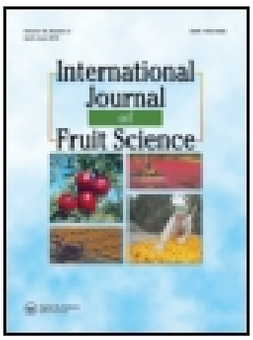

International Journal of Fruit Science

ISSN: 1553-8362 (Print) 1553-8621 (Online) Journal homepage: https://www.tandfonline.com/loi/wsfr20

Characterizing the Nutrient Composition, Physiological Maturity, and Effect of Cold Storage in Khasi Mandarin (*Citrus reticulata* Blanco)

Karishma Kashyap, Debaleena Kashyap, Mukesh Nitin, Nirala Ramchiary & Sofia Banu

To cite this article: Karishma Kashyap, Debaleena Kashyap, Mukesh Nitin, Nirala Ramchiary & Sofia Banu (2019): Characterizing the Nutrient Composition, Physiological Maturity, and Effect of Cold Storage in Khasi Mandarin (*Citrus reticulata* Blanco), International Journal of Fruit Science, DOI: 10.1080/15538362.2019.1666334

To link to this article: https://doi.org/10.1080/15538362.2019.1666334

Published online: 24 Sep 2019.

INTERNATIONAL JOURNAL OF FRUIT SCIENCE
https://doi.org/10.1080/15538362.2019.1666334

Taylor & Francis
Taylor & Francis Group

Characterizing the Nutrient Composition, Physiological Maturity, and Effect of Cold Storage in Khasi Mandarin (*Citrus reticulata* Blanco)

Karishma Kashyap[a], Debaleena Kashyap[b], Mukesh Nitin[c], Nirala Ramchiary[c], and Sofia Banu[a]

[a]Department of Bioengineering and Technology, Gauhati University, Guwahati, India; [b]Department of Biological Science, Asian Institute of Management and Technology, Guwahati, India; [c]School of Life Sciences, Jawaharlal Nehru University, New Delhi, India

ABSTRACT

Farmers often emphasize more on physical changes of color rather than physiological maturity for proper harvesting time of Khasi mandarin (*Citrus reticulata* Blanco). The study aimed to characterize the physiological maturity of the fruit across the developmental stages which has not been well reported till now. A cold temperature (4°C) treatment was also performed to evaluate its effect in the fruit postharvest. TSS (peel; pulp) reached palatable values at 230 days after flowering (DAF; 8.08 ± 0.63%, 11.06 ± 0.46%). Ascorbic acid (peel and pulp) subsequently decreasing at 230 DAF (46.05 ± 1.16; 53.43 ± 0.76 mg/100 ml). Cold storage preserved TSS/TA ratio (15.44), TSS (11.49%) in juice better.

KEYWORDS

Biochemical; cold storage; maturation; physicochemical; postharvest

Introduction

Citrus, belonging to the family Rutaceae is the third most important fruit crop in India next to banana and mango (Nath et al., 2013). It has its diverse forms originated and distributed in northeastern India (Hazarika, 2012; Sanabam et al., 2015). Among citrus, the loose-skinned Khasi mandarin (*Citrus reticulata* Blanco), a high quality seeded mandarin orange cultivar of India, is one of the most popular, financially important and worldwide accepted fruit (Singh et al., 2016). India occupies ninth position in mandarin orange production among the top countries in the world, with global production increasing to 47.8 million in 2017/18, covering an area of 3.11 lakh hectares (Jha et al., 2019). In India, Khasi mandarin constitutes about 43.6% of the total citrus fruits production, covering nearly 38.2% of the total citrus area cultivation (Tariang et al., 2018). Khasi mandarin occupies the major area in north-eastern India due to its high commercial importance in terms of production and export potentiality (Deshmukh et al., 2016). It is cultivated

CONTACT Sofia Banu sofiabanu2@gmail.com Faculty of Technology, Department of Bioengineering and Technology, Gauhati University, 781014, India

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/wsfr.

predominantly in Assam and surrounding foothills of north-eastern India with a production of 598.96 thousand tones, covering an area of 105.49 thousand hectares of total 0.31 million hectares of mandarin in India (Sangma et al., 2018). Favored by the rich agro-climatic conditions, the mandarin orchards are mostly located on hill tracts with different altitude ranging from low to high, 50 to 5000 m above sea levels in these regions (Sanabam et al., 2015).

Khasi mandarin is easy to peel, deep orange colored when ripe and popular for their dietary and nutritional properties (Hazarika, 2012). This fruit is highly consumed worldwide either as fresh produce or in the form of juice and the peel is usually discarded as primary waste in the environment (Sharma et al., 2018). In addition to the fruit pulp, the peel represents around 40% – 50% of wet fruit mass which is found to be a potential source of bioactive components such as ascorbic acid, carotenoids, phenolic compounds and pectins with the highest concentration of flavonoids. The peel has more vitamin C than its juice according to the USDA National Nutrient Database (Sharma et al., 2018). The peel exhibits significant antioxidant properties as compared to the remaining parts of the fruit. Specifically, the citrus peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils (Zema et al., 2018). The significance of this crop is multiplied not only because of its economic value but also due to its nutritional benefit and medicinal value contributing to the health of the human and domestic animals (Sharma et al., 2018). The nutritional benefits of citrus fruits in human diet shifted the focus to measure fruit maturity in order to improve nutritional values for its optimum use (Deshmukh et al., 2016). The determination of the criteria for fruit maturation is quite complex as it depends upon the internal changes occurring in the fruit flesh as well as the external fruit peel coloration which occur during fruit development, growth, and maturity (Kashyap and Banu, 2019). Therefore, visual, physical and chemical parameters such as TSS, acidity, ascorbic acid are a valuable alternative for fruit quality evaluation. However, commercial maturity indices in citrus fruit are highly variable depending on the ripening stage, environmental conditions, growing region, and varieties (Lado et al., 2014).

Khasi mandarin fruit is highly fragile in nature with a short shelf life of 1–2 weeks at ambient temperature and its rapid deterioration creates a problem for fruit postharvest management causing significant losses to the farmers and general economy (Rokaya et al., 2016). Temperature maintenance during postharvest is an important environmental criterion to preserve the overall quality of fresh citrus fruits contributing to its postharvest performance (Tietel et al., 2012). Fruits harvested at the inappropriate maturity stage develop physiological disorders during storage and affect the shelf life quality (Assumi et al., 2017). Determining the correct stage of maturity of the

fruit particularly at harvesting time is important to minimize the postharvest losses (Hossain, 2015). Proper maturity stage should be determined for optimum harvesting and maintenance of shelf life. International market emphasizes on external as well as internal quality standards for mandarin fruits (Rokaya et al., 2016).

Most of the studies available in the literature in Khasi mandarin were carried out only across ripening stages to understand the physiological maturity (Rokaya et al., 2016; Singh et al., 2016). However, till now no systematic research work has been reported before these ripening stages, throughout the development phase (right after fruit setting) in Khasi mandarin and understand the significance of the physicochemical parameters contributing toward best quality fruit. The present study was, therefore, conducted to characterize and understand the nutrient and other chemical compositions in different developmental stages of Khasi mandarin fruit. Furthermore, since, farmers and growers look for physical changes of color rather than physiological maturity, one of the objectives was also to investigate the physiological maturity and physiochemical characteristics toward the later stages of fruit development and to assess the effect of cold storage on the physicochemical parameters in the fruit postharvest.

Materials and Methods

Plant Material and Collection Site

Fruits of Khasi mandarin were harvested from May to November (during two seasons) from trees grown in a local orchard located in Kamrup (25°97′ N, 91°23′ E), Assam, India. Trees in the orchard were around 10 years old according to the grower. The samples were authenticated (accession no. 18070) at Department of Botany, Gauhati University. During two consecutive seasons, trees were randomly selected based on fruit bunches in full bloom, where fruit samples were harvested after the onset of flowering at 30 day interval period throughout the experiment based on their appearance (size and color) and health condition into the following eight developmental stages: 30 days after flowering (DAF) followed by 60 DAF, 90 DAF, 120 DAF, 150 DAF, 180 DAF, 210 DAF, with the last sampling date set at full maturity 230 DAF (Figure 1a,b). Fruits were harvested at these stages to study the physiochemical changes in fruit development as no such study has been reported. After harvesting, fruits were packed in coolers and taken for analysis to the laboratory on the same day.

During each harvest until 230 DAF, approximately 60 fruits were collected representing the population which was divided into three sets of 20 fruits each before the peel and pulp were separated and segregated. At 230 DAF, approximately 120 fruits were collected which was divided into six sets of 20 fruits. From each set of the harvested fruit, triplicate sets were used for the

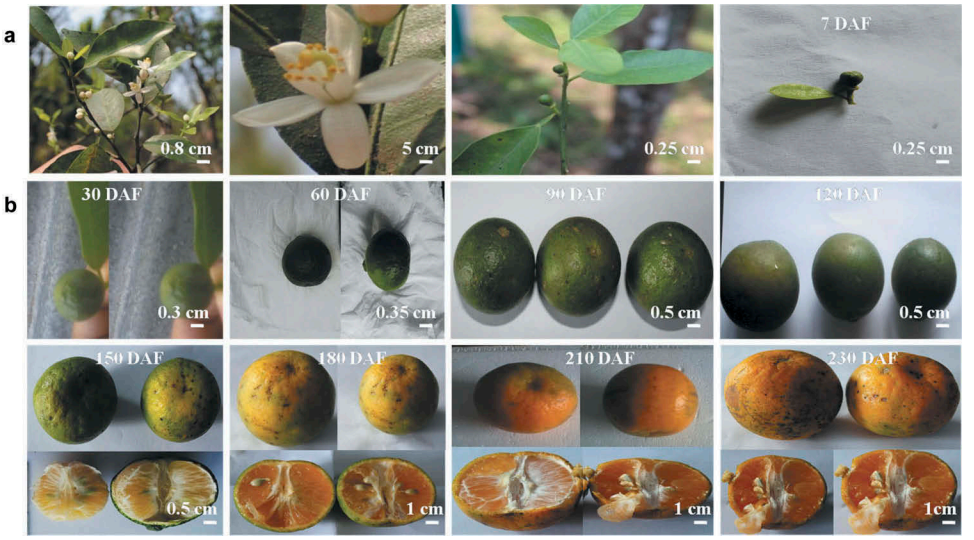

Figure 1. Developmental stages of Khasi mandarin fruit. (a): Flowering and fruit setting stages (b): Development and ripening stages in the fruit sampled from 30 DAF till 230 DAF.

study. Fruit samples collected, were cleaned with water, dried at room temperature (25 ± 2 °C) and wiped with a dry cloth. Peel and pulp tissue of the fruits were dried and powdered for the different analysis procedures. For the postharvest study, samples harvested at full maturity (230 DAF) were stored till 42 days (sixth week) and data recorded at 7th day (first week), 21st day (third week) and 42nd day at 4 °C. The following experiments were executed:

Physicochemical Parameters: Fruit Weight, Fruit Diameter, Juice pH and Juice Content

Parameter such as the fresh weight of the fruit was weighed and recorded in grams using a weighing balance (Electronic balance, BL-220H, Shimadzu, Japan). The pH of the fruit juice for each sample was determined using a calibrated pH meter (pH 700, Eutech Instruments, Thermo Fisher Scientific, Mumbai, India). The diameter for each fruit was measured in centimeter using a ruler by placing the fruit between two wooden blocks. The juice content was weighed and measured in gram (Grewal et al., 2000). The percent juice content was calculated by using the following formula as given in equation 1.

$$\%\ \text{juice content} = \text{juice weight/fruit weight} \times 100 \quad (1)$$

Biochemical Parameters

Ascorbic Acid Content and Total Soluble Solids (TSS) Content

Total ascorbic acid (vitamin C) content from dried pulp and peel extracts were determined by titration with 2, 6-dichlorophenolindophenol method (AOAC, 1995). Extractions and titrations were carried out in triplicates for each replication and expressed as mg/100 ml of sample.

TSS content in juice produced from fruits at each maturity stage was determined (triplicate for each replication and averaged) using an OPTi digital handheld refractometer (Model Brix 54, Bellingham and Stanley, UK) at room temperature as outlined by AOAC (2005) and results were expressed as % °Brix (Lacey et al., 2009).

Titrable Acidity and Maturity Index

Titrable acidity (TA) was determined by titration of 10 ml of juice against N/10 NaOH to the endpoint at pH 8.2 as per outlined by AOAC (2005) and expressed as percent citric acid (Deshmukh et al., 2016).

The maturity index was calculated as the ratio between TSS and the titrable acidity (Deshmukh et al., 2016). TSS/TA ratio was calculated by dividing the % Brix value by Percentage acid (Lacey et al., 2009). Determinations of TA and TSS/TA ratio were measured in triplicate for each replication and averaged.

Total Chlorophyll and Carotenoids Content

Chlorophyll a, chlorophyll b and total chlorophyll content was extracted from 1 g of the peel powder with 80% (v/v) acetone as described by Roongruangsri et al. (2013). Chlorophyll content was determined by measuring the absorbance at 663 nm and 645 nm. Chlorophyll a, chlorophyll b and total chlorophyll was calculated using Arnon's equations from the absorbance readings. The calculated values were expressed in μg g^{-1} Fresh Weight (FW) as given in equations 2.

$$\text{Chlorophyll a} = 0.0127A663 - 0.00269A645 \quad (2)$$

$$\text{Chlorophyll b} = 0.0029A663 - 0.00468A645$$

$$\text{Total Chlorophyll} = 0.0202A663 + 0.00802A645$$

$$\text{Where A = Absorbance}$$

Carotenoids content was measured as the method described by Wang et al. (2008) with minor modifications. 1 g of the powdered peel sample was extracted with hexane: acetone: ethanol in the ratio of 50:25:25 v/v. The top hexane layer containing the color was recovered after centrifugation at 4000 g for 10 min. Total carotenoids content was measured using an extinction coefficient of β-carotene (Sigma, Bangalore, India), E1% = 2500 at an

absorbance of 450 nm. The calculated values were expressed in mg g^{-1} FW for the peel. Carotenoids quantification was calculated as given in equation 3.

$$\text{Carotenoids content}\left(\text{mgg}^{-1}\right) = (\text{OD450 nm} \times \text{ml of n} - \text{hexane} \times 1.11 \times 100 \times \text{dilution})/(2500 \times \text{g sample}) \quad (3)$$

Where OD = Optical Density

Correlation Coefficients of Physicochemical and Biochemical Parameters

The correlation coefficient (R) was done using R software 3.2Vand p-value was used to show correlations and their significance. A probability value of $p < .05$ was adopted as the criteria for significant differences.

Statistical Analysis

The data of this investigation were subjected to means ± standard deviation of the mean (S.D.M.) of triplicates from three biological replicates for each sample. Statistical analysis of data was carried out by analysis of variance (ANOVA) to reveal significant variation for each parameter among sampling stages. For the postharvest study, two-way ANOVA analysis was carried out to reveal significant variation for each parameter among the storage days. Tukey test was used to compare the significance of difference among means using the software Graph Pad Prism 5 (GraphPad Software Inc., CA, USA). A probability value of $p < .05$ was adopted as the criteria for significant differences.

Results

Physicochemical Analysis

From the above data, significant differences between the sampling stages were observed until ripening and were consistent with the parameters at each sampling stage (Table 1). The fruit weight and diameter were found to be significantly lowest in the immature stage at 30 DAF, while pH value and juice content of the fruits were not detectable at this stage. But these parameters started increasing during maturation, as the fruits reached their maximum size and began to ripen. They reach significantly comparable values when the fruits were fully ripe at 230 DAF (Table 1). Postharvest ripening was investigated in Khasi mandarin fruits treated with low temperature sampled at three storage periods (Table 1). Low-temperature storage at 4 °C significantly reduced the losses in fruit weight, juice content and fruit p H. These parameters increased significantly till 21 days after which it started decreasing with increasing storage period.

Table 1. Physicochemical parameters in Khasi mandarin fruit during development, ripening and postharvest storage[a].

Sampling Days	Fruit Weight (g)	Fruit juice yield (%)	Fruit Diameter (cm)	Fruit pH	Total acidity (%)	TSS/TA
30 DAF	12.25 ± 0.04a	ND[†]	2.81 ± 0.01a	[†]	[†]	[†]
60 DAF	25.16 ± 0.58b	[†]	3.06 ± 0.11ab		[† †]	[†]
90 DAF	44.85 ± 0.33c	29.83 ± 0.28a	3.46 ± 0.11b	3.05 ± 0.02a	1.10 ± 0.01a	4.85 ± 0.2a
120 DAF	46.12 ± 0.08c	45.26 ± 0.25b	4.16 ± 0.15c	3.33 ± 0.11b	0.97 ± 0.01b	7.04 ± 0.1b
150 DAF	66.91 ± 0.07d	54.50 ± 1.32c	5.10 ± 0.10d	3.42 ± 0.04bc	0.93 ± 0.02c	7.67 ± 0.1c
180 DAF	128.53 ± 0.25e	70.50 ± 0.50d	5.80 ± 0.26e	3.58 ± 0.16c	0.86 ± 0.02d	10.15 ± 0.9c
210 DAF	140.22 ± 0.22f	84.80 ± 0.20e	6.60 ± 0.10f	4.25 ± 0.04d	0.84 ± 0.04e	10.16 ± 0.5d
230 DAF	144.40 ± 0.10g	92.83 ± 2.46f	7.13 ± 0.15g	4.79 ± 0.02e	0.76 ± 0.02e	14.48 ± 0.41e
7th day PHV	145.48 ± 0.22g	92.63 ± 2.34f	7.10 ± 0.17g	4.91 ± 0.01e	0.73 ± 0.02f	16.19 ± 0.63e
21st day PHV	149.17 ± 0.15h	104.80 ± 0.26g	7.86 ± 0.15h	5.17 ± 0.06f	0.74 ± 0.02f	15.68 ± 0.38e
42nd day PHV	116.00 ± 2.57i	67.93 ± 0.81d	6.76 ± 0.05fg	4.05 ± 0.01d	0.74 ± 0.00f	15.33 ± 0.05e

[a]Means followed by the same letter in each column are not significantly different based on Tukey test at 5% probability. [†]Not detected. DAF: Days after flowering. PHV: Postharvest

Biochemical Analysis

Ascorbic Acid and Total Soluble Solids

A wide variation in the levels of ascorbic acid was observed in both peel and pulp analysis. Accumulation of ascorbic acid was seen both in peel and pulp of Khasi mandarin fruits (Figure 2a). It was lowest in the immature stage at 30 DAF (25.44 ± 4.40; 25.44 ± 4.40 mg/100 ml) and was significantly highest at the breaker stage, 120 DAF (75.82 ± 3.08; 92.11 ± 0.88 mg/100 ml) of sample peel and pulp respectively, which correspond with the beginning of ripening. It started decreasing in the subsequent harvests as the fruit progressed toward maturity till 230 DAF (46.05 ± 1.16; 53.43 ± 0.76 mg/100 ml). TSS was lowest by 30 DAF (3.98 ± 0.04; 4.56 ± 0.02) % peel and pulp respectively. It was significantly highest when the fruits were fully ripe at 230 DAF (8.08 ± 0.63; 11.06 ± 0.46) % peel and pulp respectively (Figure 2b). During low-temperature storage at 4 °C, ascorbic acid content significantly decreased

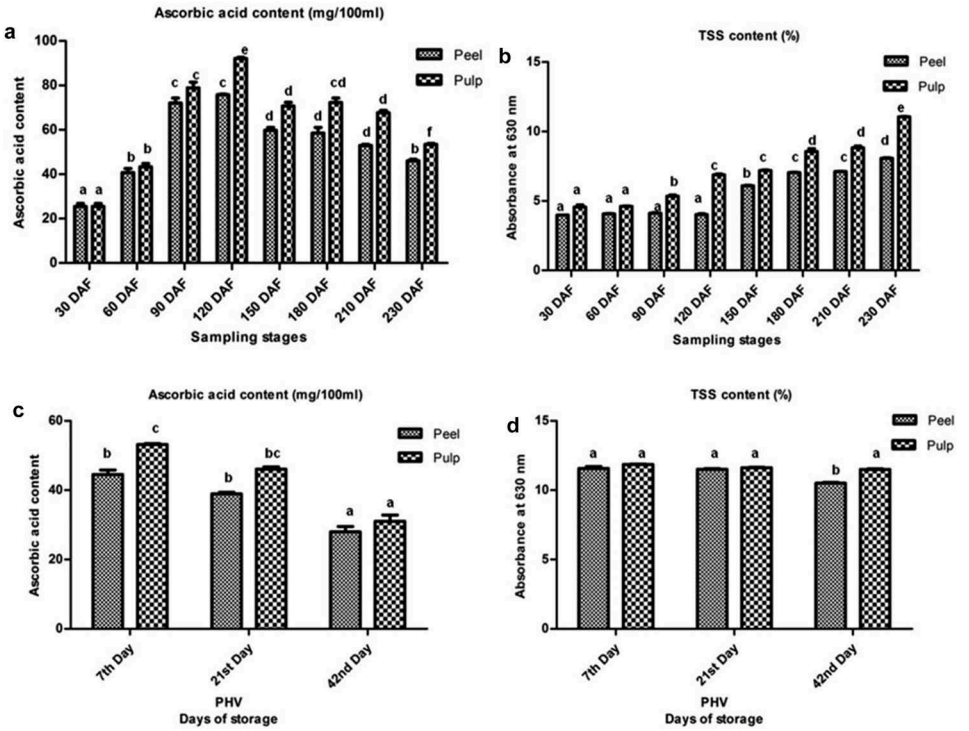

Figure 2. Ascorbic acid and TSS content in Khasi mandarin determined during developmental, ripening and postharvest stages. (a, b): during development to ripening stages; (c, d): during postharvest storage at 4ºC. DAF: Days after flowering; PHV: Postharvest. Data represent mean values (column bars) with standard deviation of n = 3 biological replicates represented as vertical lines on the column bars. Different letters on top of the bars represent significant differences between samples at 5% level of probability based on Tukey test.

till 42 days of storage (27.98 ± 2.68; 31.02 ± 2.74 mg/100 ml) of sample peel and pulp respectively (Figure 2c). During storage at 4 °C, TSS content in peel and pulp increased from 7 days to 21 days of storage (11.50 ± 0.09; 11.61 ± 0.08) %, after which it started decreasing till 42 days of storage (10.50 ± 0.07; 11.49 ± 0.08) % (Figure 2d).

Titrable Acidity and Maturity Index

The titrable acid content was significantly highest at the immature green stage, by 90 DAF, which significantly decreased toward maturity till 230 DAF. The TSS/TA ratio was recorded to be significantly lowest in 90 DAF, which gradually started accumulating during maturation and significantly reached an optimum value for consumption at 230 DAF. Low-temperature storage at 4 °C slowly decreased the titrable acid content till 42 days post-harvest storage with no significant differences between them while the TSS/TA ratio increased till 7 days postharvest storageafter which it started decreasing by the end of storage period (Table 1).

Total Chlorophyll and Carotenoids Content

Chlorophyll a, chlorophyll b and total chlorophyll content accumulated in the peel during immature stages with no significant differences between them. It was highest at 60 DAF (0.0241 ± 0.0004; 0.0436 ± 0.0007; 0.0461 ± 0.0006) μg g^{-1} fresh weight (FW) which started decreasing toward maturation till 230 DAF (0.0041 ± 0.0003; 0.0076 ± 0.0006; 0.0107 ± 0.0006) μg g^{-1} FW (Figure 3a). The lowest carotenoids content was recorded in the immature green stage at 30 DAF (1.04 ± 0.02) mg g^{-1} FW. Carotenoids content started accumulating during maturation with no significant differences between the stages but was significantly high during the fully ripe stage at 230 DAF to 4.81 ± 1.62 mg g^{-1} FW (Figure 3b). Chlorophyll a, b and total chlorophyll contents at 4 °C initially increased at 7 days which declined subsequently by 42 days of storage with no significant differences between them (Figure 3c). Carotenoids content significantly increased during the three storage periods and was highest at 42 days of storage (9.43 ± 0.81 mg g^{-1} FW) (Figure 3d).

Correlation Coefficients of Physicochemical and Biochemical Parameters

A direct correlation between fruit weight, fruit juice content and fruit diameter demonstrated high correlation coefficients at $p < .05$ (Table 2). Total carotenoids and TSS contents peel and pulp were positively correlated ($P < .05$) with each other but negatively correlated with that of ascorbic acid (Table 3).

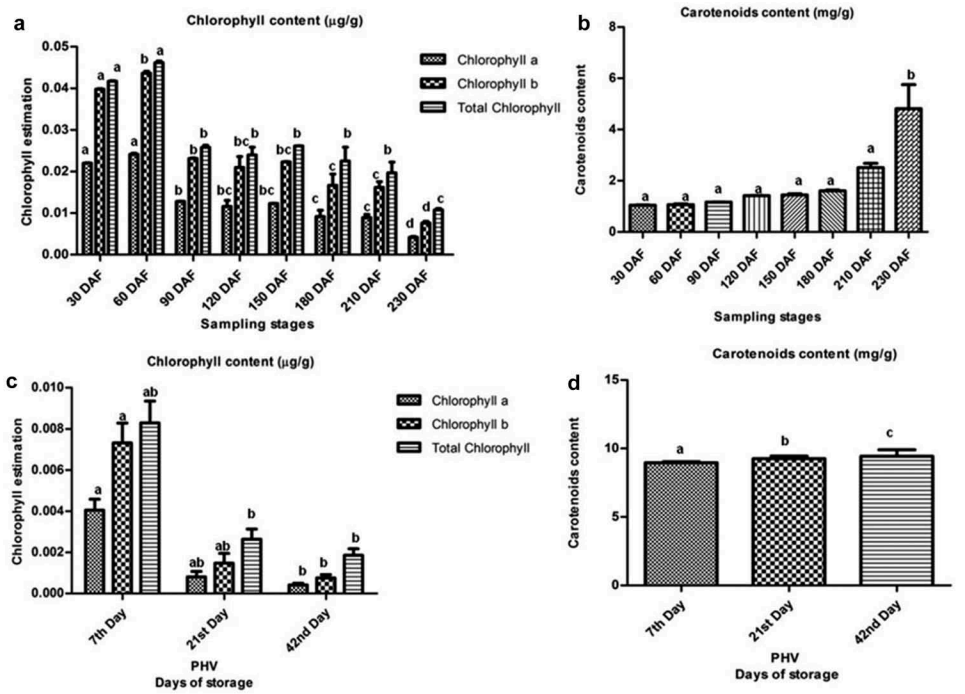

Figure 3. Chlorophyll and carotenoids content in Khasi mandarin determined during developmental, ripening and postharvest stages. (a, b): during development to ripening stages; (c, d): during postharvest storage at 4°C. DAF: Days after flowering; PHV: Postharvest. Data represent mean values (column bars) with standard deviation of n = 3 biological replicates represented as vertical lines on the column bars. Different letters on top of the bars represent significant differences between samples at 5% level of probability based on Tukey test.

Table 2. Correlation coefficients among physicochemical parameters in Khasi mandarin.

Correlations	Fruit weight	Fruit juice	Fruit diameter	Fruit p H	Citric acid	Sugar/acid ratio
Fruit weight	1.000	**0.962***	**0.969***	0.873	0.781	0.781
Fruit juice	**0.962***	1.000	**0.971***	**0.956***	0.777	0.777
Fruit diameter	**0.969***	**0.971***	1.000	0.892	0.876	0.876
Fruit pH	0.873	**0.956***	0.892	1.000	0.727	0.727
Citric acid	0.781	0.777	0.876	0.727	1.000	1.000
Sugar/acid ratio	0.781	0.777	0.876	0.727	1.000	1.000

*Correlation is significant at $p < 0.05$

Discussion

Physicochemical Analysis

Fruit weight and juice yield are important quality parameters for marketability of fruits. Fruit weight and juice content increased as it progressed toward maturity stages which could be due to the increase in cell size and accumulation of juice inside the intercellular spaces in fruits (Riaz et al., 2015). Ram and Kumar (2012) observed a gradual increase in fruit weight in

Table 3. Correlation coefficients among biochemical parameters in Khasi mandarin.

Correlations	Ascorbic acid (peel)	Ascorbic acid (pulp)	Carotenoids	Chlorophyll A	Chlorophyll B	Total chlorophyll	TSS (peel)	TSS (pulp)
Ascorbic acid (peel)	1.000	**0.986***	−0.491	0.045	0.046	**0.098***	−0.413	−0.417
Ascorbic acid (pulp)	**0.986***	1.000	−0.441	−0.034	−0.033	0.024	−0.331	−0.349
Carotenoids	−0.491	−0.441	1.000	−0.808	−0.808	−0.853	**0.944***	**0.976***
Chlorophyll a	0.045	−0.034	−0.808	1.000	**0.999***	**0.995***	−0.859	−0.893
Chlorophyll b	0.046	−0.033	−0.808	**0.999***	1.000	**0.995***	−0.860	−0.893
Total chlorophyll	0.098	0.024	−0.853	**0.995***	**0.995***	1.000	−0.879	−0.920
TSS (peel)	−0.413	−0.331	**0.944***	−0.859	−0.860	−0.879	1.000	**0.979***
TSS (pulp)	−0.417	−0.349	**0.976***	−0.893	−0.893	−0.920	**0.979***	1.000

*Correlation is significant at $p < 0.05$

Nagpur mandarin from 18.74 g (small size) to 114.14 g (mature fruits). Similar trend were also observed in Sweet Orange cv. Mosambi (Ladaniya and Mahalle, 2011). Juice contents in citrus increase toward maturity and reach a maximum value at full maturity and decreases when fruits become over-mature (Lado et al., 2014). This content varies throughout fruit development stages and slight fluctuations may be due to the time period of fruit harvest. The range of juice content in mandarin fruits acceptable for consumption should be > 49.0% (Deka et al., 2006). Juice yield of Khasi mandarin was reported to be highest at the advanced maturity stage in comparison to the development stages (Deshmukh et al., 2016). A Similar trend in juice yield with delayed harvest was reported in Khasi mandarin and Kinnow fruits (Deka et al., 2006; Grewal et al., 2000). In the study, weight loss was slow at 4°C, which was also observed with tangerine fruits stored at 5°C in comparison to storage at 25°C (Roongruangsri et al., 2009). This suggested that temperature storage can greatly influence the control of loss in fruit weight and juice content of the pulp. The pH of citrus juice is a measure of the state of acidity and basicity. Increase in pH might be due to the decrease in acidity toward maturity and decrease in pH by 42 days postharvest indicated the increased acidity of the fruit which might be due to degradation of reducing sugars resulting in the accumulation of acidic compounds (Riaz et al., 2015). Similar findings were also reported in Kinnow mandarin (Grewal et al., 2000).

Biochemical Analysis

Ascorbic Acid and Total Soluble Solids

The pulp had higher ascorbic acid accumulation than the peel during Khasi mandarin fruit development but there were no significant differences between the stages of development. According to results, significant differences were only observed for Khasi mandarin pulp at the breaker and final ripening stage (120 and 230 DAF). The data indicated that TSS in peel and pulp accumulated during maturation but there were no significant differences between the stages of maturation. Ascorbic acid content is regarded as a basic indicator for quality assessment in citrus fruits (Rokaya et al., 2016). Although the pulp segments of these fruits are recognized as a direct source of vitamin C for nutrition uptake, there are other non-edible parts of the fruit like peel which also contain this vitamin. Differences were observed in ascorbic acid contents both in the pulp and peel as it progressed toward maturity which could be due to changes in acid and sugar content. The decreasing trend in ascorbic acid content occurs because during ripened stages citrus fruits experience an increase in temperature and become susceptible to oxidation and consequently experience degradation of ascorbic acid (Lee and Kader, 2000). This decrease could also be attributed to thermal

breakdown and release in the nearby water (Lee and Kader, 2000). This finding was in accordance with the observation by Deka et al. (2006), where ascorbic acid content was decreased from 46.08 mg/100 g (green stage) to 35.74 mg/100 g (full ripe stage) in Khasi mandarin. The degradation of ascorbic acid duration the storage periods is reported to be a factor resulting in the higher loss of ascorbic acid content (Rokaya et al., 2016). This result was in accordance with the findings of Sonkar et al. (2009) in kinnow mandarin and Ladaniya et al. (2005) in Nagpur mandarin. During ripening and storage of the fruits postharvest, the degradation caused by heat and oxidation causes a decrease in ascorbic acid. Roongruangsri et al. (2013) highlighted similar trends where the ascorbic acid content of two tangerine cultivars decreased during storage at 25°C and 5°C. Moreover, Riaz et al. (2015) reported the highest amount of ascorbic acid content in immature fruits which decreased as the fruits approached maturity in Mandarins, Kinnow and Feutrell's. In the study, the increase in TSS content during early stages of fruit development could be due to breakdown of acids and formation of polysaccharides (Ram et al., 2003). It could also be due to the faster metabolic activities taking place as a result of respiratory and transpiration mechanisms (Rokaya et al., 2016). Similar results were obtained for TSS during fruit development in Nagpur mandarin where it increased from 6.45% to 11.16% (Ram and Kumar, 2012). An increase in TSS before maturity and decrease in the same when fruits became over-mature could be due to degradation of sugars and buildup of acids. Furthermore, the TSS could also be influenced by soluble components released during the degradation of cellulose, hemicelluloses and pectin from cell walls within fruit segments (Wu et al., 2014). Luo et al. (2015) highlighted the TSS and acidity patterns in spontaneous and bud mutants of Ponkan at different coloring stages harvested in November 2014, where TSS accumulated and acidity decreased in both the varieties. The increase in TSS content was slower during storage at 4°C which could be due to that fact that the production of soluble sugars from photosynthesis had stopped to take place during the storage (Echeverria and Ismail, 1987). Organic acid conversion to sugars through the gluconeogenic pathway is possible but only during the first week after harvest (Echeverria and Ismail, 1987). Organic acids decrease faster than sugars during storage of orange, so the fruits are expected to be sweeter in holding (Samson, 1986). In the study, the decrease in acid contents and an increase in TSS resulted in an increase in sugar/acid ratio during storage at 4°C. Citrus fruits are considered mature and ready for harvest when the TSS content in pulp tissue reaches at least 8.5% (Tiwari, 2006). TSS value > 10 by harvest in mandarin is an acceptable value for consumption (Deka et al., 2006). Thus, Khasi mandarin fruits harvested under tested conditions (230 DAF), exhibited optimal physical and chemical qualities.

Titrable Acidity and Maturity Index

Titrable acidity and TSS/TA ratio represent important parameter in citrus fruits as an indicator for the development of processed juices with prophylactic properties, suitable for consumer consumption (Wu et al., 2014). In the study, pulps were characterized by a decrease in titrable acidity with a concomitant increase in the TSS/TA ratio as the fruits progressed toward maturity. The decreasing trend of titrable acidity from unripe to ripening stages could be due to the breakdown of organic acids into sugars (Neves et al., 2015). Moreover, the decreasing trend of titrable acidity with the advancement of storage periods at 4°C could be due to the breakdown of acid in the Kreb's cycle during the aerobic respiration process (Rokaya et al., 2016). It could also be attributed to the utilization of acids for energy production (Cercos et al., 2006). The results are in accordance with the findings in Kinnow mandarin and Khasi mandarin (Deka et al., 2006; Sonkar et al., 2009). There are reports of declining of citric acid during low-temperature storage in mandarin, orange, and grapefruit (Ladaniya, 2008). The TSS/TA ratio presents a clear indication of the sweetness of fruits, higher the ratio, better is the taste of the fruit. This maturity indicator has been commercially accepted worldwide as an indicator of the internal quality of citrus fruits (Lado et al., 2014). TSS/TA ratio gradually increased toward maturity till postharvest storage periods but decreased toward the end of the storage periods, which are similar to results reported by Ladaniya and Singh (2006) in mandarin fruits. Riaz et al., (2015) reported a similar trend where the TSS/TA ratio increased toward maturity with a decrease toward the end of the season in Kinnow mandarin. Richardson et al., (1997) reported that the changes in the TSS/TA ratio during citrus fruit postharvest stages appear to be influenced by temperature. An increase in temperature may accelerate TSS/TA ratio whereas a decrease in the temperature level drops the rate of increase in the ratio. Similar results were reported for tangerine cultivars where titrable acid content slowly decreased and TSS/TA ratio had been reported to continuously increase during storage at 5°C (Roongruangsri et al., 2013). The optimum range of low-temperature storage required for fresh orange and mandarin is expected to operate within 1–5°C (Ladaniya, 2008). Sweet oranges, mandarins, and grapefruits are considered mature when their TSS/TA ratio and acidity have attained some optimum limits for palatability (Tiwari, 2006). Citrus fruits with a TSS/TA ratio of 8 to 16 and acidity around 0.6%- 0.7% depending on variety were considered optimum for palatibility (Lado et al., 2014; Ramful et al., 2011; Tiwari, 2006). Delayed harvest of the fruit results in the decrease in acid content followed by sugar accumulation. This results in the loss of the characteristic flavor and taste of the fruit becoming susceptible to physiological decomposition (El-Otmani and Zacarias, 2014).

Total Chlorophyll and Carotenoids Content

The loss of chlorophyll content in the fruits during maturation was noticeable, which could be due to the enzymatic conversion to carotenoids pigments during ripening stages (Soares et al., 2015). The loss of the green color could also be due to fluctuations in the temperature conditions promoting development of orange coloration on the fruit peel. Similar results were also reported by Ram and Kumar (2012) in Nagpur Mandarin and by Roongruangsri et al. (2013) in Tangerine cultivar. The differences in carotenoids content during development stages could possibly be due to the formation of chemical elements as a result of hydroxylation of carotene compounds. Moreover, carotenoids compound can trigger a conversion process through enzymatic activities during the ripening stages (Soares et al., 2015). The final concentration of carotenoids in the study might be attributed to factors, such as species origin, location, fruit maturity, growth stages, harvesting conditions and postharvest storage conditions (Soares et al., 2015). The delayed declining of peel chlorophyll and the sharp increase of the peel carotenoids were observed at low-temperature storage. Storage temperature is reported as one of the significant factors controlling carotenoids metabolism in citrus fruits through biochemical processes and hormone levels in the fruit (Zhang et al., 2011). Storage temperatures below 13 °C are reported to lead to the disappearance of chlorophyll pigments, followed by accumulation of carotenoids changing the color of citrus fruits to bright orange (Zhang et al., 2011). Similar results were reported in Satsuma mandarin by Matsumoto et al. (2009). Cold storage was suggested to be the inducement for the synthesis of a peel apocarotenoid, b-citraurin, and degradation of zeaxanthin or b-cryptoxanthin (Oberholster et al., 2001). However, a study observed an increase in the peel carotenoid content when fruits were stored in the range of 15–25°C (Rodrigo and Zacarias, 2007). In a finding in "Cara Cara" Navel Orange (*Citrus sinensis*), it was reported that total carotenoids content was higher after 21 days of storage at 20 °C compared to 4 °C (Tao et al., 2012).

Correlation Coefficients of Physicochemical and Biochemical Parameters

Our study suggested a high correlation between fruit juice content and fruit pH indicating that these parameters influence in determining the physiological maturity. In addition, correlation coefficients showed that carotenoids and TSS represent most active biochemical components as quality parameters than ascorbic acid for the indication of maturity state with respect to the sampling days in the studied citrus fruit. Chlorophyll A and chlorophyll B showed a high correlation with total chlorophyll. Alternatively, the correlation coefficients showed that ascorbic acid in pulp was positively correlated with the peel and ascorbic acid in peel was positively correlated with total chlorophyll in the peel indicating that peel is also important biochemical indicators for the maturation of the studied fruit. The hypothesis resulted that

there is significant variation in the maturity stage of the fruit with respect to days ($p < .001$; $p < .05$). The null hypothesis is accepted resulting in relative variation among maturation mean across days of sampling periods.

Conclusion

The maturity stage in the studied fruit does not seem to be characterized by a single parameter but is highly correlated to carotenoids and TSS contents. Additionally, peels are also important sources of compounds which can add to the nutrient value of the fruit. This study revealed that maturation stages affect significantly the harvesting maturity in Khasi mandarin. On the basis of the findings, it was observed that Khasi mandarin fruits had an increasing trend in the composition of juice yield, pH, fruit weight and TSS during development to maturity stages. Sugar/acid ratio and carotenoids content were increased from immature to mature fruits while there was a decrease in total acidity and ascorbic acid contents as the fruits progressed toward maturity, indicating that fruit quality is better when acidity is on the lower side. The sugar/acid ratio, TSS, carotenoids, ascorbic acid, and citric acid contents were within the optimum ranges of palatability at the fully ripe stage of the fruits. The findings of the investigation established that the best quality Khasi mandarin fruits, ideal for nutrition uptake can be produced with maximum juice yield and optimal content of bioactive compounds if they are harvested at 230 DAF stage. Consequently, postharvest management should be practiced to maximize the shelf life and quality maintenance in the fruit. Storage temperature could affect the physicochemical and biochemical constituents in postharvest Khasi mandarin fruits. Low-temperature storage at 4°C decreased the losses in fruit weight and juice content of the pulp and preserved the internal quality better. Our present study suggests that the orange cultivar can be stored for almost 42 days at 4°C, without the loss in visual appearance. The delayed declining of chlorophyll, and citric acid contents along with the gentle increases of the TSS contents were observed at 4°C storage. Low-temperature storage preserved peel carotenoids and sugar/acid ratio in juice better.

Acknowledgments

We acknowledge Miss Padma Rabha, Boko, Assam, India for the accession of samples from her orchard used in the study. This work was supported by the [Department of Science and Technology Science and Engineering Research Board (DST-SERB)] of Government of India under grant [SR/FT/LS-94/201].

Disclosure statement

The authors declare no conflict of interest.

Funding

This work was supported by the Science and Engineering Research Board [SR/FT/LS-94/201].

References

AOAC. 1995. AOAC official method, p. 981.12. In: P. Cunniff (ed.). AOAC official methods of analysis of AOAC international. AOAC International, Rockville, Maryland.

AOAC. 2005. Official methods of analysis. Association of Official Analytical Chemists, Washington, DC.

Assumi, S.R., T. Angami, H. Rymbai, A.W. Yanthan, and P.K. Sinha. 2017. Postharvest management of khasi mandarin. ICAR, Umiam, Meghalaya.

Cercos, M., G. Soler, D.J. Iglesias, J. Gadea, J. Forment, and M. Talon. 2006. Global analysis of gene expression during development and ripening of citrus fruit flesh. A proposed mechanism for citric acid utilization. Plant Mol. Biol. 62(4–5):513–527. doi: 10.1007/s11103-006-9037-7.

Deka, B.C., S. Sharma, and S.C. Borah. 2006. Post-harvest management practices of mandarin. Int. J. Hortic. 63(3):251–255.

Deshmukh, N.A., R.K. Patel, H. Rymbai, A.K. Jha, and B.C. Deka. 2016. Fruit maturity and associated changes in Khasi mandarin (*Citrus reticulata*) at different altitudes in humid tropical climate. Indian J. Agric. Sci. 86(7):854–859.

Echeverria, E., and M. Ismail. 1987. Changes in sugars and acids of citrus fruits during storage. Proc. Fla. State Hortic. Soc. 100:50–52.

El-Otmani, M., and L. Zacarias. 2014. Citrus postharvest physiology and technology, p. 17–33. In: S. Pareek, A.K. Thompson, D. Sivakumar, N. Benkeblia, and M. El-Otmani (eds.). Postharvest physiology and technology: Tropical and subtropical fruits. Wallingford: CAB International.

Grewal, A.G., I.A. Hafiz, A.H. Chaudhary, M.I. Khan, and M.I. Chaudhary. 2000. Quality estimation during marketing of kinnow and feutrell's early. Int. J. Agric. Biol. 2:328–330.

Hazarika, T.K. 2012. Citrus genetic diversity of north-east India, their distribution, ecogeograph and ecobiology. Genet. Resour. Crop Evol. 59:1267–1280. doi: 10.1007/s10722-012-9846-2.

Hossain, M. 2015. An overview on post harvest handling and commercial processing of horticultural crops in NEH region of India. Int. J. Sci. Res. 4(11):2304–2308.

Jha, P., S. Singh, M. Raghuram, G. Nair, R. Jobby, A. Gupta, and N. Desai. 2019. Valorisation of orange peel: Supplement in fermentation media for ethanol production and source of limonene. Environ. Sustainability 22:33–41. doi: 10.1007/s42398-019-00048-2.

Kashyap, K., and S. Banu. 2019. Characterizing ethylene pathway genes during the development, ripening, and postharvest response in *Citrus reticulata* Blanco fruit pulp. Turk. J. Bot. 43:173–184. doi: 10.3906/bot-1711-45.

Lacey, K., N. Hancock, and H. Ramse. 2009. Measuring internal maturity of citrus. Dept. Agric. Food 354:1–4.

Ladaniya, M.S. 2008. Citrus fruit biology technology and evaluation. Academic Press, San Diego, CA.

Ladaniya, M.S., and B.C. Mahalle. 2011. Fruit maturation and associated changes in mosambi orange (*Citrus sinensis*). Indian J. Agric. Sci. 81(6):494–499.

Ladaniya, M.S., and S. Singh. 2006. Post-harvest Management and marketing, p. 67–68. In: S. Singh and K.M. Bujarbaruah (eds.). Citrus: Production and marketing of Khasi Mandarin. Extension bulletin no. 35. NRCC, ICAR, Nagpur, India.

Ladaniya, M.S., S. Singh, and B. Mahalle. 2005. Sub-optimum low temperature of "Nagpur" Mandarin as influenced by wax coating and intermitant waxing. Indian J. Hortic. 62:1–7.

Lado, J., M.J. Rodrigo, and L. Zacarias. 2014. Maturity indicators and citrus fruit quality. Stewart Postharvest Rev. 2:1745–9656.

Lee, S.K., and A.A. Kader. 2000. Pre harvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20(3):207–220. doi: 10.1016/S0925-5214(00)00133-2.

Luo, T., K. Xu, Y. Luo, and X. Deng. 2015. Distinct carotenoid and flavonoid accumulation in a spontaneous mutant of Ponkan (*Citrus reticulata* Blanco) results in yellowish fruit and enhanced postharvest resistance. J. Agric. Food Chem. 63:8601–8614. doi: 10.1021/acs.jafc.5b02807.

Matsumoto, H., Y. Ikoma, M. Kato, N. Nakajima, and Y. Hasegawa. 2009. Effect of post-harvest temperature and ethylene on carotenoids accumulation in the flavedo and juice sacs of Satsuma mandarin (*Citrus unshiu* Marc.) fruit. J. Agric. Food Chem. 57:4724–4732. doi: 10.1021/jf9005998.

Nath, A., K. Barman, S. Chandra, and P. Baiswar. 2013. Effect of plant extracts on quality of Khasi mandarin (*Citrus reticulata* Blanco) fruits during ambient storage. Food Bioprocess Technol. 6:470–474. doi: 10.1007/s11947-012-0783-z.

Neves, L.C., P.M. Carvalho da Silva, C.G.B. Lima, V.J. Bastos, and S.R. Roberto. 2015. Study to determine the optimum harvest date of Murici (*Byrsonima coccolobifolia* Kunth.) from quality and functional attributes. Sci. Hortic. 188:49–56. doi: 10.1016/j.scienta.2015.02.039.

Oberholster, R., A.K. Cowan, P. Molnar, and G. Toth. 2001. Biochemical basis of color as an aesthetic quality in *Citrus sinensis*. J. Agric. Food Chem. 49(1):303–307. doi: 10.1021/jf0007840.

Ram, L., R.K. Godara, and S. Siddique. 2003. Primary and secondary metabolities changes of kinnow mandarin fruits during different stages of maturity. J. Food Sci. Technol. 41:337–340.

Ram, L., and D. Kumar. 2012. Effect of growth stages on the changes in bioactive compounds of Nagpur mandarin (*Citrus reticulata*) fruit of Ambia crops. Indian J. Agric. Sci. 82 (8):714–716.

Ramful, D., E. Tarnus, O.I. Aruoma, E. Bourdon, and T. Bahorun. 2011. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Res. Int. 44(7):2088–2099. doi: 10.1016/j.foodres.2011.03.056.

Riaz, M., T. Zamir, N. Rashid, N. Jamil, Z. Masood, U. Jabeen, F. Mandokhel, F. Behlil, F. Mengal, and M. Khan. 2015. Quality assessment in different stages of maturity of fruits, mandarins, kinnow and feutrell's early collected from the fruit market of Quetta city in relation to their benefits for human health. Am .Eur. J. Toxicol. Sci. 7(3):203–208.

Richardson, A.C., K.B. Marsh, and E.A. Macrae. 1997. Temperature effects on satsuma mandarin fruit development. J. Hortic. Sci. 72:919–929. doi: 10.1080/14620316.1997.11515584.

Rodrigo, M.J., and L. Zacarias. 2007. Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L Osbeck) fruit. Postharvest Biol. Technol. 43:14–22. doi: 10.1016/j.postharvbio.2006.07.008.

Rokaya, P.R., D.R. Baral, D.M. Gautam, A.K. Shrestha, and K.P. Paudyal. 2016. Effect of altitude and maturity stages on quality attributes of mandarin (*Citrus reticulata* Blanco). Am .J. Plant Sci. 7(6):958–966. doi: 10.4236/ajps.2016.76091.

Roongruangsri, W., N. Rattanapanone, and D. Boonyakiat. 2009. Evaluation of a polyethylene-candelilla coating for tangerine fruit cv. 'Sai Nam Pung'. J. Nat. Sci. 8 (1):67–76.

Roongruangsri, W., N. Rattanapanone, N. Leksawasdi, and D. Boonyakiat. 2013. Influence of storage conditions on physico-chemical and biochemical of two Tangerine cultivars. J. Agric. Sci. 5(2):70–84.

Samson, J.A. 1986. Citrus, p. 73–138. In: J.A. Samson (ed.). Tropical Fruit. Longman, London, UK.

Sanabam, R., N.S. Singh, P.J. Handique, and H.S. Devi. 2015. Disease-free Khasi mandarin (*Citrus reticulata Blanco*) production using *in vitro* microshoot tip grafting and its assessment using DAS-ELISA and RT-RCR. Sci. Hortic. 189:208–213. doi: 10.1016/j.scienta.2015.03.001.

Sangma, C., T. Anbazhagan, and D.J. Rajkhowa 2018. Influence of soil health and age of orchard on fruit quality of Khasi mandarin in Nagaland. 27th National Conference of Soil Conservation Society of India on Sustainable management of soil and water resources for doubling farmers' income, AAU, Jorhat, Assam.

Sharma, K., N. Mahato, and Y.R. Lee. 2018. Extraction, characterization and biological activity of citrus flavonoids. Rev. Chem. Eng. 35(2):1–20.

Singh, A.K., N.T. Meetei, B.K. Singh, and N. Mandal. 2016. Khasi mandarin: Its importance, problems and prospects of cultivation in North-eastern Himalayan region. Int. J. Agric. Environ. Biotechnol. 9(4):573–592. doi: 10.5958/2230-732X.2016.00076.0.

Soares, M., L. Welter, E.M. Kuskoski, L. Gonzaga, and R. Fett. 2015. Phenolic compounds and antioxidant activity of the niagara and isabel bark. Rev. Bras. Frutic. 30(1):59–64. doi: 10.1590/S0100-29452008000100013.

Sonkar, R.K., D.A. Sarnaik, S.N. Dikshit, and R.R. Saxena. 2009. Individual stretch cling film wrapped kinnow mandarin under ambient storage. Indian J. Hortic. 66:22–27.

Tao, N., C. Wang, J. Xu, and Y. Cheng. 2012. Carotenoid accumulation in postharvest "Cara Cara" navel orange (*Citrus sinensis* Osbeck) fruits stored at different temperatures was transcriptionally regulated in a tissue-dependent manner. Plant Cell Rep. 31(9):1667–1676. doi: 10.1007/s00299-012-1279-z.

Tariang, J., D. Majumder, and H. Papang. 2018. Efficacy of native *Bacillus subtilis* against postharvest *Penicillium* rot pathogen *Penicillium* sp. of Khasi Mandarin oranges in Meghalaya, India. Int. J. Curr. Microbiol. Appl. Sci 7(12):447–460. doi: 10.20546/ijcmas.

Tietel, Z., E. Lewinsohn, E. Fallik, and R. Porat. 2012. Importance of storage temperatures in maintaining flavor and quality of mandarins. Postharvest Biol. Technol. 64(1):175–182. doi: 10.1016/j.postharvbio.2011.07.009.

Tiwari, S. 2006. Mandarin: Postharvest management guide. Extension bulletin. Published under the Technology Mission on Integrated Development of Horticulture in NE states including Sikkim College of Horticulture and Forestry. Pasighat, Arunachal Pradesh, India.

Wang, Y.C., Y.C. Chuang, and H.W. Hsu. 2008. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chem. 106(1):277–284. doi: 10.1016/j.foodchem.2007.05.086.

Wu, J., Z. Xu, Y. Zhang, L. Chai, H. Yi, and X. Deng. 2014. An integrative analysis of the transcriptome and proteome of the pulp of a spontaneous late-ripening sweet orange mutant and its wild type improves our understanding of fruit ripening in citrus. J. Exp. Bot. 65(6):1651–1671. doi: 10.1093/jxb/eru044.

Zema, D.A., P.S. Calabro, A. Folino, V. Tamburino, G. Zappia, and S.M. Zimbone. 2018. Valorisation of citrus processing waste: A review. Waste Manag. 80:252–273. doi: 10.1016/j.wasman.2018.09.024.

Zhang, L.C., G. Ma, M. Kato, K. Yamawaki, T. Takagi, Y. Kiriiwa, Y. Ikoma, H. Matsumoto, T. Yoshioka, and H. Nesumi. 2011. Regulation of carotenoid accumulation and the expression of carotenoids metabolic genes in citrus juice sacs *in vitro*. J. Exp. Bot. 63 (2):871–886. doi: 10.1093/jxb/err318.