



IMPACT OF PEEL COLORATION ON INTERNAL QUALITY OF KINNOW MANDARIN (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora) AT EARLY RIPENING STAGE

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ABSTRACT

At earlier maturation, Kinnow mandarin color-break fruits were analyzed for internal quality. Ten fruits were harvested from dark green to fully red or yellow colored at field on visual observation and arranged in order of dark green to deep red or yellow through Chroma meter after being numbered as 1 to 10. Three aliquots were analyzed as per replicate. In red colored fruits juice, a decreasing trend of titratable acidity, ascorbic acid and total soluble salts were found with increasing pattern of sugars, total soluble solid and pH. Similarly in peel, low chlorophyll contents and highest carotenoids were recorded in red colored fruits. Total phenolic contents in juice were similar with declining trend in peel from green to red colored fruits. In juice both flavonoids and flavonols were constant but in peel flavonols increase were noted in red colored fruits. Total anthocyanins exhibited an increasing trend in red colored fruit for both, the peel and juice. Antioxidant activity was increased in juice and decreased in peel for red colored fruits. Nutritional values of green color peel fruits are alike those of red colored, but with reduced sugars, and sweetness were duly noted regarding their domestic consumers acceptance. Green color fruits have more ascorbic acid, polyphenols and reducing sugars are equally valuable like red colored Kinnow, thus both types are of Kinnow are best for the purpose of export.

Key word: Peel coloration; internal quality; Kinnow mandarin; early ripening.

INTRODUCTION

Although, modern food industry based on smart packaging with advanced active and intelligent materials, but appearance of either fresh or processed food is a good sensory attribute (Costa *et al.*, 2011). Color is the first visual observation to be judged for either acceptance or rejection (Leon *et al.*, 2006) as well as a good indicator of food quality (Nisha *et al.*, 2011), marketing (Pereira *et al.*, 2008) and online imaging used in distance market (Yu *et al.*, 2003). In fruit and vegetables, natural pigments produce color which change in maturation and ripening stages. Fat-soluble primary pigments are chlorophylls (green) and carotenoids (yellow, orange and red) and water-soluble secondary pigments are anthocyanins (red, blue), flavonoids (yellow) and betalains (red) to impart coloration (Barrett *et al.*, 2010). Color features can be used to judge fruit quality (Leemans *et al.*, 1998) and also serve as a good maturity indicator. Peel coloration of fruits is external maturity indicator but changes in acids, soluble solids and sugars can exactly define maturity index (El-Otmani and Zacarías, 2014). Non-climacteric fruits like citrus mature only on tree and peel color on visual observation in field is used as a tool in harvesting stage. Citrus fruit peel coloration is anciently used as sensory quality attribute like taste, sweetness and appearance (Sites and Reitz, 1950).

In domestic markets of developing nations, citrus is freshly consumed and more production and fresh consumption share is of the mandarin. In advanced world, citrus maturity standards like juice contents, soluble solids and acid ratio are

followed before harvesting can be carried out which vary in citrus as per different importing countries (E.U, 2011).

Citrus peel coloration is influenced by external environmental conditions and cultivars (Cronje *et al.*, 2013; Porras *et al.*, 2014). In warm area color-break is delayed with faint coloration (Mesejo *et al.*, 2012), but in tropic areas citrus fruit unable to develop color. In mandarin, color is not a single quality parameter to be judged for know-how of its internal properties (Tadeo *et al.*, 2008) due to high variability in TSS: acid ratio and sugars in juice. Color-break in citrus is one parameter to be followed for commercial scale harvesting. Other maturity indices like fruit size, shape, blemishes extent, biotic (pests-diseases) and abiotic (wind scars, stem injury) and physiological disorders are also used. These are external quality parameters mostly based on visual observations to judge fruit for distance marketing or export. Internal quality parameters like acids, sugars, polyphenols and antioxidants are hidden which can be estimated in laboratory with accuracy and standard protocols.

Kinnow mandarin is dominantly growing in Pakistan and major fresh fruit export of the country which starts at color-break stage during the end of November (Nawaz *et al.*, 2018). Color is main external feature to be followed for knowing the time of harvesting and spot picking of desirable fruit at earlier maturation. Most variability in peel color is found in field at early harvesting, with less color-break. Investigation regarding Kinnow fruits internal quality with respect to color change is required to judge the level of acids, sugars and polyphenols present in juice of dark green to deep red colored

fruits at harvesting. In this regard, dark green to deep red colored fruits were selected for biochemical analysis, for judging fruit internal quality with relation to peel coloration.

MATERIALS AND METHODS

The research work regarding impact of peel coloration on internal quality of Kinnow fruit was performed during growing season 2018. This study conducted at tehsil Bhalwal district, Sargodha, Pakistan.

Selection of fruit on color basis: At early maturity stage on 20th November, randomly 3 Kinnow growing plants were used for sampling. Ten fruit from each plant were harvested on the visual basis of color which are dark green to deep red peel color attained fruits were harvested and by Chroma

meter (Konica Minolta Chroma Meter, Osaka, Japan) and ranking of harvested fruit have been done by the ordering scale given by 1 to 10 order. Dark green peel color fruits were ranked in No. 1 and fully or deep red in No.10. Fruits number order 1 to 10 was arranged on peel color based (1=dark green and 10= deep red/yellow). In color based selection criteria, value of a (+red & - green) was followed to arrange fruits in 1 to 10 numbers for biochemical analysis in Post-harvest laboratory, PMAS-Arid Agriculture University, Rawalpindi, Pakistan. Chroma meter readings of both sides of fruits were taken and means data are arranged in order from more green (highest-tive value of a) to deep red (highest +tive value of a) (Table 1).

Table 1: Chroma meter based fruits color (1-10)

	1	2	3	4	5	6	7	8	9	10
Colours	DG	G	MG	LG	VLG	VLR	LR	MR	R	FR
L	59.12	48.70	47.71	68.94	53.65	63.61	63.63	71.61	73.35	70.62
a	-21.00	-18.55	-17.38	-16.44	-11.66	-10.49	-3.11	16.09	19.04	19.52
b	36.91	42.01	45.22	53.18	39.63	47.05	46.15	64.68	69.15	65.73
c	42.55	46.07	50.47	55.51	41.71	48.96	46.47	66.66	71.78	68.50
h	119.63	124.29	125.33	107.16	107.04	104.09	94.31	75.67	75.31	73.55

L= lightness (100- full lightness & 0- full darkness), a = red (+) & green (-) color attribute

B= yellow (+) & blue (-) color attribute, C = chromaticity, H =Hue angle, DG (dark green), G (green), MG (moderate green), LG (light green), VLG (very light green), VLR (very light red), LR (light red), MR (moderate red), R (red), FR (fully red).

Chlorophyll and carotenoids measurement: Chroma meter based colors (fruits 1-10) peels were used for chlorophylls and carotenoids measurement. Sample of 1 g of each fruit peel was ground in 10 mL acetones (80 % v/v) and filtered supernatants were taken (3mL) in glass cuvette. Absorption of each sample was recorded at wavelength of 663 nm, 645 nm and 470 nm by Spectrophotometer (Model: SP-3000 plus Optima, Japan). Total chlorophyll, chlorophylls a and b and carotenoids were calculated by using equation as described by [Sumanta et al. \(2014\)](#).

Total chlorophyll = $20.2 A_{645} + 8.02 A_{663}$

Chlorophyll a (C a) = $11.75 A_{663} - 2.350 A_{645}$

Chlorophyll b (C b) = $18.61 A_{645} - 3.960 A_{663}$

Total carotenoids = $1000 A_{470} - 2.270 C a - 81.4 C b/227$

Fruit Juice extraction: Juice was extracted from individual fruit of each replication through electric juicer and then filtered and used biochemical and polyphenols & antioxidant analysis. A typical citrus fruit juicer was used to avoid any contamination of seed content or seed oil in juice.

The pH, TSS and TDS measurement: Hanna pH meter (Model: HI 2211 pH/ ORP meter) was used to measure pH after being calibrated with pH buffer 7.00 and 4.00 solutions. Portable Refractrometer (Model: FG-103) was used to measure TSS (°Brix). EC meter (model: Cond.720) was used for measure TDS (g/L⁻¹) of juice.

Titrateable acidity (TA): Titration method for titrateable acidity (g/100ml of juice) as explained by [Fabro et al. \(2006\)](#) was used. Fruit juice of 10 mL quantity was taken and 40 ml distilled water add to make the volume up to 50 mL. Then 10 mL was taken from already prepared diluted 50 mL volume (1-juice: 5-dH₂O) and added 2-3 drops of phenolphthalein as

an indicator and titrated against (0.1N) Sodium hydroxide (NaOH) till light pink color developed. Sodium hydroxide (0.1N) was prepared freshly by dissolving 4 gram of pellets (NaOH anhydrous) in 1000 mL distilled water.

TA % (g/100mL) = $\frac{\text{NaOH used} \times \text{normality of NaOH} \times 0.064 \times 100}{\text{mL of juice used}}$

Ascorbic acid (AA) estimation: About 5mL juice and 5mL HCl (1%) was grounded and poured into eppendorf tube and centrifuged at 10000 rpm for 10 minutes. After centrifugation, 0.3mL mixture was taken and added 2.7mL dH₂O and measured absorbance at wavelength 243 nm on Spectrophotometer.

AA (mg/100mL) = $\frac{\text{Absorbance of sample at 243nm} \times 10}{0.293}$

Juice sugars estimation: Sugars from juice were estimated by following standard techniques described by [Khalid et al. \(2012\)](#).

Reducing sugar: A 10 mL juice was taken in 250 mL beaker and then 10 mL Potassium oxalate (7%) & 25 ml Lead acetate (2%) was added to make its volume up to 250 mL with dH₂O. Fehling solutions 10 mL (5 mL of each A & B) were used to titrate against prepared sample with gentle warm until red precipitates appeared. Fehling A was prepared by dissolving 69.3g of copper sulphate pentahydrate in 1L distilled water and fehling B by dissolving 100g of sodium hydroxide anhydrous and 345g of sodium potassium tartrate (KNaC₄O₄H₃) in 1 L of distilled water.

Reducing sugar (g/100mL) = $\frac{6.25(X)}{Y}$ (where X= volume of sample used and Y=standard sugar)

Total sugar: A 25 mL of prepared sample (250 mL of reducing sugar) was taken in beaker and added 20 mL dH₂O

and 5 mL concentrated HCl and kept in dark for overnight. Next day, 2-3 drops of phenolphthalein were added and then neutralized with NaOH and then titration against fehling solution was performed just like reducing sugar.

Total sugar (g/100mL) = $25 (X) / Y$ (where X= volume of sample used and Y=standard sugar)

Non- reducing sugar: It was calculated from total sugar to reducing sugar as given formula.

Non-reducing sugar (g/100mL) = total sugar - reducing sugar x (0.95)

Total Phenolic Contents (TPC): Total phenolic contents were measured through Folin-Ciocalteu method by following procedure of [Miliauskas et al. \(2004\)](#). 1 mL of juice was taken in volumetric flask and added 10 mL Methanol (85 % v/v)

and well shaken. After 1 hour, 1 mL of plant extract was taken and poured into another 50 mL volumetric flask and 5 mL FC Reagent (10 % v/v) was added and just after 2 to 8 minutes, 4 mL sodium carbonate (7%) was added and sample was kept for half an hour to develop blue color and absorbance was checked at 765nm. Similarly, a blank sample without plant extract (5 mL FC Reagent + 4 mL sodium carbonate) was also prepared. Different concentrations of Gallic acid were used to obtained standard curve of Gallic acid equalant (GAE) and expressed as mg of GAE/ 100mL of juice (GAE= Gallic acid equalant) (Figure 1).

TPC (mg of GAE/100mL of juice) = (Sample absorbance + 0.058)/0.102.

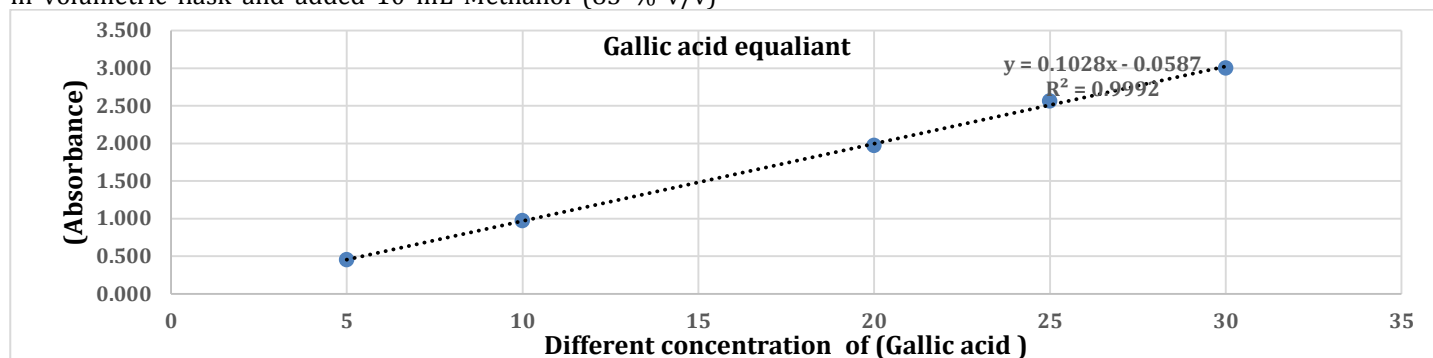


Figure 1: The GAE equalant with different concentrations of Gallic acid.

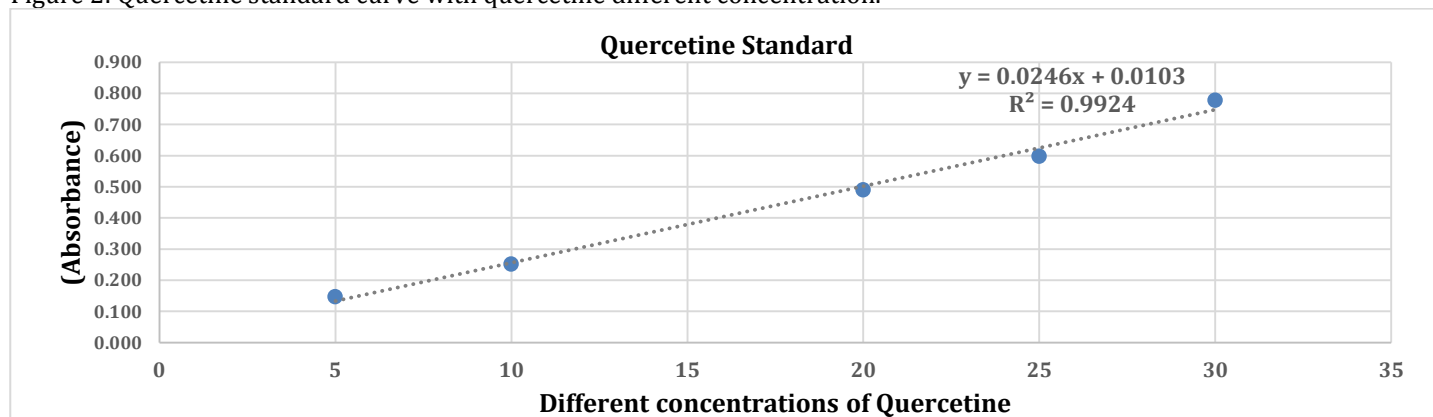
Total flavonoids contents (TFC): Total flavonoids contents were measured by following procedure of [Nongalleima et al. \(2017\)](#). Pure ethanol based plant extract (1:10) was prepared. 1 mL plant extract was taken in glass cuvette and add 0.1mL of each aluminum chloride (10%) and sodium acetate (10 %) and then 1.2 mL of dH₂O were added. Samples were kept for 2 hour in dark and then absorbance was checked at 415 nm. Similarly, a blank sample without plant extract (0.1 ml AlCl₃ + 0.1 mL sodium acetate + 1.3 mL dH₂O) was also prepared. Different concentrations of quercetine were used to obtained standard curve of quercetine equalant as shown in TF_oC.

TFC (mg of Quer.equal/100mL of juice) = (Sample absorbance +0.01)/0.024

Total flavonols contents (TFoC): Total flavonols contents were measured by following procedure of [\(Miliauskas et al., 2004\)](#). Pure ethanol based plant extract (100 µg/mL) was prepared. 0.5 mL plant extract was taken in glass cuvette and 0.5 mL of aluminum chloride (20 %) and 1.5 mL of sodium acetate (10%) were added. Samples were kept for 2.5 hour in dark and then absorbance was checked at 440 nm. Different concentrations of Quercetine were used to obtain standard curve of quercetine equalant and expressed mg of quer /100 mL of juice.

TF_oC (mg of Quer.equal/100mL of juice) = (Sample absorbance +0.01)/0.024 (Figure 2)

Figure 2: Quercetine standard curve with quercetine different concentration.



Total anthocyanins: Lee *et al.* (2005) method was used for measuring total anthocyanin. Pure ethanol based plant extract (1:10) was prepared. Potassium chloride (0.025M) with pH= 1.00 and sodium acetate (0.4M) with pH= 4.00 were prepared. 0.2 mL plant extract was taken a separate in glass cuvette and 2.00 mL of potassium chloride (pH=1.00) was added. 0.2 mL plant extract was taken in glass cuvette and 2 mL of sodium acetate (pH=4.00) was added. Both samples were checked for absorbance at 510 nm and 700 nm. In blank, simple distilled water (3.00 mL) was used and expressed as milligram of cyanidine 3-glucoside per liter (mg C3GL-1).

$$A = (A_{510} - A_{700})_{pH_{1.00}} - (A_{510} - A_{700})_{pH_{4.00}} \text{ and}$$

$$A \text{ (mg C3GL-1)} = A \times MW \text{ (DF)} \times 100 / \epsilon \times 1$$

$$\text{(where MW=449.2, D.F =10 and } \epsilon = 26900)$$

Antioxidant activity (DPPH: 1,1-diphenyl-1-2-picrylhydrazyle) Antioxidant activity (DPPH inhibition or scavenging %) was measured by standard procedure described by *Thaipong et al.* (2006). Pure ethanol based plant extract (1:2 ratio) was prepared. 0.1 mL plant extract was taken in glass cuvette and 2.9 mL of DPPH working solution was added and kept for half an hour in light on laminar chamber and then absorbance was checked at 515 nm on Spectrophotometer (Model: SP-3000 plus Optima, Japan). Stock solution was prepared by dissolving 24 mg DPPH in 100 mL of pure ethanol. Working solution was prepared by checking absorbance value 0.980 ± 0.2 at wavelength 517 nm by adding pure ethanol in stock solution until absorbance value reached at 0.980 ± 0.2 . Blank sample (without plant extract) was prepared by adding 0.1ml ethanol and 2.9 ml working solution and absorbance was checked just after preparation at 515 nm.

$$\text{Antioxidant activity (DPPH Inhibition or scavenging \%)} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}}$$

Statistical analysis: Experiment was laid out under Randomized Completed Design (RCD) with three replications. Data regarding parameters was compared by using LSD test with 5% significance level. Treatment means from average of ten replications were analyzed by standard error. Both in table and graphs were constructed with standard errors. Statistix 8.1 software was used for analysis of variance and overall significance of data used in this study.

RESULTS AND DISCUSSIONS

Data analysis regarding fruit colors development in Kinnow mandarin reflected significant change, which are explained and discussed as detailed under.

Chlorophylls and carotenoids contents in fruit peel: Chlorophyll and carotenoids contents of fruits (1-10) are presented in figure 3. Maximum total chlorophyll, chlorophyll a and chlorophyll b contents were found in fruit (1) having dark green color (3.56, 1.60 and 1.75 mg/g) and minimum in fruit (10) attained deep red colored (1.66, 0.73 and 0.83 mg/g) respectively. Opposite to chlorophylls contents, maximum carotenoids were recorded in fruit (10) having deep red colored (2.47 mg/g) and minimum in fruit (1) dark green color (0.52 mg/g). Peel chlorophyll contents in fruit

color based on chroma-meter (1-10) were observed gradually decreased in order dark green to deep red colored and carotenoids contents increased in reverse order of deep red to dark green color.

Chlorophylls contents degenerated during citrus fruit coloration phase as was seen in Kinnow fruit (1-10) with declining trend from dark green to deep red color. Carotenoid contents in peel of fruits (1-10) showed contrary trend to chlorophylls with gradually increased in fruit coloration period with maximum level was noted in red colored fruit No. (10). Chlorophylls and carotenoids are essential pigments in plants, mostly involved in photosynthetic activities (*Young and Britton, 1993*) and also protect plant parts from damaging effect of solar radiation and oxidative stress (*Gitelson et al., 2002*). Chlorophyll is an antioxidant (*Hasanuzzaman et al., 2013*) and protected citrus fruit from oxidative stress. Chlorophylls contents in peel of citrus fruit work as protection against damaging effect of heat and solar radiation (UV) (*Josse et al., 2000; Rissler and Pogson, 2001*). Similarly, carotenoids serve to stabilize protein in peel during the period of fruit growth and development (*Simkin et al., 2008*). Chlorophylls and carotenoids contents in peel also work as antioxidants and determine the age of fruits on tree. Upon touching maturation stage, citrus fruit accrues more sugars and carotenoids with reduction trend of acids and chlorophylls (*Giovannoni, 2001*) as were found in present study. In citrus fruit, carotenoids impart coloration with onset of ripening stage and accumulate more in peel with degeneration of chlorophylls (*Liu et al., 2006*). Rate of chlorophylls degeneration and carotenoids accrual determine fruit color development stage in citrus (*Baldwin, 1993*) which justified the present study having more chlorophylls at immaturity with less carotenoids.

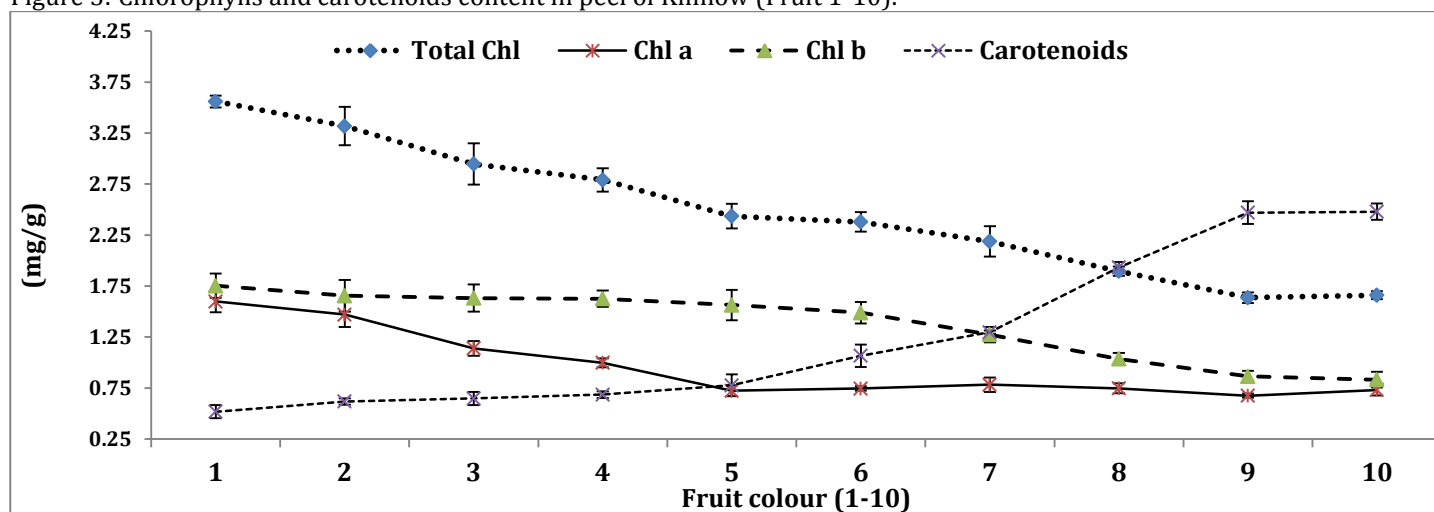
Fruit Biochemical Quality (Acids): Biochemical quality parameters are presented in table 2. Fruit juice pH was recorded maximum in fruit No. 9 (3.36) and minimum in fruit No. 1 (3.01). TSS was observed maximum in fruit No. 10 (11.67 °Brix) and minimum in fruit No.1 (9.00 °Brix). TA was found maximum in fruit No.2 (0.55 g/100mL) and gradually decreased and minimum in fruit No.10 (0.45 g/100mL). Ascorbic acid was found more in fruit No.2 (60.92 mg/100mL) and gradually reduced in color change order and less in fruit No.10 (46.45 mg/100mL). TDS was measured maximum in fruit No.1 (1628 mgL⁻¹) and minimum in red colored fruit No.10 (1279 mgL⁻¹).

Citrus fruit maturation is directly linked with peel pigmentations to represent color from dark green to red. Immature fruits having green peel color possessed more titratable acidity, ascorbic acid and TDS in juice with lower values of pH and TSS. Red colored fruits have higher pH and TSS to depict increasing trend with acid reduction. At maturity, less titratable acidity and ascorbic acid with more sugars in citrus juice were recorded (*Hardy and Sanderson, 2010*) and acidity decreased in mandarin on approaching at maturity stages (*Thapa and Gautam, 2002*) and during color

development fruit rapidly utilized acids in respiration (TCA cycle). Decreasing level of acidity resulted in ascorbic acid reduction (Rokaya *et al.*, 2016) which converted to 2-3-dioxy-L-gluconic acid by losing L-ascorbic acid (Mapson, 1970). Acids at maturity in citrus juice degenerated (Giovannoni, 2001) and reduce ascorbic acid and titratable acidity in red colored fruits with increasing trend of pH, TSS, TDS and sugars. TSS represents just 10% of acid level (mostly citric, malic and oxalic) and 10% nitrogenous compounds (amino acids) and higher percentage (70-80) of sugars (Davies and Albrigo, 1994) and red colored fruit (No. 10) possessed more TSS value which coincided with more sugars resulted in lower TDS level as was observed in present study. More non-reducing sugars, less titratable acidity and ascorbic acid in red colored fruits reduced TDS as was recorded decreasing trend

in 1 to 10 fruits. TDS represents soluble hydrogen carbonate ions, chloride salts, sulphate, calcium, magnesium, sodium, potassium (Fawell *et al.*, 2003) and it is positively correlated with acids concentration. Red colored fruit juice possessed lower titratable acidity, ascorbic acid and hydrogen ions (higher pH value) have reducing trend of TDS. Kinnow juice with more acidity and ascorbic acid and lower pH value showed more TDS than matured fruit with higher level of sugars. Juice pH represents level of acidity or alkalinity and pH value of citrus fall in acid range (Alhassan *et al.*, 2014) which was more in green color due to low pH coincided with higher value of TA and ascorbic acid and upon reaching at red color, hydrogen ions concentration reduced and pH value increased.

Figure 3: Chlorophylls and carotenoids content in peel of Kinnow (Fruit 1-10).



Biochemical properties of Juice

Fruit #	pH	TSS(°Brix)	TA(g/100ml)	AA(mg/100ml)	TDS(mgL ⁻¹)
1	3.01±0.05	9.00±0.03	0.53±0.01	60.40±3.45	1628±8.19
2	3.04±0.03	9.07±0.03	0.55±0.03	60.92±2.34	1617±13.68
3	3.13±0.08	9.27±0.03	0.54±0.01	58.33±2.75	1566±9.59
4	3.16±0.07	9.57±0.03	0.54±0.01	60.68±2.61	1358±5.04
5	3.20±0.03	9.23±0.03	0.53±0.01	54.04±2.10	1400±3.61
6	3.29±0.07	9.70±0.08	0.51±0.01	53.42±3.81	1354±5.85
7	3.26±0.02	10.53±0.03	0.47±0.01	51.43±2.75	1342±3.76
8	3.26±0.08	10.77±0.03	0.48±0.01	47.90±1.08	1355±13.11
9	3.36±0.08	11.20±0.06	0.47±0.01	44.63±2.47	1334±9.84
10	3.32±0.09	11.67±0.03	0.45±0.01	46.45±2.9	1279±9.13
Means	3.21±0.51	10.00±0.06	0.51±0.04	54.12±2.63	1423±8.18
CV	3.3	4.5	3.68	8.19	5.21

Table 2: Biochemical properties (acids) in Kinnow mandarin (Fruit 1-10) color based.

Data are mean ± standard error of triplicate determinations, TTS(total soluble solids), TA(titratable acidity), AA(Ascorbic acid), TDS(total dissolved salts).

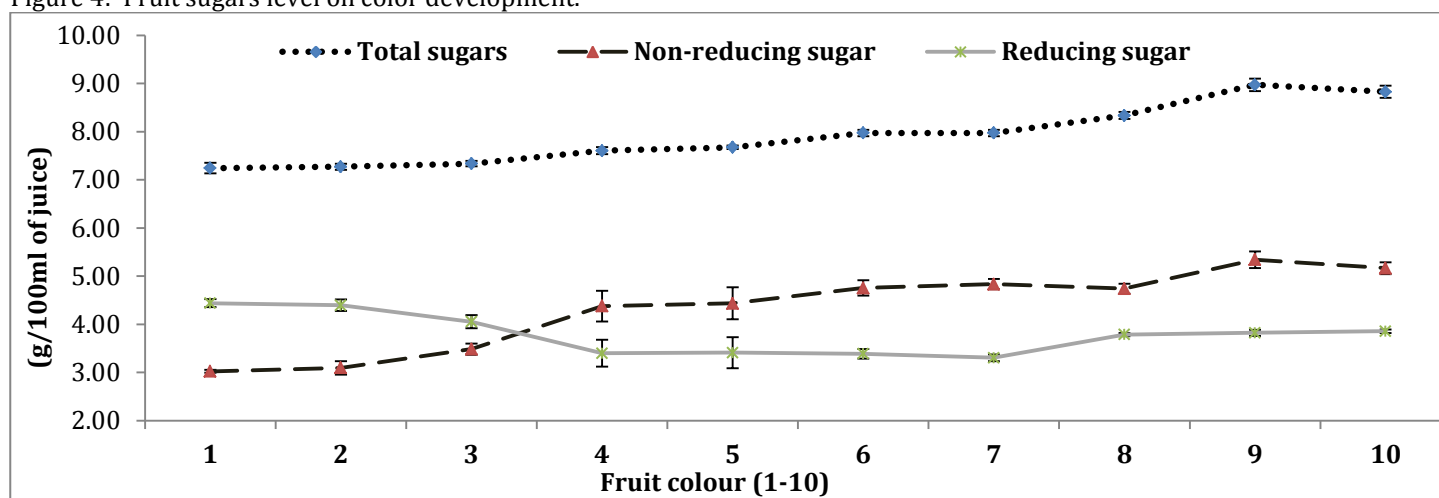
Fruit biochemical quality (sugars): Biochemical quality sugars parameters are presented in figure 4. Fruit juice total sugar and non-reducing sugar were found maximum in red or yellow colored fruit No.9 (8.97 & 5.34 g/100mL) and minimum in dark green fruit No.1 (7.24 & 3.02 g/100mL)

respectively whereas reducing sugar trend was opposite and found maximum in fruit No.1 (4.44 g/100mL) and minimum in fruit No.7 light red (3.30 mg/100mL). Total and non-reducing sugars were gradually increased and reducing sugar showed either constant or declining trend on fruit coloration

from green to red. Non-reducing sugar increased due to conversion of reducing sugar and ultimately increased total sugars. Sugar levels remained lower in citrus fruit (Hardy and Sanderson, 2010) until attained maturity. Sugar/acid ratios determine citrus fruit maturity index and upon achieving ripening stage, the level of sugars increase with reduction in acid (El-Otmani and Zacarias, 2014). In present finding, more sugars were accumulated in fruit (No.10) with less acidity. Similarly, TSS represents about 80% of sugars (glucose, fructose and sucrose) and was recorded higher in fully red colored fruit (No.10) to reinforce that color developed in peel also increased citrus fruit sugar contents. Oxidation-reduction

process during coloration of citrus fruit converted reducing sugar to non-reducing sugar. Reducing sugar has a free aldehyde or ketone group (Charlotte and Kathleen, 2013) which acts as reducing agent in fruit maturation to decrease its level by converting to non-reducing sugar. Reducing sugars was more found in dark green color fruit (No. 1) and gradually decreased in red colored fruit. Juice acids have negative correlation with sugars and as acidity decreased in present study, sugars increased. Green color fruits have more titratable acidity, ascorbic acid and total dissolved salts with low sugars and vice versa in red color.

Figure 4: Fruit sugars level on color development.



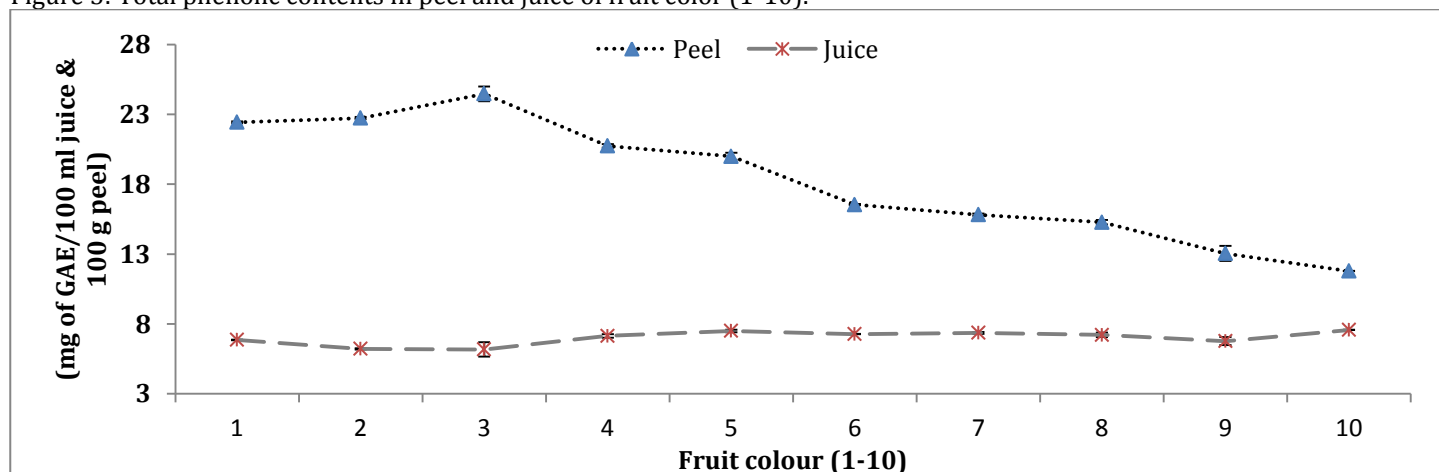
Results were reported in average with standard errors used in 3 replicates.

Total phenolic contents: Total phenolic contents are presented in figure 5. Maximum total phenolic contents were recorded in peels of fruit No.1 and 3 (23.97 mg of GAE/100ml each) and minimum in peel of fruit No.10 (11.28 mg of GAE/100ml) whereas in juice contents of fruit No. 5 possessed maximum TPC (7.79 mg of GAE/100ml) and minimum in fruit No. 3 (5.66 mg of GAE/100mL each).

In peel of fruits (1-10) has shown decreasing trend of TPC at red colored due to declining of chlorophylls and accumulation of anthocyanins. Juice TPC remained non-significant in dark green to fully red colored fruits. In juice, TPC remained at lower in immature fruit and reached at constant concentration in medium ripening fruit to declining trend in fully matured fruit with acid reduction. These findings are in line with the work of Mokhtar et al. (2014).

Total phenolic contents were reported different in citrus fruits attaining maturity index in different times (Webb et al.,

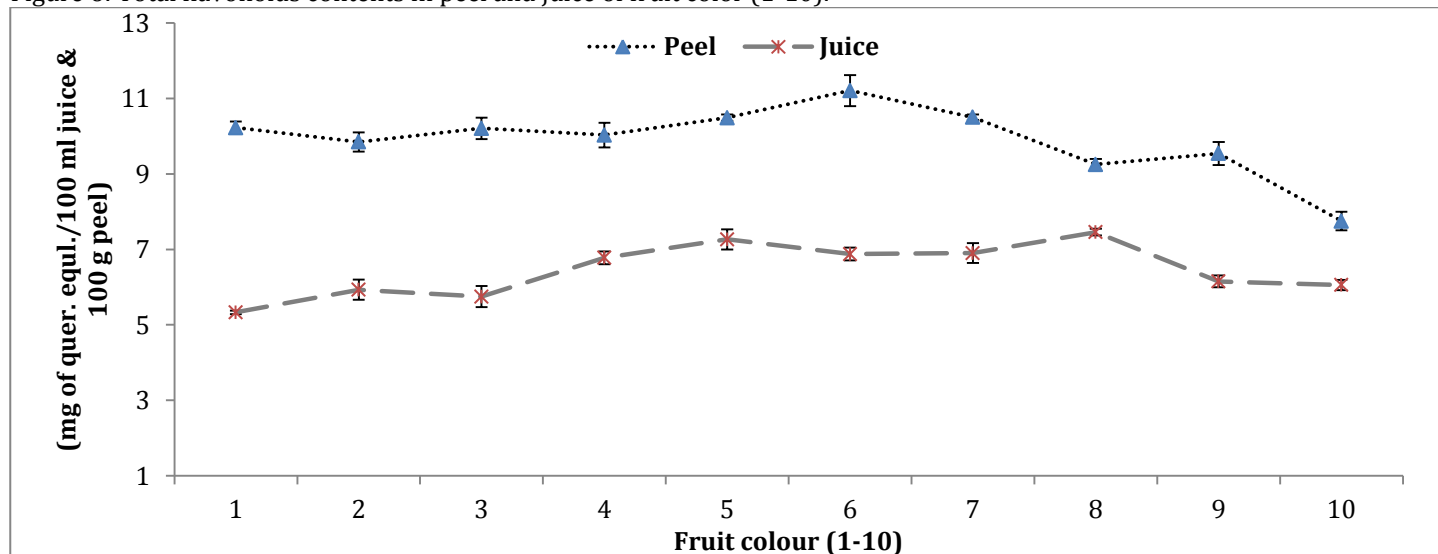
Figure 5: Total phenolic contents in peel and juice of fruit color (1-10).



Total flavonoids contents: Total flavonoids contents are presented in figure 6. Maximum total flavonoids contents were recorded in peels of fruit No.6 (11.21.0 mg of quer.equil./100mL) and minimum in peel of fruit No.10 (7.71 mg of quer.equil./100mL) whereas in juice contents of fruit No.8 possessed maximum TFC (7.46 mg of quer.equil./100mL) and minimum in fruit No.1 (5.33 mg of quer.equil./100mL).

Total flavonoids contents (TFC) were marginally fluctuated in peel and juice of fruits (1-10) with increasing trend in juice and decreasing in peel of red colored fruits. Citrus fruits showed different contents of polyphenols during ripening stages (Ferguson *et al.*, 1999) and slight different were observed in juice of Kinnow mandarin from green to red colored fruits. In peel, more TFC were found in green color than red. Comparatively citrus peel has more TFC than juice (Lattanzio, 2003). Deep red colored fruit (No.10) possessed higher TFC in juice. The citrus flavonoids have three types namely hesperidin (quercetine or rutin), flavonols (quercetin) and flavones (tangeritin) and protect against oxidative stress

Figure 6: Total flavonoids contents in peel and juice of fruit color (1-10).



Total flavonols contents: Total flavonols contents are presented in figure 7. Maximum total flavonols contents (TFoC) were recorded in peels of fruit No.6 (7.10 mg of quer.equil./100mL) and minimum in peel of fruit No. 2 (5.56 mg of quer.equil./100mL each) whereas in juice contents non-significant different in TFoC in all fruits. However, maximum TFoC were found in fruit No. 1(3.26 mg of quer.equil./100mL) and minimum in fruit No. 7 (2.26 mg of quer.equil./100mL).

Total flavonols contents in juice and peel showed mix trend with slightly differences in fruits (1-10). In fruit juice, flavonols function as antioxidants to protect ascorbic acid from oxidative stress (Herrmann, 1976) and more found in green fruits juice having more ascorbic acid in this study. Flavonols contents in fruits influenced by plant growth habit, seasonal variability and maturing phases (Aherne and O'Brien, 2002). Flavonols contents estimation in Kinnow fruits were based on color with same level of maturity

and solar radiation (UV) harmful effects and also serves as chemical messengers, physiological regulators and cell cycle inhibitors (Galeotti *et al.*, 2008). Polyphenols possess antioxidant activity or scavenging of free radicals. Flavonoids have more capability to capture electrons and scavenge or inhibit free radicals (Benavente-García *et al.*, 1997). In fruit color (1-10) showed different concentrations of TFC in peel and juice. More TFC in peel at green color fruits served more protection in extreme weather events in summer and gradually decreased in maturing. In juice with declining acidity, more TFC accumulates in red colored fruits. Flavonoids in plants serve as protection against drought and heat stress and freezing injury and functioning as temperature balancing (Samanta *et al.*, 2011). More TFC in Kinnow mandarin peel imparted more resistance against heat stress in summer months that gradually decline on coloration phase. In juice of red fruits, sugars accumulate and acids decrease with increasing trends of TFC that is opposite to peel.

Figure 6: Total flavonoids contents in peel and juice of fruit color (1-10).

showed slight differences in fruit juice and peel. Flavonols contents work as antioxidants against reactive oxygen species and reduce oxidative stress in plants (Nakabayashi *et al.*, 2014) and were found more in citrus peel than juice to protect fruit during whole growing season against vagaries of weather.

Total anthocyanins: Total Anthocyanins are presented in figure 8. Maximum anthocyanins were recorded in peels of fruit No. 10 (7.29 mg C3GL-1) and minimum in peel of fruit No. 2 (2.62 mg C3GL-1) whereas in juice contents of fruit No. 10 possessed maximum (2.56 mg C3GL-1) and minimum in fruit No. 1 (1.73 mg C3GL-1).

Total anthocyanins showed more significant increasing trend in peel from green color (fruit No. 1) to red colored (fruit No.10) whereas slight differences in color change in juice content. Maximum total anthocyanins contents were reported in matured fruit (Arakawa *et al.*, 1985) as showed in fully red

colored fruit (No.10) in present findings and more in color fruits like oranges (Lee, 2002) than mandarin. More pigmentation in peel is due to more anthocyanins and increased in coloration of peel (Rapisarda et al., 2009). In citrus more anthocyanins are found in peel than juice with rising trend upon reaching at maturity. More anthocyanins were found in peel of red than green color fruits. Citrus fruit has relatively less anthocyanins in juice and also less in immature fruit juice. Anthocyanins enrich fruit peel with red and yellow color and more found in fruit red or orange or

yellow outer skin. They are categorized in polyphenols mostly accumulated in fruit peel, leaves and flowers petals (Winkel-Shirley, 2001). In citrus, more anthocyanins exist in blood oranges whereas mandarin peels accrue higher contents at full red or yellow color stage. In Kinnow mandarin, maximum anthocyanins were found in full color stage than immature green fruit. It is found mostly in peel pigments and changes fruit color development to produce yellow or red colored fruit juice and peel.

Figure 7: Total flavonols contents in peel and juice of fruit color (1-10).

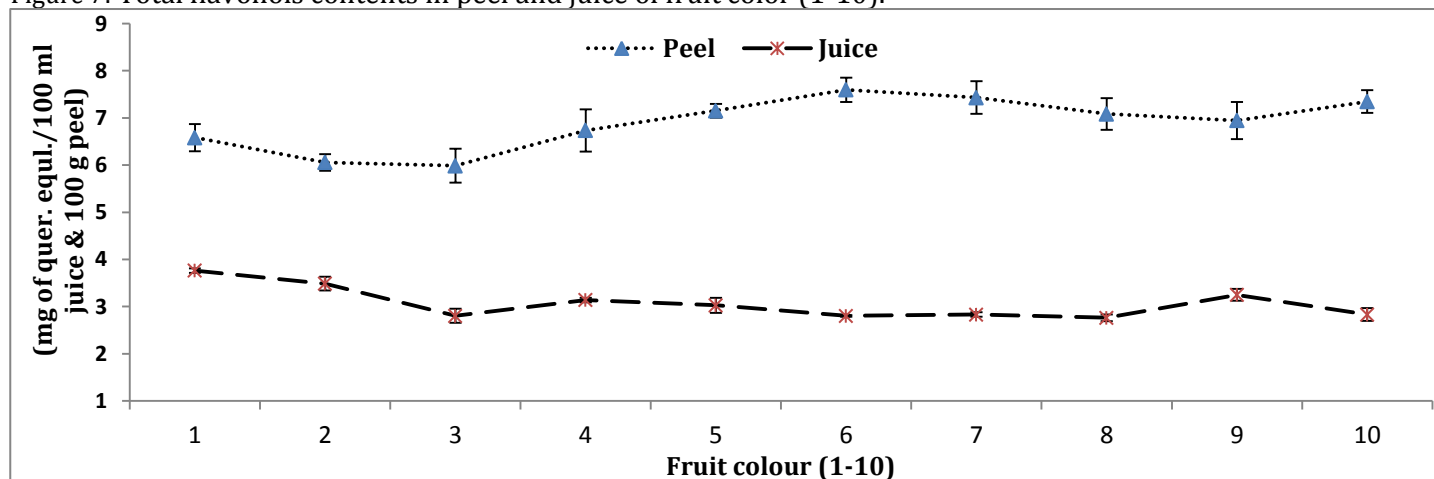
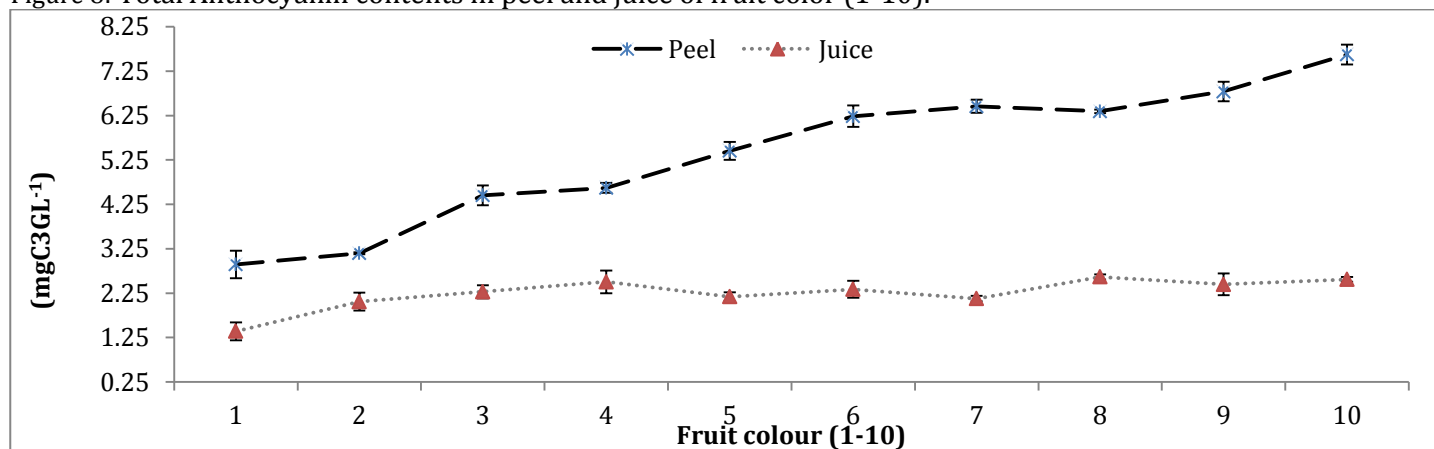


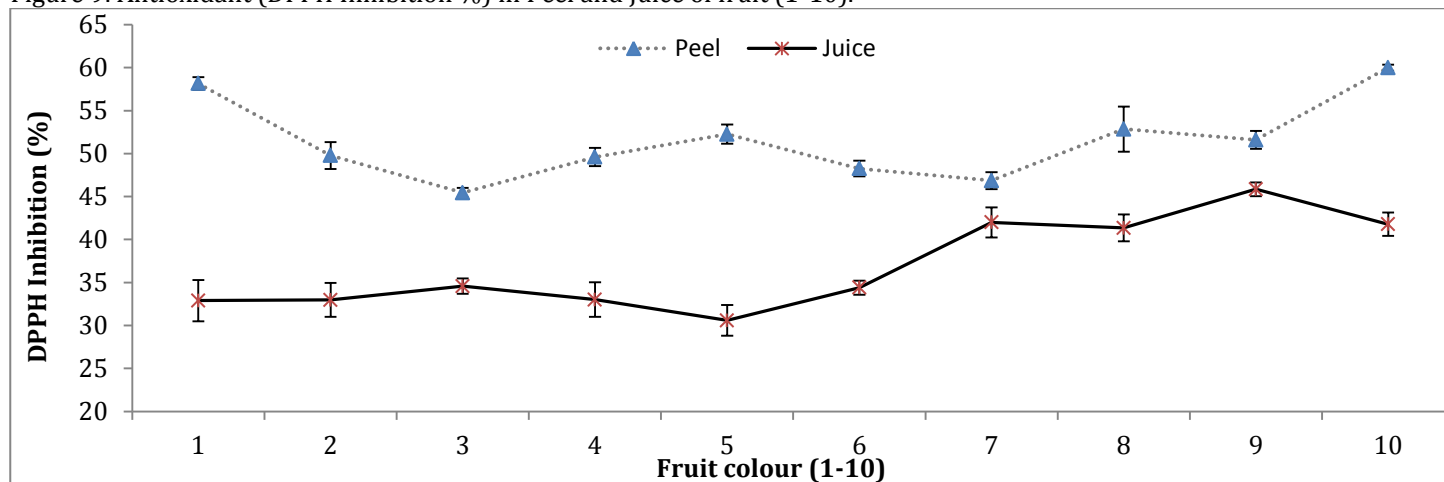
Figure 8: Total Anthocyanin contents in peel and juice of fruit color (1-10).



Antioxidant activities: Antioxidant activities are presented in figure 9. Antioxidant activity (DPPH inhibition or scavenging %) was recorded maximum in fruit No. 10 (60%) and found minimum in fruit No. 3 (45%) whereas, in juice maximum inhibition percentage was recorded in fruit No.9 (46%) and minimum in fruit No.5 (31%). Antioxidant has the capability to prevent or reduce oxidation process. Antioxidant activity (DPPH inhibition %) showed decreasing trend in peel while increasing in juice of Kinnow mandarin upon achieving red color in fruit (1-10). Antioxidant activity and polyphenols showed variation at maturing stages in citrus fruits (Webb et al., 2007) while in present study at same time of early maturation, fruits

possessed variability in scavenging or inhibition of oxidation on peel color basis. Polyphenols have positive correlation with antioxidants and possessed variation in coloration stages (Ferguson et al., 1999) and citrus fruits showed variability in green to red colored Kinnow mandarin. Reduction in ascorbic acid during color change as seen in fruit (1-10) has not influenced on antioxidant due to unchanged in polyphenols contents and more anthocyanins. Chlorophylls are antioxidants (Hasanuzzaman et al., 2013) and more found in green color fruits to reinforce present work with more antioxidant in green fruit peel. These findings were in line with results of Gardner et al. (2000), Zvaigzne et al. (2009) and Rekha et al. (2012).

Figure 9: Antioxidant (DPPH Inhibition %) in Peel and Juice of fruit (1-10).



CONCLUSION

Although, citrus peel color is used for maturity indicators, but it not depicts fruit internal quality. Color-based fruit assessments showed more sugars with sweetness in tasting point of view, but not for fruit acidity and its nutritional values. Fruit nutritional values in Kinnow mandarin were not changed in green to red colored fruits at early maturation. Green to light green colored fruits are equally valuable for consumers whose preference is based on acidity and nutritional values. Present findings will be useful for early picking of Kinnow fruits either for domestic consumption, or for exporting them to markets where more acidity and nutrition based consumers' acceptance is found.

ACKNOWLEDGEMENT

HEC Pakistan for awarding indigenous PhD fellowship and Agriculture Department, Punjab Government on granting study leaves for PhD degree.

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Date Published (D-M-Y): 15-04-2019