Research Journal of Agriculture and Biological Sciences, 5(6): 892-898, 2009

Relationship Between Mineral Composition of the Flavedo Tissue of 'Marsh' Grapefruit and Chilling Injury During Low Temperature Storage

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Abstract: In this study, the behavior of certain macro- and micro-elements in the flavedo tissue of grapefruit were studied. The fruits were harvested in December and January and stored for 6 weeks at 4.5 C Significant differences were found as to Ca, Mg, K, Fe, Cu, Zn, and Mn content between the initial (zero time) and diseased flavedo tissues in the two harvest dates. Ca and Mg increased while K and Fe decreased with the increase of the severity of injury (darkness of browning). Cu, Zn, and Mn did not show consistent patterns of response with the storage period but were always lower in the stained portions than in the healthy ones in both harvest dates. Quality of the same fruits were evaluated by determining its content of ascorbic acid (vitamin C), TSS and acidity. Significant differences were found in all these constituents between the two harvest dates. Correlation coefficients between pitting severity (browning) and both the mineral content and fruit quality were determined. Positive correlations were found between severity of browning and both pitting area and Ca and Mg content. Negative correlations were found for K, Fe, Cu, Zn, and Mn except for Mn in the first harvest date and Cu in the second where the correlation was positive. Negative correlations were found between pitting severity and ascorbic acid and acidity while positive correlation was found for TSS.

Key words: Mineral Composition; Grapefruit; Chilling injury; Harvest date

INTRODUCTION

Grapefruit are subject to chilling injury (CI) when stored at temperatures below 12 C[16,36,22] reported that pitting was the common symptom. Damaged fruits showed depression in the flavedo (pitting) which had generally rounded areas that started initially pink and then turned dark brown. These areas may cover a high percentage of the surface according to the severity of the injury[23,30,32].

Storage conditions seem to be the major external factor influencing the incidence of these disorders. However, many post-harvest problems are attributed to cultivation procedures such as fertilization and spraying with minerals[34,32] studied CI in limes and grapefruit on different root stocks and fertilizing with NPK, Mg and several CaCO3 levels. They found at the end of cold storage (@ 6 C and 10 C) a relationship between CI and mineral content in the flavedo tissue. In addition,[27] related susceptibility of grapefruit to CI to the harvest date.

Still, it was felt that there was lack of information in relating several important parameters together. Therefore, this investigation was an attempt to find the effect of harvest date, storage time, storage temperature (4.5 C) and mineral content in relation to pitting severity (browning) in the flavedo tissue of grapefruit throughout the storage period. This would enable us to draw conclusions and define clear indicators as to fruit quality and signs of damage before it reaches to the point of being uncorrected.

MATERIALS AND METHODS

This investigation was carried out during the 2007/2008 and 2008/2009 seasons on Marsh grapefruit *(Citrus paradisi)* grafted on sour orange root stock. Fruit of this variety were harvested on December 7 for 2007 and 5 for 2008 and January for 17, 2008 and15 for 2009 from selected trees grown in a private orchard at El- Taarh village, El-Behera Governorate. The orchrd soil was clay loam; the trees were budded on sour orange rootstock, fertilized with 15 cubic meters of organic manure per feddan in winter, and with ammonium nitrate (33% N) at a rate of 2 kg per tree in three equal doses; March, May and July.

Round fruit free of rind punctures and of similar size were chosen, transported by car to the Researching laboratory of faculty of Agriculture Saba Bacha, University of Alexandria, washed in tap and deionized

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water and left to dry in air. The fruit from the first harvest date were divided into six groups and those from the second were divided into seven groups. All groups consisted of nine fruit.

Fruit were stored at 4.5 ^oC for 6 weeks for both harvest dates in the two experimental seasons. The storage temperature was proposed by[7,27] and was intended to maximize the development of fruit peel pitting, which is the symptom of chilling injury.

Nine-fruit samples (replicates) were taken at random biweekly from the two harvest dates, respectively. The fruit were peeled and juice was extracted from each. Healthy (zero time) and pitted flavedo tissues were carefully removed with a stainless steel knife. Samples were collected until the end of storage from healthy fruit as well as from fruit with different degrees of damage for the purpose of comparison. The degree of damage was scored from 1 to 9 depending on the severity (discoloration) of browning using a hedonic scale previously developed for rating the severity of discoloration in lettuce by assigning 1 = None; 3= slight; 5=moderate; 7=sever and 9=extreme[18]. Additionally, unpitted flavedo tissues from chilled and unchilled fruit were collected at the end of experiment.

Samples were oven-dried at 70 ^oC and then ground to 20 mesh size. The dried, ground flavedo of each sample (replicate) was digested with sulfuric acid and hydrogen peroxide according to[12]. A suitable aliquot from the digested solution of each sample was taken for mineral composition determination. Macro-elements (calcium, magnesium and potassium) and micro-elements (iron, copper, manganese and zinc) were determined by Jarrell Ash ICAP-61E Spectrophotometer[17]. The levels of macro-nutrients were expressed in percent and those of micro-nutrients in mg/l on dry weight basis.

The juice extracted from the fruit was analyzed for total ascorbic acid by the dinitrophenylhydrazine method of[33]. The concentration of total ascorbic acid was calculated per 100 ml of juice from absorbance measured at 540 nm using a standard curve. Total soluble solids (Brix) were determined with an Abbe refractometer. Titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator according to the[1]. The results were expressed as percent citric acid.

The data collected throughout the course of this study were statistically analyzed using the Statistical Analysis System computer package[29]. Initial analysis of the data for the combined harvests by analysis of variance (ANOVA) indicated a significant harvest effect. Subsequently, data for the different harvests were analyzed separately. Significant differences among the constituents and fruit quality between the two harvest dates were detected using the F-test.

RESULTS AND DISCUSSION

The data listed in Table (1 and 3) showed that there was no significant difference between the two harvest dates in pitted area. It may point out that the two harvest dates fall in the early season where fruits are known to be sensitive to chilling[25]. The same data showed the mineral element content in nonchilled (zero time) and chilled flavedo tissues in grapefruit in both harvest dates. There were significant changes in Ca, Mg, K, Fe, Cu, Zn, and Mn content as the period of chilling exposure increased. This result is in line with that reported by[32]who studied the behavior of certain macro- and micro-elements in grapefruit and lime juice and in the flavedo tissues of healthy and pitted grapefruit stored at 6 ^oC and 10 ^oC from trees that received different CaCO3 levels.

Calcium and magnesium concentrations increased while that of potassium diminished with increased storage time. These results are in line with trends reported by[32] who indicated that Ca concentration increased and K concentration decreased in the lime and grapefruit flavedo at the end of the cold storage period at 6 ^oC.

Since these minerals sometimes act interdependently, it may be more useful to express their contents in terms of their ratios rather than their individual concentrations[8,32]. In both experimental seasons, an examination study of the ratios of K/Ca and K/Mg indicates that these ratios were apparently lower in diseased tissue than in healthy tissue. In the first season, the ratios were 1.30 to 0.76 and 11.67 to 8.37 for K/Ca and K/Mg, respectively, for the first harvest date. For the second harvest date, the same ratios ranged, respectively, from 1.14 to 0.55 and 10.13 to 7.00. The Ca/Mg ratio increased from 8.94 in healthy fruits to 11.01 in damaged ones for the first harvest date, while it increased from 8.91 to 12.75 for the second harvest date. It is possible that these ratios may be used as indicators of severity of fruit damage from chilling injury.

The same trend was observed to the second experimental season. Whereas, the ratios were 1.33 to 0.79 and 12.26 to 8.55 for K/Ca and K/Mg ratio, respectively, for the first harvest date, and 1.14 to 0.52 and 9.95 to 6.69 for the second harvest date, for K/Ca and K/Mg ratio, respectively. The Ca/Mg ratio increased from 9.25 in healthy fruits to 10.76 in damaged ones for the first harvest date, while it increased from 8.72 to 12.96 for the second harvest date.

Table 1: Effect of harvest date and storage time on mineral content and fruit quality of Marsh grapefruit stored at 4.5 C in 2007/2008 season.

Harvest Date	Weeks @4.5 C	Minerals % Ca	Mg	K	mg/l Fe	Cu	Zn	Mn	AA (mg/100ml)	TSS %	Acidity %
7-Dec	0	0.751	0.084	0.980	37.67	17.67	27.33	5.33	40.28	8.13	1.33
	2	0.815	0.081	0.936	27.67	17.67	24.89	4.22	38.92	8.46	1.30
	4	0.958	0.085	0.844	33.33	15.44	26.00	5.00	36.37	8.57	1.23
	6	0.980	0.089	0.745	28.44	16.66	26.78	4.77	34.29	8.60	1.17
	Mean	0.876	0.085	0.876	34.28	16.86	26.25	4.83	37.47	8.44	1.26
17-Jan	0	0.829	0.093	0.942	36.67	16.67	28.67	5.33	42.19	7.90	1.30
	2	0.890	0.092	0.932	34.67	17.33	26.44	4.44	38.96	8.06	1.27
	4	1.048	0.097	0.777	29.11	15.33	26.44	5.11	32.57	8.10	1.19
	6	1.300	0.102	0.714	24.00	16.89	27.56	4.89	31.62	8.20	1.08
	Mean	1.017	0.096	0.841	31.11	16.56	27.28	4.94	36.33	8.07	1.21
L.S.D.	0.036	0.004	0.033	1.56	1.21	1.05	0.62	2.52	0.14	0.02	0.05
ANOVA H	***	***	***	***	N.S.	***	N.S.	**	***	***	
W	***	**	***	***	**	**	**	***	***	***	
H*W	***	***	***	***	***	N.S.	***	***	***	***	

N.S.=not significant; *,**,*** Significant at 0.05. 0.01, or 0.001, respectively.

Table 2: Correlation coefficients between pitting severity (browning) and measurements in 2007/2008 season.

Harvest Date	Measurement: Pitted area (cm^2/fr)	Macro-elements (%) Ca	Mg	Micro-elements (mg/l) K	Fe	Cu	Zn	Fruit quality Mn	AA (mg/100ml)	TSS (%)	Acidity (%)
7-Dec	0.873	0.867	0.527	-0.859	-0.813	-0.148	-0.065	0.052	-0.440	0.682	-0.880
	***	***	***	***	***	N.S.	N.S.	N.S.	**	***	***
17-Jan	0.759	0.867	0.512	-0.764	-0.763	0.013	-0.058	-0.046	-0.614	0.440	-0.767
	***	***	***	***	***	N.S.	N.S.	N.S.	***	*	***

N.S. not significant; *, ** , *** Significant at 0.05, 0.01, 0.001, respectively.

In light of the fact that Ca plays a retarding role against physiological (storage maladies) and pathological disorders[2,6] and prevent physiological disorders, delay ripening and improve fruit quality [21,31,9] and that it is one of the most immobile elements, the question arises, then, why does concentration in fruit flavedo increase with pitting severity (browning)? In answering this question, it may be suggested that Ca in the albedo may react with organic acids (malic, oxalic and citric) present in the albedo underneath the stained flavedo portions and thus be transformed into a mobile form. Next, it may move to the stained areas in the flavedo in response to chilling injury. [32] also implied that there may be translocation of Ca from other portions of the fruit when chilling injury occurred in lime and grapefruit at low temperature storage.

At the end of the storage period, higher contents of Ca were found in the unpitted flavedo of chilled as well as unchilled fruit. The SD was 0.622±0.08 1 and 1.094±0.103 for the flavedo of pitted and unpitted fruit, respectively, in the first season, while, 0.601±0.078 and 1.090±0.099 respectively, in the second season. It may be argued that the unchilled fruit received more Ca content through the absorption and translocation more than that in chilled fruit. Higher content of Ca was received when the fruit (unchilled) were attached to the tree due to its position on the canopy. This result can be supported by arguments presented by[20] pointed out that a high proportion of total Ca in plant tissue was located in the cell wall. This unique distribution of Ca was mainly the result of abundance of binding sites of Ca in the cell wall. Additionally,[5] reported that the typical symptoms of Ca deficiency were the disintegration of cell wall and the collapse of the affected cells.

Data also showed a slight increase in Mg may be explained by arguing that Mg is more mobile than Ca. As for K, its concentration diminishes as a result of the antagonist relationship between it and both Ca and Mg[15]. The trends presented above for the ratios of K/Ca and K/Mg are clearly due to increases in both Ca and Mg along with a reduction in K.

As for Fe, it seemed to give a similar pattern of response as that observed herein for K (Table 1 and 3). It may be possible to relate the decline in Fe in pitted flavedo to the respiration depression in such fruits due to dead cells, bearing in mind that Fe is present in the molecules of respiratory enzymes and peroxidases[24].The other micro-elements (Cu, Zn and Mn) did not show consistent patterns of response although their concentration in damaged fruits was consistently lower in stained portions than in healthy ones in both harvest dates. The role of Zn is to provide resistance to cold temperature and that of Mn is to resist physiological disorders as reported by[32,35]. explained the mechanism of Zn by suggesting that the metabolic functions of Zn were based on its strong tendency to form tetrohedral complexes with N-, O-, and particularly S-lignands. Thus, it plays both a functional (catalytic) and structural role in enzyme reactions. As for Mn,[3] indicated that it was a cofactor, activating about 35 different enzymes, which catalyzed oxidation-reduction, decarboxylation and hydrolytic reactions.

Comparing the two harvest dates in mineral content, data in Table (1 and 3) showed that the concentrations of Ca and Mg were significantly higher in the second harvest date than in the first as a result of the accumulation of both by the absorption and translocation. However, K concentration was significantly lower in the second harvest date due to the antagonist relationship between Ca and Mg on one hand and K on the other. As for microelements, Fe content was significantly higher in the first harvest date while for Cu, Zn, and Mn, no differences were found. The juice quality of the same fruit used for flavedo mineral analysis was evaluated by determining the contents of ascorbic acid, TSS and acidity. Ascorbic acid decreased with storage time in both harvest dates. This may be attributed to the oxidation it undergoes with time[13,14,19,28]

In both harvest dates, TSS increased with storage time. This increase is probably not a result of an increase in the content; rather, it is likely due to water loss during storage resulting in an apparent increase in TSS concentration[26]. Further, it was suggested that cell wall polysaccharides are degraded by galactosidases and glucosidases found in citrus juice vesicles[4] and thus contribute to increasing TSS concentration[10]. However, the same data showed that acidity declined with storage time which may be attributed to the use of acids as substrates for respiration[1].

Significant differences were found in all these constituents between the two harvest dates. Although TSS was higher in the first harvest date, the TSS/Acidity ratio was the same for the two harvest dates.

The data in Table 2 and 4 show that for both harvest dates that there were highly significant positive correlations between the pitted area and severity of browning. The same applies to Ca and Mg concentrations in the pitted flavedo. However, highly negative correlation was found between pitting severity (browning) and K and Fe concentrations. As for Cu, Zn and Mn, correlations were nonsignificant. For the same fruits, significant negative correlations were found between pitting severity (browning) of the flavedo and ascorbic acid content and acidity in the fruit juice, while a positive correlation existed for TSS. All these correlation results are consistent with the trends presented in Table (1 and 3).

Conclusions: It is evident that under these experimental conditions the mineral composition of grapefruit may be related to pitting during cold storage. This could be due to difference in mineral composition of healthy (unchilled) and stained (chilled) portions of fruit. A balanced fertilization program is highly recommended because minerals play an important role in reducing chilling symptoms in low temperature storage.

The mineral analysis of fruit tissue and calculating the ratios of mineral elements could be used as a good indicator of physiological changes. Finally, it may point out that, CI does not necessarily affect the internal fruit quality but may result in increasing fruit susceptibility to pathogen invasion and reduced marketability. Therefore, it may be recommended that chilled fruit may be used for juice production.

Table 3: Effect of harvest date and storage time on mineral content and fruit quality of Marsh grapefruit stored at 4.5 C in 2008/2009 season.

		Minerals									
		%			mg/l				AA	TSS	Acidity
Harvest Date	Weeks @4.5 C	Ca	Mg	K	Fe	Cu	Zn	Mn	(mg/100ml)	%	%
5-Dec	0	0.740	0.080	0.981	36.61	17.20	28.41	5.11	42.66	8.23	1.39
	2	0.795	0.083	0.930	32.44	18.11	25.72	4.37	37.25	8.49	1.28
	4	0.889	0.086	0.871	30.15	17.42	25.21	5.01	35.10	8.62	1.21
	6	0.925	0.086	0.735	27.51	17.51	26.43	4.52	28.39	8.68	1.15
	Mean	0.837	0.084	0.879	31.68	17.56	26.44	4.75	35.85	8.51	1.26
	0	0.820	0.094	0.935	37.31	16.58	27.39	5.21	44.18	8.01	1.36
	2	0.886	0.093	0.926	33.25	17.13	25.93	4.63	40.61	8.09	1.32
15-Jan	4	1.142	0.099	0.759	28.63	16.41	25.84	5.21	34.21	8.24	1.20
	6	1.400	0.108	0.723	23.01	15.94	27.71	5.37	30.22	8.35	1.03
	Mean	1.062	0.099	0.836	30.55	16.52	26.72	5.11	37.31	8.17	1.23
L.S.D.	0.043	0.006	0.040	1.00	1.53	1.66	0.94	2.34	0.18	0.02	0.05
ANOVA H	***	***	***	***	N.S.	N.S.	N.S.	**	***	***	
W	***	**	***	***	**	**	**	***	***	***	
H*W	***	***	***	***	***	N.S.	***	***	***	***	

N.S.=not significant; *,**,*** Significant at 0.05. 0.01, or 0.001, respectively

Table 4: Correlation coefficients between pitting severity (browning) and measurements in 2008/2009 season.

	Measurement										
Harvest Date	Pitted area (cm^2/fr)	Macro-elements (%)			Micro-elements (mg/l)				Fruit quality		
		Ca	Mg	K	Fe	Cu	Zn	Mn	AA (mg/100ml)	TSS (%)	Acidity (%)
5-Dec	0.854	0.822	0.527	-0.865	-0.804	-0.155	-0.054	0.062	-0.434	0.662	-0.874
	***	***	**	***	***	N.S.	N.S.	N.S.	**		
15-Jan	0.788	0.831	0.499	-0.770	-0.771	0.015	-0.062	-0.052	-0.620	0.470	-0.733
	**	***	***	***	***	N.S.	N.S.	N.S.	***	**	***

N.S. not significant; *, ** , *** Significant at 0.05, 0.01, 0.001, respectively.

ACKNOWLEDGMENT

The authors would like to thank the private orchard.

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