

Characterization and comparative nutritional study of three strains of Kinnow Mandarin (*Citrus reticulata* Blanco)

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Abstract

The high number of seeds per fruit is a major hindrance in export, fresh fruit consumption as well as in the processing industry. A seedless trait in Kinnow is the utmost desirable characteristic to accelerate the local and export markets. In this comparative study, three strains of Kinnow (normal seeded Kinnow, low seeded and seedless Kinnow) grafted on Rough Lemon rootstock were evaluated for various physico-chemical attributes and nutritional parameters at gene pool center of Citrus Research Institute, Sargodha, Pakistan. Even though conventionally used normal seeded Kinnow performed better in various aspects like fruit size, firmness, acidity, and vitamin C contents. Two new strains of Kinnow mandarin delineate promising nutritional value regarding various physicochemical and proximate analysis parameters like TSS, total sugars, Beta carotene, fat, and ash percentage (11.95%, 7.84%, 63.92 g/100ml, and 2.03% respectively). Seedless Kinnow and low seeded Kinnow strains portray similar results in various citrus quality and nutritional value aspects, anticipating a healthier and progressive domestic and export market.

Keywords: Citrus, Seedless, Vitamin-C, Physio-chemical attributes, Proximate analysis, Fruit quality

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Introduction

Citrus is considered one of the major fruit crops cultivated in several tropical or subtropical climates

(Kamal et al., 2011). Citrus fruits are a valuable source of minerals, vitamins, carbohydrates, dietary fibres, and physico-chemical features (Prasad et al., 2015; Hayat et al., 2017; Wu et al. 2018). Citrus



belongs to the rutaceae family comprising of 140 distinct genera and 1300 species, which includes essential crops like mandarin, orange, limes, lemons, grapefruits and pummelos etc (Bora et al., 2020).

The citrus industry of Pakistan, driven by Kinnow (*Citrus reticulata* Blanco), is now supported by two new Kinnow strains (low seeded and seedless Kinnow) to meet the increasing domestic and international market demand. Kinnow has a vigorous, large plant with dense foliage bearing medium-large fruit with a typical orange yellowish color of rind and flesh, very juicy having rich and aromatic flavor with high nutrients (Singh, 2018). Normal Kinnow has numerous seeds, a polyembryonic, and a strong tendency to alternate bearing. Kinnow is a hybrid that originated from King and Willow Leaf cultivars and belongs to family Rutaceae (Frost, 1935). There are many problems in citrus export; most prominent of them is high seediness in citrus fruit (Mallick et al., 2017). Mandarins are rich with vitamins, natural antioxidants, physicochemical properties, and sensory attributes. Due to its richness, it is appreciated all over the world (Chopra et al., 2004; Altaf and Khan, 2008; Shireen et al., 2018).

Pakistan has taken a prominent position in the citrus industry among leading Kinnow exporting countries in the world, with an average production of 2.132 million tons, and the cultivated area is 199.9 thousand hectares (Ghani et al., 2016). Citrus is grown all over Pakistan, and more than 95% of citrus is grown in Punjab- Pakistan. Out of 95% of citrus, 70% Kinnow mandarin is grown in Punjab Province (Khan et al., 2010). Kinnow stands first in the citrus industry of Pakistan because of wider climatic adaptation, high yield potential, a good blend of sugars and acids, consumer preference, and highest economic return (Shah, 2004). In addition to this, Kinnow is considered as a low-value export fruit because of a high number of seeds per fruits and problematic in the juice extraction and processing industry as seeds cause bitterness (Shahid et al., 2016).

The seediness in Kinnow mandarin is due to various natural factors like pollen self-incompatibility or ovule and pollen incompatibility (Altaf et al., 2004). Seedlessness is closely associated with triploidy, while spontaneous polyploidy is also found in nature (Jaskani et al., 2005; Usman et al., 2008). Crossing tetraploid and diploid cultivars can produce triploid cultivars (Krug and Bacchi, 1943). Kinnow has extreme variability because of its hybrid nature. Variability in seed number per Kinnow fruit was 0-52

(Khan, 1992; Sharma and Thind, 2005). Furthermore, Altaf and Khan (2008) found 0-3, 2-6 developed seeded branches. The seedless trait was 0-6 seeds, and the most common was 14-32 in seeded background.

Simple Sequence Repeats (SSR) markers are not available for Kinnow, which made it look into cross genera marker polymorphism and characterization of these clones (Mallick et al., 2017). So, genetic improvement within Kinnow cultivar is by selection and vegetative multiplication of natural mutant branches. Kinnow mandarin has different descriptive morphological characteristics, i.e., height, spread and canopy of plant and physico-chemical analysis of fruit like physical maturity of the fruit, total soluble solids (TSS) and acid ratio, size, and shape, juice percentage, number of seeds per fruit, peel adherence and fruits holed on the tree, etc. plants holed the fruit (Altaf et al., 2014). So, there is a dire need for the characterization of Kinnow (Agbayayo et al., 2018). The goal of this study was to characterize and compare three different strains of Kinnow mandarin (based on the high number of seeds to zero number of seeds per fruit), physico-chemical, nutritional, and proximate analysis of fruits.

Material and Methods

Plant material

The trial site for this experiment was selected at the orchard of Citrus Research Institute, Sargodha, Pakistan (Latitude 32°51'N longitude 72°40'E). During growing season 2017 & 2018, 'Kinnow' mandarin strains (*Citrus reticulata* Blanco) (seedless, less seeded and normal seeded as explained in table 1 below) plants grafted on Rough Lemon rootstock (*Citrus jambhiri* Lush).

Table-1: Seed characteristics of three Kinnow strains in agro-climatic conditions of Sargodha

Kinnow strains	The average number of seeds/fruit
Seedless	0- seeds
Less seeded	2-7 seeds
Normal seeded	15-27 seeds

The plants spaced about 18 ft. × 18 ft, and grown in same agro-climatic conditions following recommended agronomic practices and inputs during the study period were used. Statistical analysis was done using RCBD with three treatments and three



replications per treatment. Mature fruits were selected randomly for further physical and biochemical analyses.

Physical characteristics of fruits

A representative sample of twenty fruits was taken at the time of fruit maturity to measure the fruit size, fruit weight, juice percentage, peel thickness, and other physico-chemical parameters. Fruit diameter and peel thickness were measured by digital caliper, and fruit weight was measured by the use of the digital scale. Fruit firmness average data was collected in Kg by the use of a digital penetrometer; the measuring force required to penetrate the probe to a depth of 2 cm randomly at eight different locations in the mesocarp layer of the fruit.

TSS was measured by the help of a digital refractometer, which was provided with automation temperature correction. The pH of the fruit juice was expressed by the use of a bench-type digital calibrated pH meter at 20 °C following the Anwar et al. (2007). The amount of citric acid expressed as juice acidity was calculated by titrating the juice against a standardized solution of 0.1 N NaOH.

Bio-chemical characteristics of fruits

Determination of vitamin C and sugars

The amount of vitamin C in the juice was calculated as described by Ruck (1961). Sugar in the juice was determined as described by Hortwitz 1960. Total sugar and reducing sugars were determined through titration against Fehling solution, while non-reducing sugars were calculated by subtraction reducing sugar from total sugars.

Proximate analysis

Proximate analysis for the determination of crude fat, crude fiber, total ash, and crude protein was carried out as described in AOAC 2006. Crude fat was analyzed by Soxhlet's apparatus in which petroleum ether was used to extract the sample, which was afterward dried and weighed in a petri dish till constant reading.

The crude fiber was determined by digesting sample with H₂SO₄ and NaOH and incinerating the residues in a muffle furnace at elevated temperature of 550 °C till grayish-white ash, cooled weighed and calculated as below;

Crude fiber % = Weight loss on ignition (g)/ Weight of original sample (g) x 100

Total ash determination was carried out by taking dried samples in a muffle furnace. The sample was added into a crucible, and the blue flame burner was used to ignite it. Then again, muffle furnace was used to reheat on a temperature of 500 °C ± 5 °C for 1-2 hours. Then weighing of the sample was done after cooling the sample in the desiccator.

For the determination of protein, the digestion mixture was prepared by CuSO₄ + K₂SO₄ + selenium oxide. This mixture was mixed with 5 g sample in cupped filter paper and put in Kjeldahl's flask. After adding 25 ml of H₂SO₄ (Conc.) in the flask, contents were heated so that frothing would minimize and boiled to clean the solution thoroughly, then makeup with distilled water and cool in 100 ml volumetric flask. NaOH 10 ml (40% solution) was also added to it. Free ammonia was collected in boric acid 25 ml (4% solution) with added indicator.

$$\text{Nitrogen Percentage} = \frac{A-B \times 0.001}{W} \times 100 \frac{V}{V_1} \times 100$$

A = 0.1 N H₂SO₄ used for sample

B = 0.1 N H₂SO₄ used for blank

W = Sample weight (2 g)

(V) = Volume made (100 ml)

(V₁) = Aliquot distilled (10 ml)

Statistical analysis

Randomized Complete Block Design (RCBD) was used for statistical analysis of the data. Treatments responses on fruit quality and nutritional value were statistically analyzed with software Statistix 8.1. The least significant difference (LSD) and multiple comparison tests at $P < 0.05$ (Steel et al., 1997) were used to evaluate the mean comparison among the treatments. Graphical presentation of data and standard error were calculated using Sigma Plot software (SigmaPlot, 2008).

Results and Discussion

Physical characteristics

A completely randomized design ANOVA was conducted to evaluate the three different strains of Kinnow mandarin on average fruit weight, fruit size, juice, peel thickness, TSS and firmness (Table 2A). The effect of Kinnow strains on average fruit weight, K (2, 4) $F = 81.3044$, $p = 0.0000$, fruit size, K (2, 4) $F = 119.306$, $p = 0.0002$, juice, K (2, 4) $F = 26.8536$, $p = 0.0085$, peel thickness, K (2, 4) $F = 0.21941$, $p = 0.0000$, TSS, K (2, 4) $F = 1.34543$, $p = 0.0000$ and



firmness, $K(2, 4) F = 0.11231, p = 0.0000$) were significant (Table 2A). Fruit size has a vital significance for market demand. Data regarding fruit size depicted maximum fruit size (76.0 mm) in the case of normal seeded Kinnow, while a minimum fruit size of 63.44 mm was recorded in seedless Kinnow strains (Table 2B). The result of per fruit weight showed significant differences among all three Kinnow strains. Maximum weight per fruit (176.69 g) was recorded in normal seeded Kinnow, whereas the minimum fruit weight was observed (166.28 g) in seedless Kinnow (Table 2B). All three treatments varied in the quality of juice recovered from fruit. The results regarding juice percentage depicted significant differences among various Kinnow strains. Maximum juice (44.29%) was found in normal seeded Kinnow, followed by less seeded Kinnow (41.35%), and the minimum juice (38.30%) was recorded in seedless Kinnow (Table 2B). Peel thickness was a variable trait among citrus strains. The results for peel thickness described significant differences among different Kinnow strains. Maximum peel thickness was observed in the fruit of normal seeded Kinnow (3.01 mm) followed by less seeded (2.86 mm), while minimum peel thickness (2.48 mm) was recorded in the seedless Kinnow fruits (Table 2B). Fruit firmness is an important criterion to assess the quality of citrus fruits. Normal seeded Kinnow showed significantly higher fruit

firmness (1.90 kg) as compared to other treatments, and the lower fruit firmness (1.55 kg) was recorded in less seeded fruits (Table 2B). Kinnow has extreme variabilities in seed number per fruit and fruit quality (Altaf and Khan, 2009). Kinnow has various polyembryonic strains that grow to apomictic seeds (Altaf et al., 2004). Kinnow has low seeded/seedless fruits, and that was utilized for developing new strains (Altaf et al. 2004). The normal seeded plants were producing more fruits compared to low seeded plants, and perhaps the seed formation helps in a better fruit set (Altaf and Khan, 2009). Jaskani et al. (2002) observed that the seed number and peel thickness of diploid Kinnow trees were more than tetraploid Kinnow trees. In the present study, normal seeded Kinnow increased the peel/rind thickness, weight of fruit as compared to less seeded and seedless Kinnow fruits (Table 2B). Total soluble solids (TSS) define quality. TSS is an indicator of sweetness mostly imparted by the sugar present in the juice. The significant difference in TSS among all three contestants Kinnow strains was noticed. In the present study, significant differences of TSS ($^{\circ}$ Brix) were observed among various Kinnow strains. Maximum TSS was observed in seedless Kinnow (11.95%) followed by normal seeded Kinnow (11.40%), while lowest TSS (10.62%) was found in the less seeded Kinnow fruits (Table. 2B).

Table-2A. ANOVA analysis of three different strains of Kinnow mandarin on physical characteristics of fruits

SOV	df	MS					
		Average fruit weight (g)	Fruit size (mm)	Juice (%)	Peel thickness (mm)	TSS (%)	Firmness (kg)
Replication	2	7.0019	0.532	1.0412	0.00338	0.00103	0.00101
Kinnow strains	2	81.3044**	119.306**	26.8536**	0.21941**	1.34543**	0.11231**
Error	4	0.2766	0.764	1.3663	0.00024	0.00007	0.00001
Total	8						

**Highly Significant

Table-2B. Comparison of three different strains of Kinnow mandarin on physical characteristics of fruits

Treatments	Average fruit weight (g)	Fruit size (mm)	Juice (%)	Peel thickness (mm)	TSS (%)	Firmness (kg)
Normal seeded	176.69 a	76.00 a	44.29 a	3.01 a	11.40 b	1.90 a
Less seeded	171.66 b	68.66 b	41.35 ab	2.86 b	10.62 c	1.55 c
Seedless	166.28 c	63.44 c	38.30 b	2.48 c	11.95 a	1.59 b
LSD	1.1923	1.9816	2.6498	0.0354	0.0185	0.007

Different letters in columns show significant difference among means of genotypes following Tukey LSD, where $P \leq 0.05$ showed significant difference



Bio-chemical characteristics of the fruit

A completely randomized design ANOVA was conducted to evaluate the three different strains of Kinnow mandarin on vitamin C, beta carotene, reducing sugars, non-reducing sugars, total sugars and acidity (Table 3). The effect of Kinnow strains on vitamin C, K (2, 4) $F = 12.0823$, $p = 0.0004$, beta carotene, K (2, 4) $F = 0.82654$, $p = 0.0002$, reducing sugars, K (2, 4) $F = 0.01141$, $p = 0.0002$, non-reducing sugars, K (2, 4) $F = 0.0006$, $p = 0.0000$, total sugars, K (2, 4) $F = 0.09954$, $p = 0.0000$ and acidity, K (2, 4) $F = 0.00760$, $p = 0.0011$ were significant (Table 3). Kinnow fruit is rich with Vitamin C and is the essential quality parameter for Kinnow fruit. Maximum vitamin C (38.5 mg/100 ml) was recorded in normal seeded Kinnow fruits, while minimum (34.80 and 35.43 mg/100ml) was observed in less seeded and seedless strains, respectively (Fig. 1A). The higher β -carotene content (63.15 and 63.92 $\mu\text{g}/100\text{g}$, respectively) were recorded in normal seeded and seedless Kinnow strains, while minimum (62.92 $\mu\text{g}/100\text{g}$) were found in less seeded Kinnow strains (Fig. 1B). For reducing sugars, the minimum percentage (3.35%) was acquired by seedless Kinnow fruits whereas, the maximum percentage (3.48%) was found with less seeded Kinnow fruits (Fig. 2A). Maximum non-reducing sugar percentage (3.52%) was attained by normal seeded Kinnow fruits followed by less seeded Kinnow fruits (3.44%). In contrast, the seedless Kinnow fruits resulted in lowest values of non-reducing percentage (3.38%) (Fig. 2B). While data regarding total sugars showed that minimum total sugar content (7.50%) was recorded with less seeded Kinnow strains and the maximum amount of total sugars (7.84%) was found in seedless Kinnow strains (Fig. 2C). Acidity is another important citrus quality parameter. The maximum juice acid content (0.60% and 0.54%, respectively) was found in normal seeded and seedless Kinnow,

while minimum (0.50%) was found in less seeded Kinnow fruits (Fig. 2D). Juice recovery is the most desirable trait by processors, which is influenced by scion/rootstock combinations (Shireen et al., 2018). Jaskani et al. (2002) reported that diploid trees had significantly higher juice percentage, TSS, and the number of developed seeds compared with tetraploids. Characteristics of seedlessness in Kinnow is because of pollen self-incompatibility, while parthenocarpy, defective ovules, and embryo abortion. It is also stated that Kinnow plants having dominant characteristics of cultivar King can easily survive in hot temperatures like Pakistan (35-51°C in summer) instead of those having dominant characteristics of Willow Leaf cultivar. Kinnow fruits have variable characteristics with regards to various parameters like seed number, fruit size, color shape, peel thickness, firmness, aroma, acidity, sweetness and juice contents etc. (Altaf and Khan, 2008). Singh (2018) reported that Kinnow cultivar has wide adaptability due to being a high yielder with abundant juice contents but has a problem of a high seed number. The high seed number is a hindrance in the adaptation of Kinnow as a table fruit, and seeds impart bitterness to juice in the processing industry. Seedless cultivars are preferred for international trade.

Proximate composition

A completely randomized design ANOVA was conducted to evaluate the three different strains of Kinnow mandarin on crude protein, crude fat, crude fiber and ash (Table 4). The effect of Kinnow strains on crude fat, K (2, 4) $F = 0.00101$ $p = 0.0325$, crude fiber, K (2, 4) $F = 0.06981$, $p = 0.0000$ and ash, K (2, 4) $F = 0.03303$, $p = 0.0013$ were significant while crude protein, K (2, 4) $F = 0.0003$, $p = 0.2215$ had non-significant (Table 4).

Table-3. ANOVA analysis of soil applied boron on plant height, leaf area, number of bolls per plant, boll size and weight and seed cotton yield per plant of cotton cultivars

SOV	df	MS					
		Vitamin C (mg/100mL)	Beta carotene ($\mu\text{g}/100\text{g}$)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Acidity (%)
Replication	2	5.0833	0.01281	0.00101	0.00083	0.00381	0.00043
Kinnow strains	2	12.0823**	0.82654**	0.01141**	0.01343**	0.09954**	0.00760**
Error	4	0.1180	0.00518	0.00008	0.00017	0.00001	0.00013
Total	8						

**Highly Significant



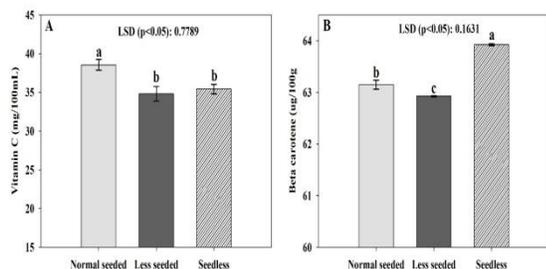


Figure-1. Comparison of three different strains of Kinnow mandarin on vitamin C and beta carotene contents of fruits.

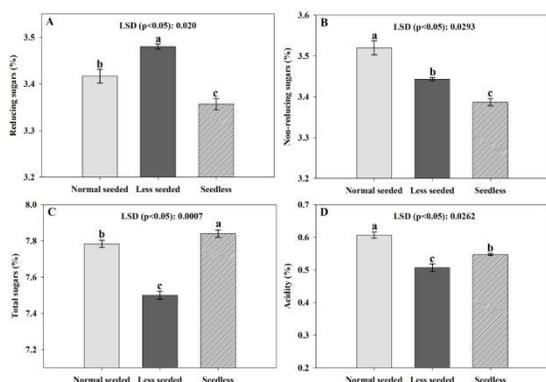


Figure-2. Comparison of three different strains of Kinnow mandarin on biochemical characteristics of fruits.

Intake of supplements in specific populations groups is recommended to meet the nutritional inadequacies but in general whole foods are preferred source of nutrients as compared to supplements (USA, 2010). Thus consumption of wholesome citrus fruit may have more nutritional benefits as compared to its juices or any sort of supplements which is evident from example of orange fruit providing better quantities of fiber and carotenoids as compared to its juice (US Department of Agriculture, Agriculture Research Service). Macronutrients including water (moisture), total carbohydrates, total lipids (fat), protein and ash are referred as proximate components of a food (USDA, 2015). Plethora of health benefits can be related to individual compounds or synergistic effects of various nutrients, proximate components and phytochemicals found in citrus fruit exerting antioxidant activities and anti-proliferative activities curing diabetes, cancer, various types of stresses improving cardiovascular health, blood pressure and cholesterol levels (Sun et al., 2002; Harats et al.,

1998; Ghanim et al., 2010). It was also reported that efficacy of antioxidant was affected through the Interaction between proteins and flavonoids (Arts et al., 2002). Data on crude protein content showed non-significant differences among all three various Kinnow strains (Fig. 3A). There were significant differences among different Kinnow strains with respect to crude fat. The highest crude fat (0.180%) was detected in seedless Kinnow strain followed by less seeded (0.163%), whereas minimum (0.143%) crude fat was found in normal seeded Kinnow fruits (Fig 3B). Citrus fruit contain minimal quantities of fat and cholesterol contrary to energy-dense foods causing obesity and cardiovascular disorders. Consumption of citrus fruit aids in controlling the effects of diabetes due to having relatively low glycemic index. Although a difference was found in fat percentage among three strains but due to minimal quantities the difference is not significant for nutritionists. The maximum percentage of crude fiber (2.60%) was found in seedless Kinnow fruits, and a minimum percentage (2.31%) was observed in normal seeded Kinnow fruits (Fig. 3C). Dietary fiber is not digested and absorbed in body mainly consists on edible carbohydrates and lignin found in plants while fiber in citrus fruit is predominantly comprise on pectin (65-70%) which is a soluble fiber. Fibers reduce absorption of bile acids, fat, glucose and cholesterol reducing risk of cardiovascular disorders promoting satiety, laxation and healthy intestinal fermentation (Brown et al., 1999; Dikeman and Fahey, 2006; Economos and Clay, 2012).

Table-4. ANOVA analysis of soil applied boron on plant height, leaf area, number of bolls per plant, boll size and weight and seed cotton yield per plant of cotton cultivars

SOV	df	MS			
		Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)
Replication	2	0.00003	0.00014	0.00101	0.00023
Kinnow strains	2	0.0003 ^{NS}	0.00101*	0.06981**	0.03303**
Error	4	0.0001	0.00011	0.00003	0.00062
Total	8				

**Highly Significant NS = Non-significant

Significant percentage of crude fiber found in seedless Kinnow fruit is an optimistic development for health conscious consumers. For ash, minimum



ash percentage was acquired by less seeded (1.82%) and normal seeded Kinnow (1.88%) whereas maximum ash percentage was found in seedless Kinnow fruits (2.03%) (Fig. 3D).

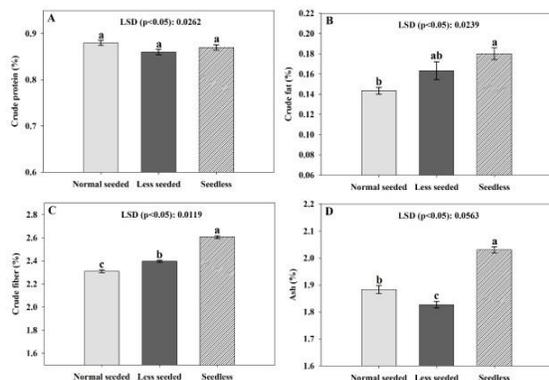


Figure-3. Comparison of three different strains of Kinnow mandarin on proximate analysis of fruits

Conclusion

This study was undertaken to evaluate two new strains, i.e., low seeded and seedless Kinnow with normal seeded Kinnow for various biochemical quality parameters. Results indicated that two new strains did not differ much for fruit quality characteristics and nutritional value. While seedless and less seeded can add to varietal diversification to the citrus industry of Pakistan and can accelerate the fruit demand in local as well as the nutritional market as a table fruit and for processing as well.

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