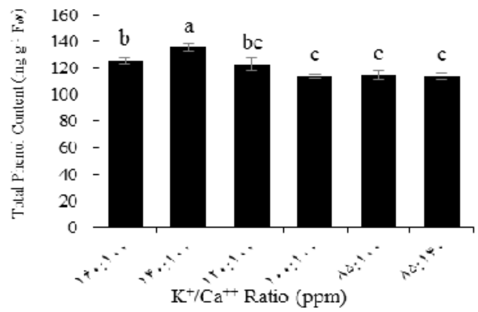

Fig. 1. Effect of K^+/Ca^{++} ratio on strawberry fruit total phenol.

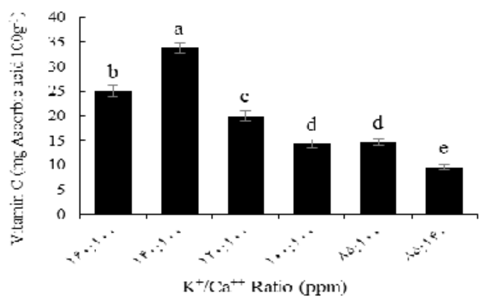

Fig. 2. Effect of K^+/Ca^{++} ratio on vitamin C content of strawberry 'Selva'.

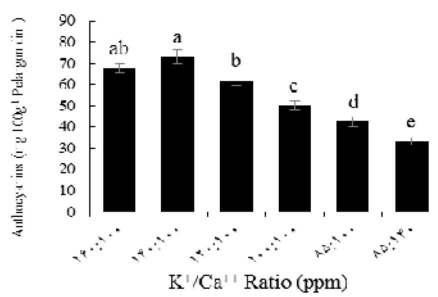

Fig. 3. Effect of K^+/Ca^{++} ratio on strawberry fruit total anthocyanin.

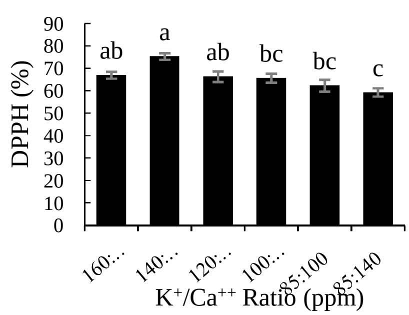

Fig. 4. Effect of K^+/Ca^{++} ratio on antioxidant capacity of strawberry 'Selva'.

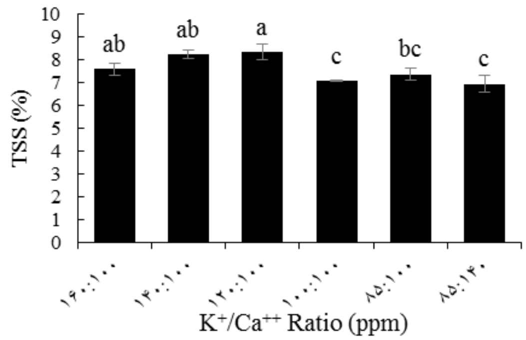

Fig. 5.Effect of K^+/Ca^{++} ratio Total Soluble Solids of strawberry 'Selva'.

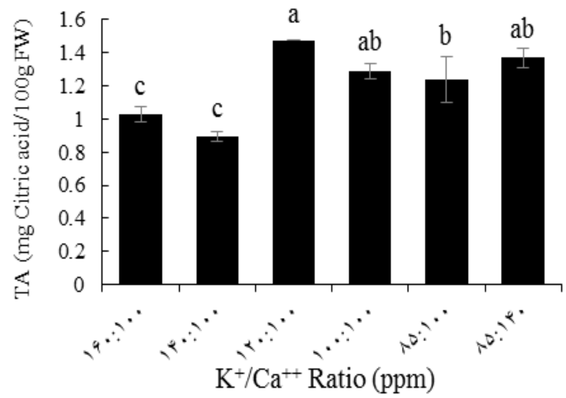

Fig. 6. Effect of K^+/Ca^{++} ratio titratable acidity of fruit juice strawberry.

Also the highest content of titratable acidity such as TSS was in K/Ca (120:100) ratio and lowest content unlike TSS was in K/Ca (100:140 and 100:160) ratios. The balance of sugar and acid in strawberry fruit will led to the high quality and better fruit taste. The best fruit taste based on TSS/TA was in K/Ca (140:100) and lowest taste considering high acid content was in K/Ca (85:140, 85:100, 100:100 and 120:100) which was about 40% less comparing K/Ca (140:100) ratio. (Fig. 5, 6 and 7). Fruit productivity was also affected by different K/Ca ratio ($P < 0.01$), (Table 2). Increasing in K/Ca ratio toward K content (160:100) led to the highest productivity with 160.2 gr/plant which was about 58% higher comparing K/Ca (85:140) as the least productive treatment. (Fig. 8).

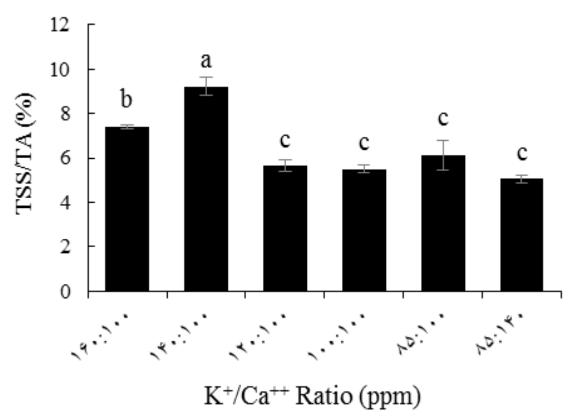

Fig. 7. Effect of K^+/Ca^{++} ratio strawberry fruit taste index (TSS/TA).

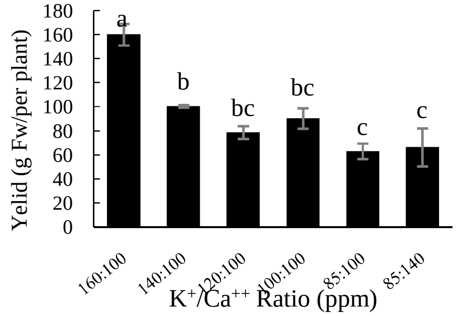

Fig. 8. Effect of K^+/Ca^{++} ratio strawberry plant performance.

DISCUSSION

Considering K+ as a vital and essential element in metabolism of plants (Taiz and Zeiger, 2010), in this experiment, high proportion of potassium ($K^+ > Ca^{++}$ 140: 100) increased antioxidant compounds (Wang and Lin, 2000), including total phenolic (Chen and Ho, 1997), vitamin C (Odriozola-Serrano *et al*., 2007) and anthocyanin (Wang and Lin, 2009). The antioxidant capacity (Phuong *et al*., 2010), which was affected whit high ratio of K/Ca (Fig. 4), has a special role in anti-microbial, anti-allergy, cardiovascular diseases, and inhibition of some physiological enzymes activity (Santos-Buelg *et al*., 2000). Soaring popularity and increasing per capita consumption of strawberry fruit, is related to appearance, taste and flavor that is mainly measures by the ratio of sugar/acid (Rutkowski, 2006; Vicente *et al*., 2009).

Taste, fruit quality (Tagliavini *et al*., 2005; Macit *et al*., 2007), as well as plant productivity are highly influence by fertilization management (Abdi *et al*., 2006). Therefore providing the optimal balance of minerals, especially potassium and calcium in the root zone will led to the high productivity (Ahmad *et al*., 2014) (Figure 8), acids and sugars contents of strawberry (Fig. 5 and 6). Also increasing the sugar/acid ratio increases the taste index (Figure 7), which is in agreement whit Lieten *et al* (1993) and Morgan (2005) which stated that balance of organic acids and sugars, also fertilizers management especially K^+ increase strawberry taste. Considering the upward trend in greenhouse production, management nutrient solutions necessity and fertilizer formulations (Hewitt, 1966) to achieve the greenhouse with high quality products and healthy food is inevitable.

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