DOI : 10.5958/0974-0112.2019.00039.2

Indian J. Hort. 76(2), June 2019: 253-258

Environmental impact on biochemical parameters during developmental stages of *Citrus* fruit

Dinesh Kumar*, Lallan Ram, M. S. Ladaniya, Archana Khadse and Sunil Kumar**

ICAR- Central Citrus Research Institute, Nagpur 440033, Maharashtra

ABSTRACT

Influence of climate (temperature and rainfall) on biochemical and antioxidant capacity of Nagpur mandarin (*Citrus reticulata* Blanco) fruit was studied during the two consecutive years. At fruit maturity stage higher vitamin C was recorded as 36.49mg/100ml at minimum temperature level at location 2 and total soluble solid contents was found to be 8.28°B at fruit maturity stage at location 1. Pectin estimated as calcium pectate was recorded as 2.76% in the fruit peel and 1.16% in the fruit juice in the maturity stage at location 1. PME activity was found higher (4.21 U/ml) in the mature fruit juice of Nagpur mandarin at location 1. Total phenol and total flavonoid content were recorded as 7.35 mg/100ml and 8.16 mg/lit respectively in fruit juice at location 1. The findings indicated that varies temperature and rainfall pattern at a moderate rainfall zone (Nagpur district referred as location 1) significantly influence the biochemical parameters and antioxidant activity in Indian citrus (*Citrus reticulata* Blanco) at maturity stage.

Key words: *Citrus reticulata*, mandarin, PME, flavonoid, antioxidant activity.

INTRODUCTION

Citrus is vulnerable to high temperature. Fruit growth is determined as a function to the daily average temperature and an estimate of daily evaporative demand which is calculated from maximum temperature, which range over the day and solar radiation. Citrus fruit are well recognized worldwide for their flavour and health properties since they contain a wide collection of secondary metabolites that originally address different plant needs but possess crucial nutritional properties. The fruits and juices of citrus species are found to be rich in source of bioactive compounds including antioxidants such as flavonoids, phenolic compounds, ascorbic acid and pectins which are important to overall human growth and nutrition (Jayprakash and Patil, 3). Nagpur mandarin (*Citrus reticulata* Blanco) belongs to the family of Rutaceae is one of the prominent fruit crop of citrus group, grown throughout the India. Increased interests in human health, nutrition and disease prevention, consumers have increased their demand for Nagpur mandarin fruits. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Sarkar *et al.*, 11).

Vitamin C being highly sensitive to oxidation and as leaching into water soluble media in storage, the retention of it is used as an approximation for the overall nutrient retention of food products. After harvest, it degrades immediately and also degrades steadily during the prolonged period of storage. Analytical results showed that the lower the temperature the better the concentration of vitamin C in fruit juice. Higher temperature does not favour vitamin C (Njoku *et al.*, 7).

The content and stability of phytochemicals is affected by many pre-harvest factors such as climate, maturity, crop genotype variation, temperature, carbon dioxide and light. In addition, maturity of fruit crops also influences the antioxidant capacity significantly and considerably influenced with different levels of maturity. The different types of phytonutrients are synthesized in parallel with the maturation of fruits and overall development. Therefore with different stages of maturity of fruits the antioxidant capacity also varies considerably. High temperature growing conditions (25/30°C) significantly enhanced antioxidant activity, as well as anthocyanin and total phenolic content. As, the lowest antioxidant activity is seen in fruits grown on plants in a cool day & night temperature (18/12°C) (Wang and Zheng, 15).

The knowledge of the changes, occurring during growth and maturation, holds great significance from both dietary and nutritional point of view. It is therefore, imperative to study the changes in the content of biochemical parameters as influenced by varied temperature, relative humidity and rainfall during their maturity stages. There are no more reports available on the secondary metabolites and antioxidant content in Nagpur mandarin fruit,

*Corresponding author's E-mail: dineshscn@gmail.com

**Central Institute of Horticulture, Medziphema, Nagaland

even the influence of climate (viz. temperature and rainfall) on Nagpur mandarin in this two different zones are limited. With this aim the study was carried out on Influence of varied temperature and rainfall pattern on bio-chemical parameters during developmental stages of Indian citrus fruit (*Citrus reticulata* Blanco) during the year 2012-13 and 2013-14 at ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India.

MATERIALS AND METHODS

According to agro-climatic zones in Maharashtra, India two zones were selected on the basis of rainfall and vegetation, which are Moderate Rainfall Zone and Assured Rainfall Zone. Nagpur mandarin fruits were collected from citrus orchards of these two zones, which referred as Location 1 (Nagpur, India) and Location 2 (Amravati, India). Fruits were harvested at different fruit growth stages i.e. 30 days interval up to fruit maturity. The fruits were washed and cleaned with distilled water to remove surface residues and juice was squeezed from the fruits for physico-chemical and biochemical analysis in PHT and Processing laboratory of ICAR- Central Citrus Research Institute, Nagpur, India

Estimation of ascorbic acid in the fruit juice, pectin as calcium pectate in peel and juice of the fruit were estimated as per the method given by Association of Analytical Chemists. Activity of enzyme pectin Methylesterase (PME) in the fruit peel and juice was measured as per the method given by Carbonell *et al.* (1). Total Soluble Solid (TSS) was recorded by thermostatic based at 25°C, refractometer (A. Kruss Optronic, Germany) which expressed in °B.

Total phenols content (mg/100ml) in Nagpur mandarin juice and peel was determined by the Folin-Ciocalteu reagent by using spectrophotometer (Singleton *et al.*, 12). In which 0.5 ml juice and 1g peel sample were extracted (25 ml 80% methanol with filter paper No. 4), aliquot of volume 0.1 ml juice and 0.2 ml peel extract were diluted (0.9/0.8 ml distilled water, respectively). Then, in aliquot 1 ml of Folin Ciocalteu reagent (1:1 diluted with distilled water) and Na_2CO_3 (2 ml, 20 %) were added. The mixture was boiled at 100°C for 1-2 min, then cool it and made a volume upto 10ml with distilled water. That mixture was centrifuge at 8000 rpm for 10 min and read at a wavelength 650 nm.

Total flavonoid (mg/lit) was estimated from juice and peel by the method given by Zarina and Tan, (16), in which 0.5 ml juice and 0.5g peel sample were extracted (25ml 80% methanol with blotting paper), volume of 2ml of juice and peel sample solution were accurately measured in a test tube. Secondly, added 0.6ml (5%) sodium nitrite ($NaNO_2$), shake properly and leave it for 6 min. Then added 0.5ml (10%) aluminum nitrate ($AlNO_3$) and shake well and again leave it for 6 min. Finally, added 3ml of (4.3%) sodium hydroxide (NaOH) and shake it properly. Make up volume of solution up to 10ml with distilled water and left to stand for 15 min, same procedure was run with distilled water and treated it as a blank. Finally read at 500nm wavelength on Shimadzu UV-1650 PC UV-Visible spectrophotometer, concentration of total flavonoid in the sample was determined using the standard graph plotted using standard solution of rutin (Sigma-Aldrich Corporation).

In this experiment three replicates were analyzed per treatment. Total phenol and flavonoid content in fruit juice and peel were correlated with rainfall. The experimental data was statistically analyzed and the data is pooled for two years 2012-13 and 2013-14. All statistical analyses and differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The physicochemical and bio-chemical parameters were recorded at regular interval from fruit set to till attending the maturity of the fruit. The Vitamin C content varies from 30.94 to 36.49 mg/100ml at the time of fruit maturity in both locations. At the fruit maturity stage minimum temperature were recorded at location 2 which significantly increased vitamin C content as 36.49mg/100ml in the fruit. The vitamin C level is especially influenced with temperature and climate (Padayatty *et al.*, 9). Environmental conditions which increase the acidity of citrus fruits is responsible for increasing the vitamin C content. Climatic conditions including light and average temperature have a strong influence on the chemical composition of horticultural crops. Similar, finding was observed by Spiegel-Roy and Goldschmidt (13) that internal quality is also affected by climate. Total soluble solid increased during fruit developmental stages in both the locations, in which fruits of location-1 were recorded 8.28^{0}B at fruit maturity stage (Table 1). Fruit developing in a hot, tropical climate tends to have high total solids content, which is an advantage for the processing industry. Fruit developing under warm climate reach marketable sugar/acid ratios sooner than cooler locations (Fig. 1 & 2).

The fruit peel pectin content remains constantly higher throughout the fruit development stage and it remains higher 2.76 % at fruit maturity stage and also the pectin content was recorded higher 1.16 % in juice of matured fruit at location 1, having the higher temperature during the fruit maturation stage of Nagpur mandarin. Pectin content was estimated as calcium pectate and which was also found highly significant having a positive correlation (r = 0.88)

Table 1. Pooled data of bio-chemical parameters of Nagpur mandarin fruit peel & juice.

Fruit growth period (months)	Vitamin C (mg/100ml)		Total soluble solid (°B)		Fruit Pectin (%)		Juice Pectin (%)		Fruit PME (U/ml)		Juice PME (U/ml)	
	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2	L-1	L-2
August	31.21	31.52	8.16	6.21	2.10	1.75	0.65	1.53	0.91	0.41	4.81	4.72
September	35.63	30.94	8.51	8.60	3.11	2.72	1.03	1.22	0.63	0.46	7.79	5.52
October	30.90	37.19	7.52	8.42	5.15	1.96	0.31	1.13	0.16	0.18	5.65	3.44
November	31.04	36.49	8.28	8.97	2.76	0.92	1.16	0.28	0.10	0.61	4.21	3.45
CD (0.05)	2.471	2.890	0.251	0.335	0.407	0.211	0.245	0.201	0.211	0.122	0.362	0.271

L-1: Nagpur District, L-2: Amravati District

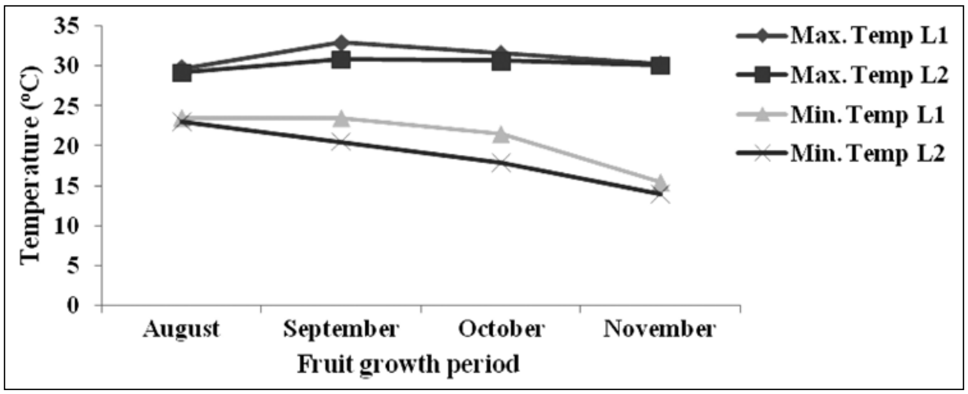

Fig. 1. Maximum and minimum temperature of two locations of Nagpur mandarin orchards during fruit growth period.

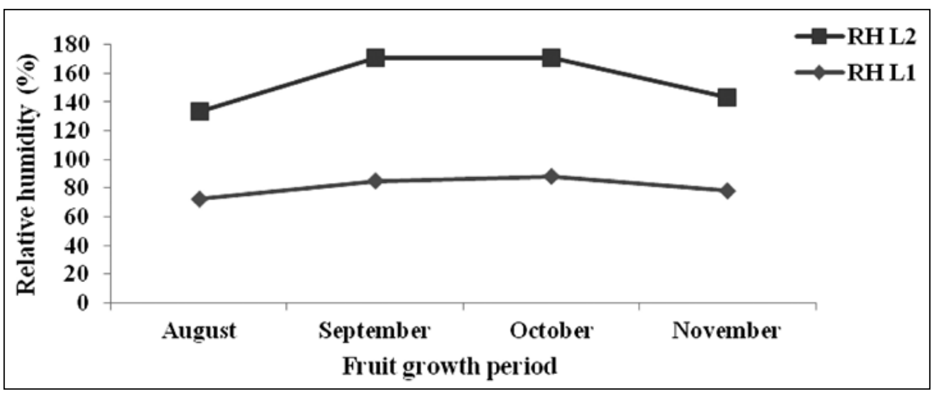

Fig. 2. Relative humidity of two locations of Nagpur mandarin orchards during fruit growth period.

with the relative humidity, which was recorded higher at location 1 during the fruit maturity. Pectin methyl esterase (PME) activity varied with the development stages of the fruit. PME activity was found higher (4.21 U/ml) in the juice of mature fruit of Nagpur mandarin at location 1. Consequently, maximum temperature has also shown a highly significant, positive correlation (r = 0.93) with the PME activity at location 1 (Table 1).

The biochemical parameters were also recorded as total phenol and total flavonoids contents in the fruit peel and juice (Fig. 3-6). The peel showed the higher amount of phenol as compared to the juice in accordance with previous studies and expressed as mg catechin equivalent per 100 ml. During the fruit growth development stage, the bio-chemical parameter varies. It has been found that total phenol content (7.35 mg/100ml) was recorded higher in the juice of fruit maturity at location 1. At the early growth stages, total phenol content was higher, then it decreased and at maturity again

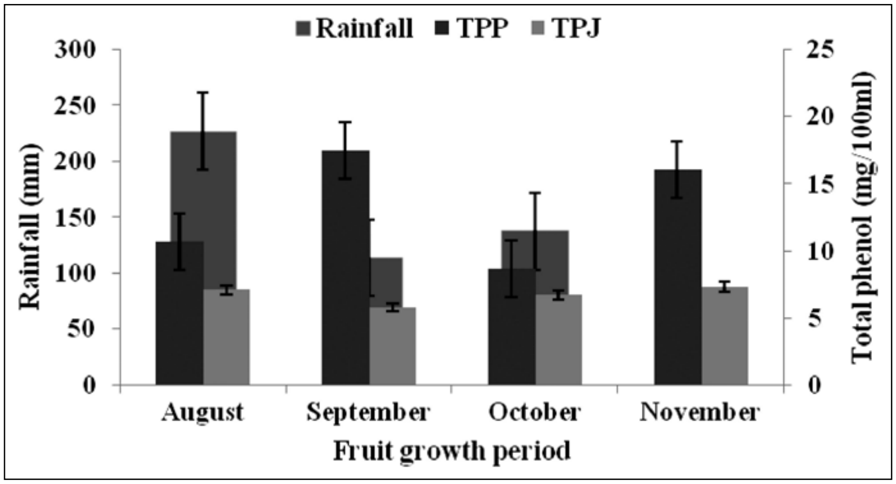

Fig. 3. Effect of rainfall on total phenol content of Nagpur mandarin fruit in location 1.

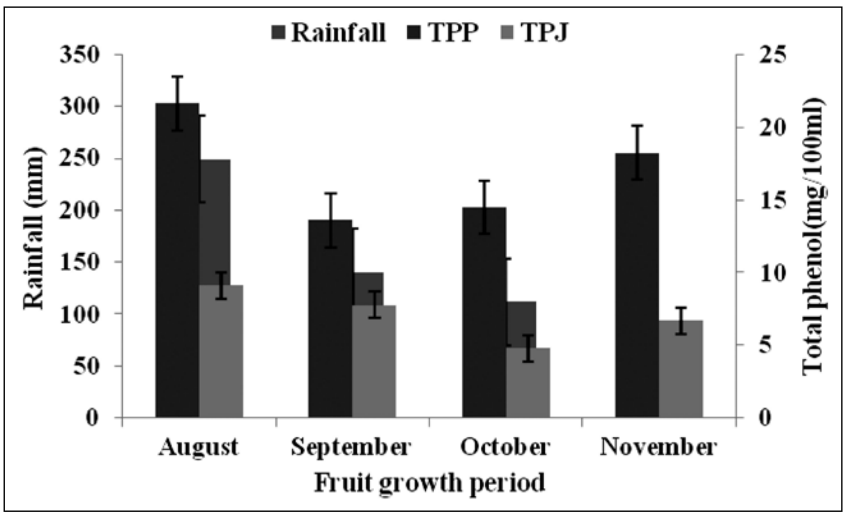

Fig. 4. Effect of rainfall on total phenol content of Nagpur mandarin fruit in location 2.

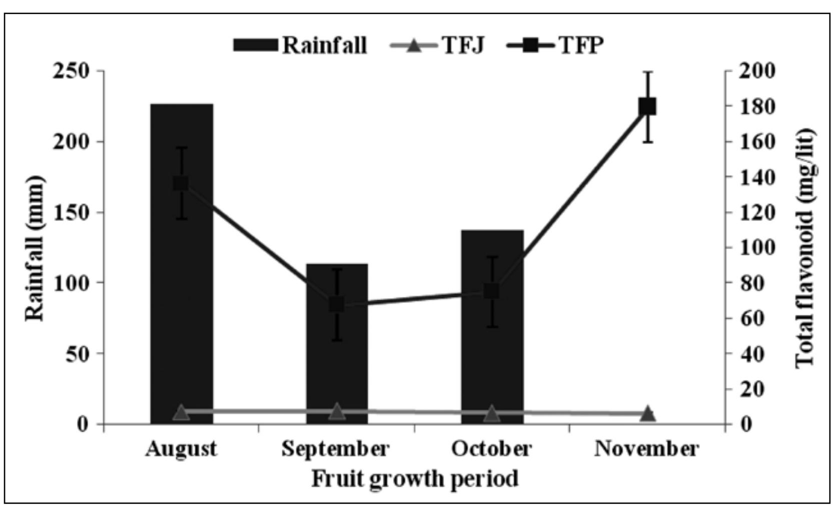

Fig. 5. Effect of rainfall on total flavonoid content of Nagpur mandarin fruit in location 1.

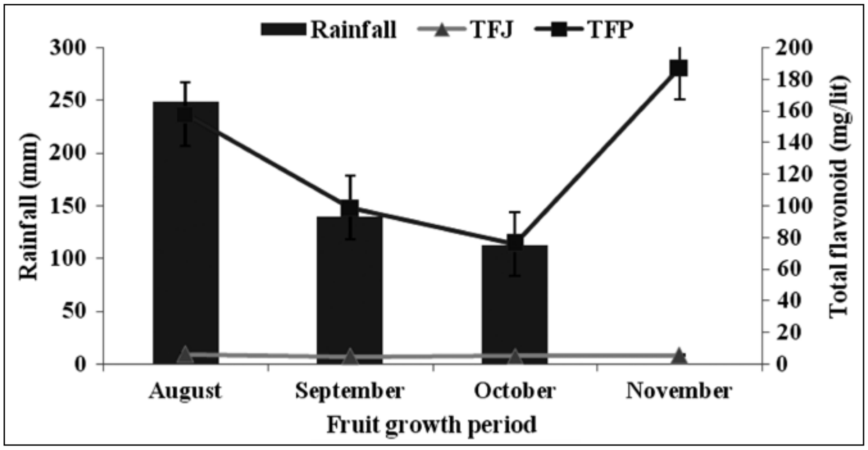

Fig. 6. Effect of rainfall on total flavonoid content of Nagpur mandarin fruit in location 2.

concentrations of the content get increased. Similar, findings was also reported by Kondo *et al.* (5) that the composition and concentration of phenolic compounds in the fruit change during development and ripening. In most cases, the phenolic compounds concentration increases during fruit development and later decreases or remains constant during ripening. Total phenols have reportedly been linked with the antioxidant capacity of fruits. Increasing the total phenol content also increased the antioxidant efficacy in fruits (Proteggente *et al.*, 10). Similarly, Gorinstein *et al.* (2) reported that high correlation of the total phenolic and antioxidant activity of citrus extracts. Total phenols were reported to be the major antioxidant of citrus fruits. Total polyphenol contents were the highest in the late August and decreased with ripening. Similarly, unripe fruit juices have displayed stronger antioxidant activity when compared to ripe fruit juices. The reduction in the total phenolic content and ascorbic acid during ripening is responsible for lower antioxidant activity of ripe fruit juices. Rainfall has shown a positive correlation (r = 0.85) with the total phenol in the juice. Temime *et al*. (14) recorded that a positive linear relationship (r = 0.796) existed between phenol content and precipitation for the virgin olive oils from Chétoui.

The higher total flavonoid content (8.16 mg/lit) was recorded in the juice of fruit maturity at location 1. Kim *et al.* (4) observed that flavonoid contents in the citrus fruit juices were the highest during early maturation and decreased rapidly while ripening. Similarly, the accumulation of flavonoid content in various citrus species is related to certain stages of fruit growth. Three different stages of citrus fruit growth are: logarithmic phase stage (50 days after full bloom); linear growth stage (50 to 150 days after full bloom); and fruit maturation stage. The highest levels of hesperidin is detected in very young tissues (logarithmic phase) of the fruit (Omidbaigi and Nasiri, 8). The expressions of genes and activities of enzymes related to flavonoid biosynthesis and metabolism can be regulated and altered in different cultivation regions under different temperatures, precipitations, and sunshine exposures, which eventually impact the accumulation of the flavonoids in fruits. Interestingly, total flavonoid in the fruit peel found highly significant having a positive correlation (r = 0.99) with the rainfall at location 1 (Fig. 5).

It has been found that cultivars varied greatly in composition and level of metabolites and was independent of species and geographical locations. Similar, result was recorded by Kumar *et al.* (6) that the expression of unique variation in phytochemical and bioactive compounds in pummelo influenced by distinct climate and geographical condition.

From the experimentation it can be inferred that varied temperature and rainfall pattern on Moderate Rainfall Zone which is the agro-climatic zone in Maharashtra (Nagpur district-referred as location 1) shown significant influence on the biochemical parameters and antioxidant activity in Indian citrus fruit (*Citrus reticulata* Blanco) at fruit development stages and fruit maturity stages. Total phenol found to be increased according to fruit development stages, similar trend was observed in related to antioxidant content. Based on which the fruit development and subsequently the shelf life of Nagpur mandarin fruit with desired quality get affected.

ACKNOWLEDGMENTS

The research reported in this manuscript has been funded through the "National Initiative Climate Resilent Agriculture (NICRA)". We authors gratefully acknowledge the guidance and necessary facilities provided by the Director, ICAR-Central Citrus Research Institute, Nagpur and also for his constant encouragement during the course of investigation and last but not the least the necessary help rendered by supporting staff members of processing laboratory in successfully carrying out the experiments is duly acknowledged.

REFERENCES

1. Carbonell, J. V., Contreras, P., Carbonell, L. and Navarro, J. L. 2006. Pectin Methylesterase activity in juices from mandarins oranges and hybrids. *European Fd. Res. Technol*. **222:** 83-87.

2. Gorinstein, S., Zachwieja, Z., Katrich, E., Pawelzik, E., Haruenkit, R., Trakhtenberg, S. and Martin-Belloso, O. 2004. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. *Lebensmittel-Wissenschaft und-Technol*. **37:** 337-43.

3. Jayaprakasha, G. K. and Patil, B. S. 2007. In vitro evaluation of the antioxidant activities in fruit extracts from citron and blood orange. *Fd. Chem* .**101:** 410-18.

4. Kim, Y. D., Ko, W. J., Koh, K. S., Jeon, Y. J. and Kim, H. S. 2009. Composition of flavonoids and antioxidative activity from juice of jeju native citrus fruits during maturation. *Korean J. Nutr*. **42:** 278-90.

5. Kondo, S., Tsuda, K., Muto, N. and Ueda, J. 2002. Antioxidative activity of apple skin or flesh extracts associated with fruit development on selected apple cultivars. *Sci. Hort.* **96:** 177-85.

6. Kumar, D., Lamer, H., Singh, I. P., Ladaniya, M. S. and Sthapit, B. 2015. Phytochemical evaluation of pummelo fruits (*Citrus grandis*) in India for enhancing marketing opportunities. *Indian J. Pl. Genet. Resour*. **28**: 50-54.

7. Njoku, P. C., Ayuk, A. A. and Okoye, C. V. 2011. Temperature effects on vitamin C content in citrus fruits. *Pakistan J. Nutr*. **10:** 1168-69.

8. Omidbaigi, R. and Nasiri, M. F. 2004. Quantitative distribution of hesperidins in Citrus species during fruit maturation and optimal harvest time. *Natural Product Radiance*. **3**: 12-15.

9. Padayatty, S. J., Katz, A., Wang, Y., Eck, P. and Kwon, O. 2003. Vitamin C as an antioxidant: Evaluation of its role in disease control. *J. American Nutr*. **22:** 18-35.

10. Proteggente, A. R., Saija, A., De, A., Pasquale, A. D. and Rice-Evans, C.A. 2003. The compositional characterization and antioxidant activity of fresh juices from Sicilian sweet orange [*Citrus sinensis* (L) Osbeck] varieties. *Free Radical Res*. **37:** 681-87.

11. Sarkar, N., Srivastava, P.K. and Dubey, V.K. 2009. Understanding the language of vitamin C. *Curr. Nutri. Fd. Sci*. **5:** 53-55.

12. Singleton, V., Orthofer, L. R. and Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol*. **299**: 152-78.

13. Spiegel-Roy, P. and Goldschmidt, E. E. 1996. Biology of Citrus. Cambridge University Press, pp. 230.

14. Temime, S. B., Wael, T., Bechir, B., Leila, A., Douja, D. and Mokhtar, Z. 2006. Changes in olive oil quality of Chétoui variety according to origin of plantation. *J. Fd. Lipids*. **13**: 88-99.

15. Wang, S.Y. and Zheng, W. 2001. Effect of plant temperature on antioxidant capacity in strawberry. *J. Agric. Fd. Chem*. **49:** 4977-82.

16. Zarina, Z. and Tan, S. Y. 2013. Determination of flavonoids in Citrus grandis (pomelo) peels and their inhibition activity on lipid peroxidation in fish tissue. *Int. Fd. Res. J.* **20**: 313-17.

Received : June, 2018; Revised : April, 2019;
Accepted : May, 2019