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Evaluation of chlorophylls activity, carotenoids content and total anthocyanin Changes of fruit in different aspects and fruit location within orange tree canopy

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ABSTRACT: Orange (*Citrus sinensis*) is a semitropical plant which grows in areas with semi cold to tropical climate. Radiant climate can effect on physical and biochemical properties of fruit. Therefore, purpose of this research is to study effect of geographical aspect and position of fruit in canopy of tree on qualitative properties of Sangin orange. In this research, random complete blocks in form of divided patches were used. Two north and south part as main patches and surface or depth of awning from position of fruit in awning as divided patches were used. This research was performed with three frequencies in Semeskandeh village in Sari on 2012. The results show that anthocyanin content and carotenoides of the fruit increases with approaching to time of ripening the fruit. Geographical aspect and place of fruit in canopy of tree has a significant effect on anthocyanin and carotenoid content. chlorophyll a, b and ab activity of the fruit decreased with approaching to time of fruit ripening. The parts had not a significant effect on pH, TSS, percent of TA and relation of TA/TSS during measurement.

Keywords: semitropical, radiant, north, climate , chlorophyll, ripening.

INTRODUCTION

Harvesting of the fruit in different times has a considerable effect on qualitative properties of the fruit. Harvesting of the fruit before complete ripening is very important for increasing time of storing and protecting qualitative properties of the fruit. The fruits which are harvested very soon, are smaller and their color, taste and essence are low. Therefore, sunlight as key and the most important factor effect on quality of the fruit during growing. Qualitative indexes of the fruit such as size of the fruit, soluble solids, anthocyanin, starch, pH, surface color of the fruit all are affected by light.

Generally, flux of light shined to the fruit on tree is the function of canopy position. Flux of light and canopy position is closely linked to each other. Flux of light shined to the fruit is variable with the shade of the fruit on the tree in a canopy. And also, geographical position of canopy effects on portion of flux of light shined to the fruit. Putting the fruit exposed to the light has an important effect on growth of the fruit but transmittance of the light shined to canopy is more important than direct light. The light shined to the fruit in internal and external parts of canopy is not similar to it in lower and upper parts of canopy. Physiological maturation of the fruit may be postponed in lower part of canopy in comparison with upper part of canopy. It is possible to gain the highest quality of the fruit by increasing flux of light shined to the fruit. Size of tree, stance and form of canopy effect on transmittance of the light shined to the tree. Pruning of the tree can also increase the light shined to the fruit and leaves. Pruning increases the light by optimization of photosynthesis efficiency. Covering fruit trees with textile awning decreases quality of the fruit with decrease of available light. Awning of trees delays harvesting of the fruit, decreases soluble solids and firms the fruit.

Relocation of photosynthesis materials from sunlight parts of canopy to shadowy parts of the tree is important for growth of the fruit. Purpose of this research is to study effect of geographical direction and position of fruit in canopy of tree on qualitative properties of Sangin orange.

MATERIALS AND METHODS

A garden in Semeskadeh village in Sari was sampled for studying biochemical properties of Sangin Moro orange. In this research, a plan of divided patches in form of complete random patches was used. Studying treatments were four geographical directions (north, south, east and west) as main patch and position of the fruit inside canopy as divided patch. This test was performed with three frequencies in summer and autumn of 2012. Some oranges were randomly harvested from geographical aspects, inside and surface of canopy in every sampling. The fruits were taken to laboratory of agricultural meteorology in Sari University of agricultural sciences and natural sources for measuring physical and qualitative factors of the fruits. The frits were harvested in three times, 20 days before complete ripening, ripening time and 20 days after ripening. Content of total soluble solids (TSS) of orange juice was measured by refractometer in room temperature and represented in percent. titratable acid (TA) was measured in presence of PhenolPhthalein (pH=8.2) and represented in percent of acid citric. Orange juice pH was measured by pH meter. Contents of anthocyanin in samples were calculated by absorption variance method in differential pH. In this method, absorption of samples was measured in buffers of pH1 and pH 4.5 from Cyanidin 3-glucoside pigment in wavelength of 510 nm by using spectrophotometer. Then, total content of anthocyanin was calculated by equation (1).

Equation (1):

$$C(mg/100ml) = \frac{\Delta A}{\varepsilon L \times M \times D}$$

Where, C: molar density, Ɛ: molar absorption, D; dilution factor, ΔA: difference between buffers' absorption in pH1 and pH4.5, M: molecular weight of Cyanidin 3-glucoside, L: passage way of cell of spectrophotometer (usually 1cm). for determining content of chlorophyll and Carotenoides, at first, 0.5 g sample is grinded at presence of acetone 80%, then, the solution is centrifuged in 5000 cycles for 5 minutes. Then, amount of absorption was recorded by spectrophotometer in 480, 510, 645, 663nm wavelength. Acetone 80% was used as evidence of spectrophotometer. Content of chlorophylls a, b and ab and Carotenoides were calculated by the following equations:

$$Chloa(mg/g.f.w) = 12.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000} \times W$$

$$Chlob(mg/g.f.w) = 12.9(A_{645}) - 4.68(A_{663}) ' \frac{V}{1000} ' W$$

$$Chloa*b(mg/g.f.w) = 20.2(A_{645}) + 8.02(A_{663}) ' \frac{V}{1000} ' W$$

$$Carotenoeid(mg/g.f.w) = 7.6(A_{480}) - 1.49(A_{510}) ' \frac{V}{1000} ' W$$

Where, A: wavelength (nm), V: final volume of solution (ml), W: weight of sample (gr). The information got at the end of test was analyzed by using SAS software and student-Nueman-Kouel (SNK) test was used for comparing averages.

RESULTS AND DISCUSSION

Anthocyanin

Position of fruit in canopy of tree has meaningful effect on content of total anthocyanin of fruit in all time efficiency of harvesting ($p< 0.01$). According to findings of the research, the fruits which have received more light on canopy of tree have more total anthocyanin toward the frits inside the awning. Before ripening the fruit, geographical aspect of canopy of tree had not effect on content of total anthocyanin (table 1). The fruits in south of canopy had the most content of total anthocyanin both at the time of ripening and after it. There wasn't any meaningful difference in content of anthocyanin I three directions of north, east and west (table 1). Also, transactional effect of geographical aspect and position of fruit, in shadow or sun, on content of total anthocyanin was not significant. Content of total anthocyanin in red oranges showed its high quality. One of the most important environmental factors effective in synthesis of anthocyanin is light that impress gens associated with biosynthesis of anthocyanin. Density of anthocyanin is different in stages of fruit development, position of fruit on tree and different parts of a fruit. In hull of apple, sunlight is one of the most important external factors in synthesis of anthocyanin. According to report of Ju et al. (1997), bagging the fruit increases sensitivity of fruit to light while synthesis of anthocyanin is stimulated by removing the cover and placing the fruit subject to sunlight. Therefore, any anthocyanin is not stockpiled in hull in lack of light in any stages of growth. Then the reason of increasing total anthocyanin in the fruits which were in south of canopy and subject to more light can be decreasing flux of light.

Table 1. The total anthocyanin content in different of geographical aspects along different harvest times (pre-maturity, maturity, post-maturit) on orange fruit

Aspects	Pre- maturity	Maturity	Post-maturity
North	13.78^{a}	32.3^{b}	45.5^{b}
South	14.46^{a}	41.7^{a}	60.5^{a}
East	12.78^{a}	33.3^{b}	45.0^{b}
Wrest	12.91^{a}	33.8^{b}	45.2^{b}

Numbers with the same letters have no significance difference (5%)

Content of chlorophyll

The data of the research showed that geographical aspect of canopy has significant effect on activity of chlorophylls (a, b, ab) before ripening. Content of chlorophyll decreased significantly at the time of ripening (table 2). The least content of chlorophyll occurred at the eastern aspect of canopy before and at the time of ripening which has meaningful difference with other three directions. This difference shows that the fruits discolor soon due to higher flux of light at eastern aspect. At the time of complete ripening, there wasn't meaningful difference between two aspects that shows decrease of chlorophyll. At the time of ripening, effect of geographical aspect on activity of chlorophyll a was significantly ($p< 0.01$) (figure 2) while position of being sunshiny or shadowy of the fruit was not significant in its activity.

Table 2. chlorophyll a ,b and a*b activity in different along different harvest times (pre-maturity, maturity, post-maturit) and different geographical aspects on orange fruit

Harvest times	Aspects	Chlorophyll a	Chlorophyll b	Chlorophyll a*b	
Pre-maturity		South	0.44^{a}	0.31^{a}	0.44^{a}
		North	0.38^{ab}	0.25^{ab}	0.38^{ab}
		East	0.30^{b}	0.18^{b}	0.30^{b}
		West	0.41^{ab}	0.31^{ab}	0.41^{ab}
Maturity		South	0.26^{a}	0.17^{a}	0.26^{a}
		North	0.18^{b}	0.16^{b}	0.18^{b}
		East	0.26^{a}	0.14^{a}	0.26^{a}
		West	0.30^{a}	0.17^{a}	0.30^{a}
Post-maturity		South	0.16^{a}	0.10^{a}	0.16^{a}
		North	0.11^{a}	0.07^{a}	0.11^{a}
		East	0.18^{a}	0.07^{a}	0.11^{a}
		West	0.14^{a}	0.09^{a}	0.14^{a}

Numbers with the same letters have no significance difference (5%)

Also effect of interaction of geographical aspect and content of light on activity of chlorophyll a, b and ab were significant at the time of ripening and before that while position of fruit on canopy and geographical aspect of tree had not significant effect on activity of chlorophyll a, b and ab after ripening (table 3). According to report of Turgeon and Webb (1975), maximum amount of chlorophyll a, b and ab of the leaves was at the last stage of harvesting and their activity depends on need of the plant. By increasing photosynthesis products in the fruit, leaves produce more chlorophyll for synthesis of Carbohydrates. At the early growth, due to being more growing part, huge synthesized materials are stored in growing organs by leave. But by growing the fruit, more photosynthesis products are stored in fruits. Therefore, content of chlorophyll has decreased along with ripening the fruit. Usually at the end of ripening, enzymes of analyzing chlorophyll become active and chlorophyll is synthesized to other materials for increasing quality of the fruit.

Table 3. comparison of the chlorophyll a ,b and a*b activity in different along different harvest times (pre-maturity, maturity, post-maturit), different geographical aspects and fruit position in the canopy on orange trees

Harvest times	Aspects	Position**	Chlorophyll a	Chlorophyll b	Chlorophyll a*b
Pre- maturity	South	Light	0.44^{a}	0.55^{a}	0.50^{a}
	North	Light	0.28^{abc}	0.40^{a}	0.35^{a}
	East	Light	0.33^{ab}	0.43^{ab}	0.38^{ab}
	West	Light	0.17^{ab}	0.36^{ab}	0.31^{ab}
	South	Shade	0.15^{bc}	0.33^{ab}	0.28^{ab}
	North	Shade	0.24^{c}	0.36^{ab}	0.31^{ab}
	East	Shade	0.18^{abc}	0.40^{b}	0.35^{b}
	West	Shade	0.11^{a}	0.25^{a}	0.20^{a}
Maturity	South	Light	0.29^{a}	0.25^{a}	0.33^{a}
	North	Light	0.20^{abc}	0.23^{a}	0.22^{a}
	East	Light	0.11^{ab}	0.14^{bc}	0.29^{bc}
	West	Light	0.06^{ab}	0.14^{bc}	0.27^{bc}
	South	Shade	0.07^{bc}	0.09^{c}	0.18^{c}
	North	Shade	0.04^{c}	0.10^{c}	0.15^{c}
	East	Shade	0.09^{abc}	0.19^{bc}	0.31^{bc}
	West	Shade	0.04^{a}	0.15^{ab}	0.25^{ab}
Post- maturity	South	Light	0.25^{a}	0.10^{a}	0.16^{a}
	North	Light	0.24^{a}	0.07^{a}	0.11^{a}
	East	Light	0.23^{a}	0.08^{a}	0.13^{a}
	West	Light	0.23^{a}	0.09^{a}	0.15^{a}
	South	Shade	0.21^{a}	0.09^{a}	0.16^{a}
	North	Shade	0.23^{a}	0.06^{a}	0.11^{a}
	East	Shade	0.22^{a}	0.06^{a}	0.11^{a}
	West	Shade	0.23^{a}	0.08^{a}	0.13^{a}

Numbers with the same letters have no significance difference (5%)
** fruit position in the canopy

Content of Carotenoid

The findings of the test show that position of fruit on canopy has significant effect on activity of Carotenoid (table 4). The findings of the test show that position of fruit on canopy has significant effect on content of Carotenoid ($p< 0.01$) at all times of harvesting. Also, geographical aspect had significant effect on content of Carotenoid. At all times of study, the most content of Carotenoid and the least one was at south aspect of canopy and east aspect respectively at the time of ripening and after that and it was seen on north, west and eastern aspects before ripening (table 4).

Table 4. The carotenoid content in different of geographical aspects along different harvest times (pre-maturity, maturity, post-maturity)

Aspects	Pre- maturity	Maturity	Post-maturity
North	0.16^{b}	0.19^{a}	0.24^{b}
South	0.28^{a}	0.21^{a}	0.34^{a}
East	0.16^{b}	0.14^{b}	0.21^{b}
Wrest	0.16^{b}	0.09^{c}	0.14^{c}

Numbers with the same letters have no significance difference (5%)

effect of interaction of cares was not significant at ripening and after that, while, it was significant after ripening (figure 5). Carotenoids are a group of yellow and orange pigments that are found in most of creatures that photosynthesize. These pigments are available in green color but their color appears after decomposing chlorophyll at autumn. As Carotenoid absorbs blue and violet color of light (430-490 nm), its spectrum of absorption is different from molecule of chlorophyll. Carotenoid and pigments of chlorophyll has important role on restraining free radicals due to power of their oxidation. This property of Carotenoid causes insistence of cell against environmental tensions. High content of Carotenoid and anthocyanin at first stage may be due to small size of fruit and sending carbohydrates to fruit and being excess of carbohydrates in fruits has caused to use saccharine compounds at the way of synthesis of Carotenoid and anthocyanin pigments. Finding of this research is similar to results of Ghasemi et al (2009) in the field of increasing content of Carotenoid and anthocyanin in peach during ripening.

Table 5. Comparison of the carotenoid content in different of geographical aspects and fruit position in the canopy along post-maturity of harvest time

Aspects	Position**	Carotenoid content
South	Light	0.37[a]
North	Light	0.35[a]
East	Light	0.38[a]
West	Light	0.24[b]
South	Shade	0.16[bc]
North	Shade	0.14[cd]
East	Shade	0.05[d]
West	Shade	0.05[d]

Numbers with the same letters have no significance difference (5%)
** fruit position in the canopy

pH, titration acid (TA), total soluble solids (TSS), ratio of TA/TSS

The findings of the research showed that geographical aspect and position of fruit in canopy had not significant effect on pH, TA, TSS and TA/TSS. Findings of pH, TA and TSS of this research was similar to findings of Anor et al (1999) in some items and findings of Elsheikh and Abu- Goukh (2008) and Jang et al (2005) for effect of methods of harvesting and storing on quality of grapefruit. Also Erturk et al (2010) studied operation and quality of types of strawberry and noted that there wasn't any significant difference between type and content of TSS and pH during studying.

CONCLUSION

Base on the results, the geographical position of fruit tree canopy, during the harvest times hade a significantly effect on the biochemical properties of fruit. So that the total anthocyanin and carotenoid fruit nearing harvest maturity increased and decreased at the end of fruit development. As well, the amount of chlorophyll a, b and ab pace with the maturation and ripening of fruits decreased. Usually the final stage on chlorophyll- degrading enzymes and to improve fruit quality, reduce the amount of chlorophylls. In all the harvest times, fruits were located in the sun lights compared to the shade canopy, hade a significantly effect on the biochemical properties of fruit. However, experimental treatments on pH, TA, TSS and TA/TSS did not change was relatively constant.

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