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Maturity indicators and fruit quality attributes of mandarin orange varieties

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Abstract

Mandarin orange is an important export oriented horticultural commodities in Bhutan. The maturity and harvest time is based on fruit colour and harvesting calendar which may not be accurate in terms of ensuring market quality. Hence, the study was conducted at Citrus germplasm block at ARDC Wengkhar from 2015 to 2017 to determine maturity indicators and fruit quality attributes of three mandarin orange varieties. Three uniform trees for each of Clementine Nules (Wengkhar Tshelu Dukchu) Tarku (Wengkhar Tshelu Ngarm) and Local Mandarin Selection (49-50) were used as treatment varieties. Five fruits from each sample tree of each variety were collected for fruit quality analysis. The fruit quality analysis was done at fortnightly interval starting from 3rd November to 23rd January in each year. Different quality attributes such as Total Soluble Solids (TSS), Acid content, TSS: Acid ratio and Juice content were determined. The data was analyzed using Analysis of Variance technique on Statistical Package for Social Sciences (SPSS) 20 for Windows and fruit quality attributes was compared with standard in export market using One Sample t-test. The variation in TSS: Acid ratio and juice content were highly significant between the varieties at the 0.05 level of significance. The trend analysis showed that Clementine Nules (Wengkhar Tshelu Dukchu) can be harvested from 3rd week of December while Local Mandarin Selection (49-50) and Tarku (Wengkhar Tshelu Ngarm) can be harvested from 4th week of January under Wengkhar condition. Further, the fruit quality attributes observed in three mandarin varieties was compared with quality standard in export market. All three varieties showed significantly higher TSS and

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Clementine Nules exhibited significantly higher TSS: Acid ratio than export market at the 0.05 level of significance. However, it may be important to introduce and evaluate non-destructive method to predict fruit maturity accurately and minimize unnecessary loss along the supply chain.

Keywords: *Maturity indicators, Total Soluble Solids, Acid, TSS: Acid ratio, Non-destructive*

1. Introduction

Citrus is one of the important export oriented horticultural commodities in Bhutan. Citrus is considered not just a commodity but as a source of livelihood and employment opportunities (Joshi & Gurung, 2009) and as such about 60% of the adult population are dependent on citrus production for their livelihood (Gyeltshen, Griffith, Dorji, & Lakey, 2015). According to Agriculture Statistics (2016), mandarin orange is cultivated in over 15 districts with a production of about 42,003 Metric tons (Department of Agriculture, 2016). According to the Bhutan Trade Statistics (2018), about 348 MT and 10739 MT were exported to India and Bangladesh earning revenue of Ngultrum 5.12 Million and 349.8 Million. Therefore, the stage of harvesting to provide quality fruits into these markets is a compromise between the agro-climatic conditions and management practices being followed by our growers and the fruit quality standards established by India and Bangladesh.

The genus citrus comprises of different species and cultivars that display unique quality attributes. Amongst the citrus, mandarin orange (*Citrus reticulata* Blanco) is the predominant citrus type grown in Bhutan (Dorjee, Dukpa, Bockel, Punjabi, & Chhetri, 2007; Gyeltshen et al., 2015). Mandarins form an important group of citrus fruits, together with pummelo and citron, they are considered to be original citrus ancestors, from which all other citrus species have evolved (Barkley, Roose, Krueger, & Federici, 2006). According to Hodgson's classification, the mandarin group comprises various natural sub-groups, including common mandarin (*C.reticulata* Blanco), Satsuma mandarin (*C. unshiu* Marcovitch), King mandarin (*C. nobilis* Loureiro), Mediterranean mandarin (*C. deliciosa* Tenore), small-fruited mandarin (*C. indica*, *C. tachibana* and *C. reshni*), and mandarin hybrids. Based on the above classification, Tarku (Wengkhar Tshelu Ngarm) and Local Mandarin Selection (49-50) falls under common mandarin group but Clementine (*C. clementina* Hort.ex.Tan) is considered to be a unique mandarin sub-group with 79 % pure mandarin (Goldenberg, Yaniv, Porat, & Carmi, 2018).

The fruits of different species and varieties share common mechanisms and biochemical pathways during growth, development, maturation and ripening, that are critical factors for determining maturity and harvest time (Lado, Rodrigo, & Zacarías, 2014). The fruit quality of mandarin orange like other fresh fruits depend on its external attributes such as colour and firmness and internal quality attributes such as Total Soluble Solids (TSS) or Brix, Acid, Brix: Acid ratio and Juice content (Riaz et al., 2015; Sinclair & Ramsey, 1944). Further, Riaz et al. (2015) noted that fruit quality attributes of mandarin orange depend on type of cultivar and stage of maturity. In this connection, Lado *et al.* (2014) reported that determination of harvest time is difficult and relies on growing areas and market requirement. For example, the minimum fruit quality standards established for fresh fruit mandarin in Indian market Total Soluble Solids (TSS) of more than 8.5% and TSS: Acid ratio of 6.5 (Lado et al., 2014). However, for countries preferring sweet taste, TSS: Acid ratio of 14 is desirable. The appropriate harvest decision prevents the unnecessary loss that might occur along the supply chain and ensures that production meets the quality requirement of market as over-or immature fruits have a lower or even no retail value and represent significant income loss and a waste of resources (Li, Lecourt, & Bishop, 2018).

On the contrary, earlier studies on Clementine have shown that acidity below 0.8% is not preferred for its insipid taste and susceptibility to decay (Chahidi, El-Otmani, Luro, Srairi, & Tijane, 2007). Moreover, citrus fruit show a non-climacteric ripening behavior and should be harvested when the internal maturity has been achieved since no further relevant changes occur in fruit flesh composition after harvest (Lado et al., 2014; Rokaya, Baral, Gautam, Shrestha, & Paudyal, 2016). Hence, the determination of maturity indices for mandarin fruit maturation is complex as it depends on internal changes taking place in the fruit flesh and external colour modifications taking place in the fruit peel (Lado et al., 2014). As such, the common mandarins, sweet oranges and grapefruits are considered to be mature when their external coloration, juice content and TSS: Acid ratio and other internal constituents have reached a minimum level of visual acceptance or palatability.

Similarly, other studies have shown that accurate estimation of maturity is crucial as over-mature fruits often become more susceptible to damage during postharvest handling and immature fruits will lead to unmarketable produce (Rokaya, Baral, Gautam, & Paudyal, 2015; Rokaya et al., 2016). There are many ways to determine maturity and predict harvest time. Essentially, the citrus maturity can be determined by destructive (Lado et al., 2014; Rokaya et

al., 2016; Singh, Aulakh, & Rattanpal, 2017) and non-destructive methods (Antonucci et al., 2011; Olmo, Nadas, & García, 2000; Raji & Alamutu, 2005; Theanjumpol et al., 2012; Valero, Crisosto, Garner, Bowerman, & Slaughter, 2003; Yamakawa, Khot, Ehsani, & Kondo, 2012; Zhang, Peng, Zhang, Song, & Ma, 2017).

The destructive method of determining maturity indices based on the fruit quality attributes such as Total Soluble Solids (TSS) content, Acidity level, Juice content and TSS: Acid ratio (Lado et al., 2014; Olmo et al., 2000; Rokaya et al., 2015; Rokaya et al., 2016) are time consuming and inflicts damage to the fruits while the non-destructive method through use of colorimetry and visual imaging allow rapid assessment and inflict minimal or no damage to the fruits (Li et al., 2018). Of the two methods, the destructive method is the most widely used in determining citrus maturity and TSS: Acid ratio is widely used by growers and trading companies worldwide as an indicator of fruit maturity (Goldenberg et al., 2014; Goldenberg et al., 2018).

However, currently, growers and exporters usually consider mandarin orange fruits to be mature when their external coloration has reached a minimum level of visual acceptance which may not be accurate to ensure optimum market quality (Dorjee et al., 2007). Thus, there is no systematic method of determining optimum maturity of mandarin fruits based on internal fruit quality changes such as TSS content, Juice content, Acidity level and TSS: Acid level is hardly practiced in our country. Therefore, this study was carried out mainly to assess maturity stage in relation to fruit quality attributes and compare with the quality standards in export market.

2. Materials and Method

2.1.Experimental Site

The study was carried at Citrus germplasm Block of Agriculture Research and Development Centre (ARDC), Wengkhar. The site is located at about 1600 m above mean sea level (amsl). The area falls in warm temperate zone which is characterized by heavy rainfall during July to September and scanty rainfall during rest of the year.

2.2. Experimental Design

Three cultivars of mandarin namely, Clementine Nules (Wengkhar Tshelu Dukchu), Tarku (Wengkhar Tshelu Ngarm) and Local mandarin Selection (49-50) were used as treatment

varieties. All three varieties were grown on Trifoliate orange rootstock (*Poncirus trifoliata* L., released for commercial use in the country as Wengkhar Tshelu Rhato 1). Three trees of each variety with uniform size, age and vigor were selected randomly and trial was set up on Completely Randomized Design (CRD) (Lacey, Hancock, & Ramse, 2009)

2.3. Sample collection

Five fruits from each sample tree of each variety were collected for fruit quality analysis. The fruit quality analysis was done at fortnightly interval starting from 3rd November to 23rd January in each year. Different quality attributes such as Total Soluble Solids (TSS), Acid, TSS-Acid ratio and juice content were determined and calculated.

2.4. Determination of fruit quality attributes

The Total Soluble Solids (TSS) of the fruit juice was measured as ^{0}Brix by using digital refractometer (ATAGO, Japan). It is often expressed as percentage of Total Soluble Solids. Similarly, the acidity of the juice was determined through acid base titration method. The percentage acid was calculated by using the formula given by Lacey et al. (2009).

% Acid = Volume of Sodium hydroxide (NaOH) used for titration (ml) x Titre value.

Accordingly, the TSS-Acid ratio was calculated by using the formula;

TSS Acid ratio = TSS content (^{0}Brix)/ Acid content.

Similarly, the juice content was weighed and recorded in grams by using fruit juice squeezer. Accordingly, the percent juice content was calculated by using the formula given by Rokaya et al. (2015).

Juice content = Juice weight (grams)/ Fruit weight (grams) x 100

2.5. Statistical Analysis

The data was compiled in Microsoft (MS) Excel and analyzed by using the Analysis of Variance (ANOVA) techniques. Tukey's Highly Significant Difference was used to compare the significant differences among the means at 95% Confidence Interval. Statistical Package for Social Sciences (SPSS) 20 for Windows was used for analysis. The trend analysis was done using Microsoft Excel to assess the fruit quality variation over time. Eventually, the

fruit quality attributes of three mandarin cultivars were compared with the minimum fruit quality standards in export market using One Sample T-test.

3. Results and Discussion

3.1.Fruit quality attributes

The mean fruit quality attributes of the three varieties tested are shown in Table 1. The TSS: Acid ratio and juice content were highly significant between the varieties at the 0.05 level of significance (Table 1). Results show that Clementine Nules (Wengkhar Tshelu Dukchu) contains significantly higher TSS: Acid ratio and Tarku (Wengkhar Tshelu Ngarm) contains significantly higher quantity of juice than other two varieties tested (Table 1). These fruit quality attributes are considered to be important indicators of determining maturity in mandarin orange

Table 1. Mean fruit quality attributes of three mandarin varieties

Cultivars	TSS (^{o}Brix) Mean ± SEM	Acid (%) Mean ± SEM	TSS: Acid Ratio Mean ± SEM	Juice Mean ± SEM
49-50	12.31±0.50	2.30±0.21	7.63^{a}±0.72	34.98^{a}±1.41
Clementine	12.32±0.43	1.86±0.13	9.55^{b}±0.65	29.11^{b}±0.92
Tarku	12.75±0.77	2.17±0.16	8.76^{c}±0.93	36.44^{c}±1.36
P-Value	0.836	0.19	<0.001	<0.001

SEM, Standard Error of Means; Means in columns with different superscripts differ significantly (Tukey's Highly Significant Difference (HSD) at the 0.05 level of significance)

3.2. Variation in fruit quality attributes over time

3.2.1. TSS content

The TSS content of all three varieties showed steady increase from 3rd November which tends to decrease after 15th January. However, Tarku (Wengkhar Tshelu Ngarm) exhibited abrupt increase in TSS content from 1st to 30th December, which tends to decrease afterward (Figure 1). This trend clearly shows that fruit ripening starts with rapid break down of carbohydrates thereby increasing the TSS content (Riaz et al., 2015; Zhang et al., 2017). Further, the past studies have indicated that TSS content tend to decrease when the fruit reaches maturity (Goldenberg et al., 2014).

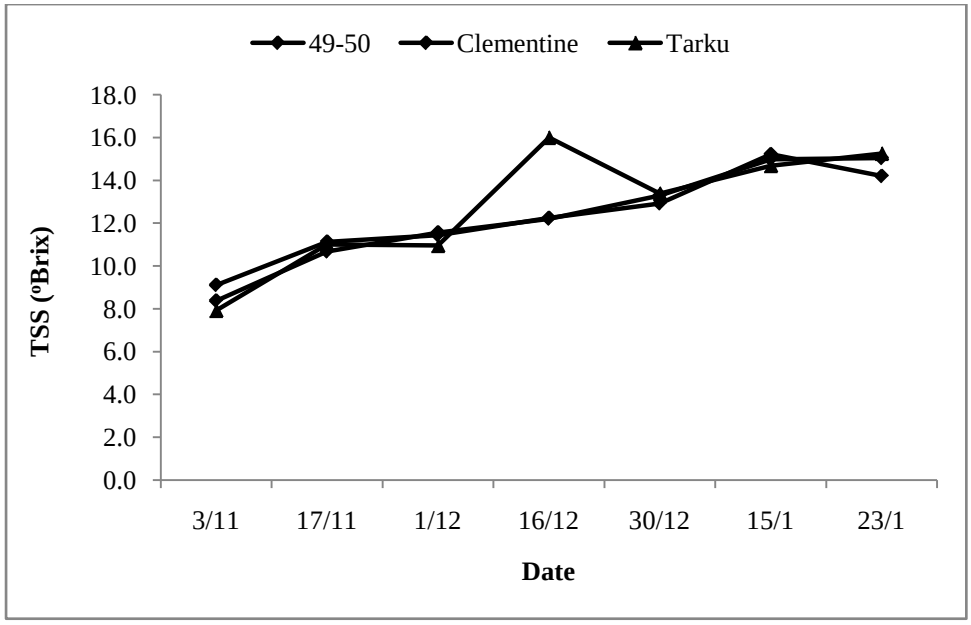

Figure 1. Change in TSS content of three mandarin orange varieties over time

3.2.2. Acid content

Figure 2 shows the variation in Acid content between three mandarin varieties ($F = 19.09$, $P<0.001$). The acid content of three mandarin varieties showed decreasing trend from 3rd November to 30th December after which the acidity increased rapidly until 15th January and then declines rapidly which indicates the maturity of fruits (Figure 2). Citrus mainly contains citric acid and fruit taste depends on proper blend of Total Soluble Solids (TSS) and Acid (Goldenberg et al., 2018)

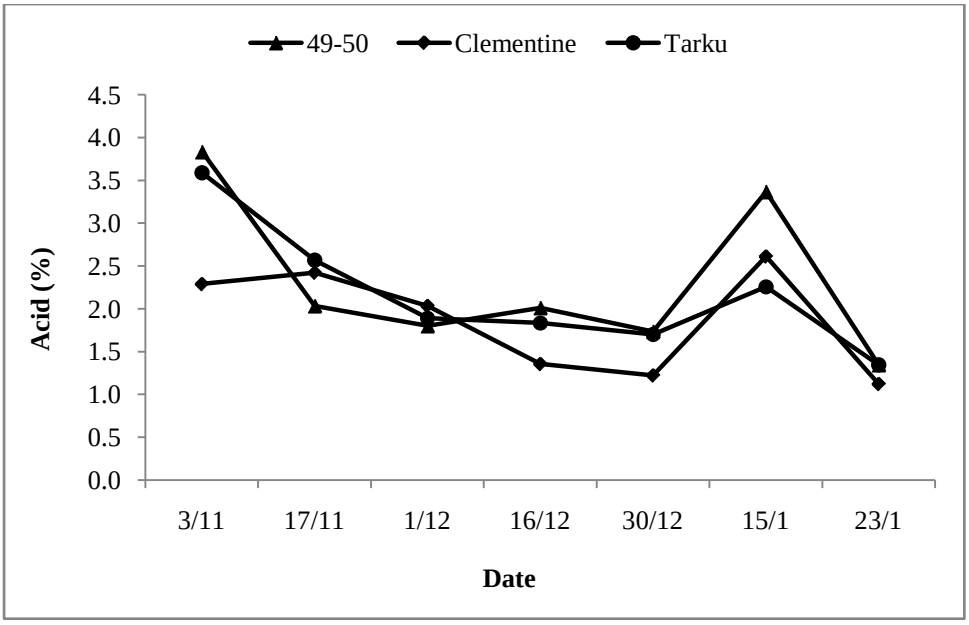

Figure 2. Change in Acid content of three mandarin orange varieties over time

3.2.3. TSS: Acid ratio

The TSS: Acid ratio of Clementine Nules (Wengkhar Tshelu Dukchu) exhibited increasing trend, which tends to decline rapidly from 30th December. On the other hand, Local Mandarin Selection (49-50) showed sharp increase in TSS: Acid ratio from first to second week of November with steady increase in between, which increased sharply from 15th January onward. Similarly, Takru (Wengkhar Tshelu Ngarm) showed highly variable trend in TSS: Acid ratio, which tends to decrease after 15th January (Figure 3). This trend clearly indicates that Clementine Nules is early maturing than Local mandarin and Tarku varieties. This means that former can be harvested from 2nd week of December under Wengkhar condition whereas latter two varieties can be harvested from 4th week of January. Nevertheless, the time of harvest would depend on growing area and market requirement.

In this respect, the past studies have also observed high variability in TSS: Acid ratio among mandarin varieties belonging to various sub-groups, which tends to decrease when the fruit attains maturity (Goldenberg et al., 2018; Lado et al., 2014). Further, Goldenberg et al. (2018) noted that TSS: Acid ratio or fruit-ripening ratio is the most important fruit quality attribute which determines fruit taste although the taste and unique flavor is attributed to not just one but combination of many volatile compounds (Goldenberg et al., 2018). Hence, the ratio is widely used as one of the key indicators of fruit maturity worldwide.

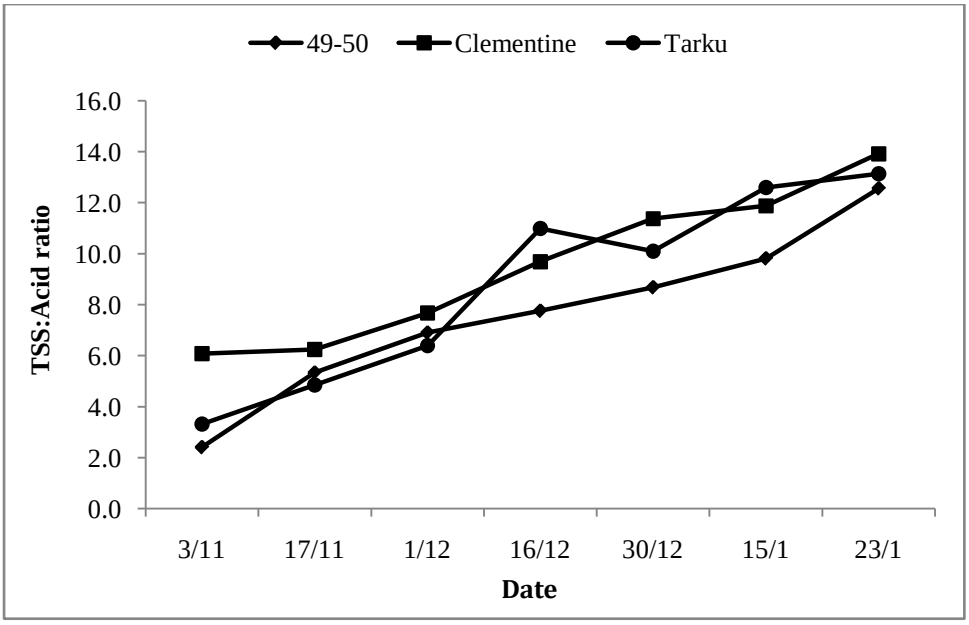

Figure 3. Change in TSS: Acid ratio of three mandarin varieties over time

3.2.4. Juice content

Figure 4 shows the variation in juice content of three mandarin varieties. The trend analysis shows that juice content increases rapidly from 17th November until 1st December after which the juice content more or less shows a declining trend from 1st December onward (Figure 4). Juice is another important maturity indicator, which contains many flavor imparting volatile compounds. The evaluation of juice content of mandarin varieties belonging to different sub-groups by Goldenberg et al. (2018) reported that juice of Common and Clementine mandarin contains distinct set of aroma volatile compounds. Hence, the three mandarin varieties being evaluated also possess a unique fruit taste and flavor.

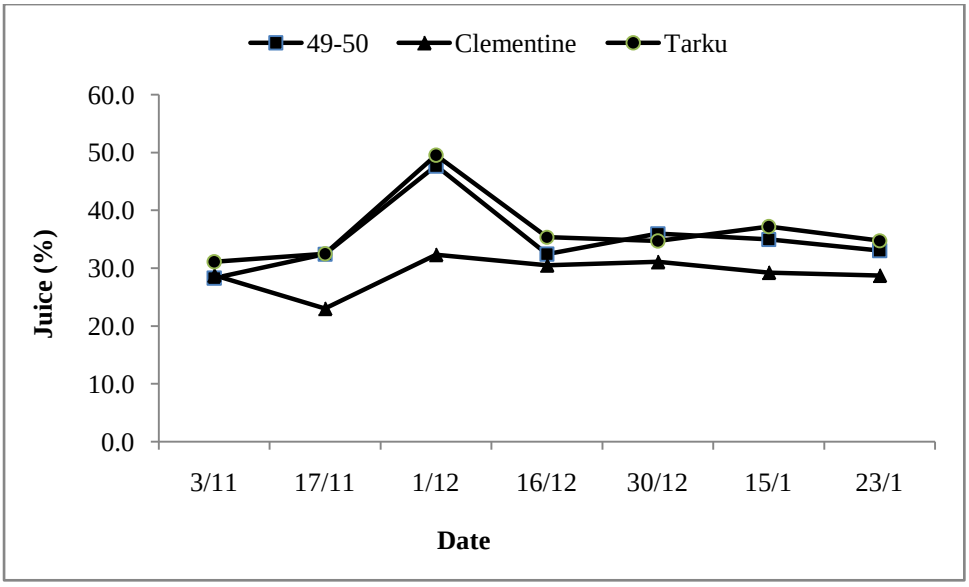

Figure 4. Change in Juice content of three mandarin orange varieties over time

3.3.Comparison of fruit quality attributes with export market standard

Bhutanese mandarin is mainly exported to India and Bangladesh. The fruits should be harvested when the quality attributes meet the market requirement. Hence, the fruit quality attributes of three mandarin varieties were compared with the requirement in Indian market using One Sample t-test. All three mandarin varieties showed significantly higher TSS than market standard. However, Clementine Nules exhibited statistically significant TSS: Acid ratio at the 0.05 level of significance (Table 2). The findings clearly show the potential of including Clementine mandarin in the export basket.

Table 2. Comparison of fruit quality attributes with market standard

Outcome	Mean	Standard deviation	No. of samples	Comparison value	95% CI for Mean Difference	t-value	df	p-value
TSS (%)								
49-50	12.31	2.39	7	8.5	1.60, 6.02	4.22	6	0.006
Clementine Nules	12.33	2.03	7	8.5	1.95, 5.70	4.98	6	0.003
Tarku	12.75	2.9	7	8.5	1.56, 6.93	3.87	6	0.008
TSS: Acid ratio								
49-50	7.64	3.26	7	6.5	-1.87, 4.16	0.93	6	0.39
Clementine Nules	9.56	3.01	7	6.5	0.27, 5.84	2.69	6	0.036
Tarku	8.77	3.89	7	6.5	-1.32, 5.87	1.55	6	0.173

df – degree of freedom

4. Conclusion

The harvest time of mandarin orange is mainly estimated by counting of days after flower (harvesting calendar), subjective tasting, or visual assessment of fruit colour. All of these methods on their own or in combination are time consuming and not necessarily accurate. Hence, the determination of mandarin fruit maturity based on destructive assessment of fruit quality attributes is more reliable as it takes into account the internal changes taking place during the fruit maturity. However, the agro-climatic condition of a growing area, varieties and market requirement would determine the harvest date.

This study showed that TSS: Acid ratio of Clementine Nules (Wengkhar Tshelu Dukchu) exhibited increasing trend, which tends to decline rapidly from 30th December. On the other hand, Local Mandarin Selection (49-50) exhibited sharp increase in TSS: Acid ratio from first to second week of November with steady increase in between, which increased sharply from 15th January onward. Similarly, Takru (Wengkhar Tshelu Ngarm) showed highly variable trend in TSS: Acid ratio, which tends to decrease after 15th January (Figure 3). This trend clearly indicates that Clementine is early maturing than Local mandarin and Tarku varieties. This means that Clementine can be harvested from 2nd week of December under Wengkhar condition, whereas other two varieties can be harvested from 4th week of January. Nevertheless, the time of harvest would depend on growing area and market requirement.

As such the comparison of the fruit quality attributes of three mandarin orange varieties with Indian market standard indicate that the quality attributes observed in our mandarin varieties are superior. This clearly shows that mandarin varieties other than local mandarin could also find a place in export market. Nonetheless, the manual measurement of fruit quality attributes are destructive methods and are often time-consuming due to the large number of samples required to take account of the within-field variability. Thus, in order to predict fruit maturity accurately and minimize unnecessary loss along the supply chain, there is a need to introduce and evaluate non-destructive technique of predicting fruit maturity using colorimetry, visible imaging, visible and near-infrared spectroscopy, hyper-spectral and multi-spectral imaging, among others. Unlike destructive methods, the fruit quality attributes obtained from non-destructive methods can, however, be modelled to predict the optimum time of mandarin fruit harvest taking into the environmental conditions.

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