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Original article

Agro-climatic conditions affect fruit quality of mandarin (*Citrus reticulata* Blanco) cultivars

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Summary

Introduction – **Citrus occupies a prominent position in the world fruit industry. Quality of citrus fruits is affected by various biotic and abiotic factors. Among these, climatic factors may have some significant influence that this study investigated.** *Materials and methods* – **The fruits of two mandarin cultivars ('Kinnow' and 'Feutrell's Early') grown under different agro-climatic conditions of the Province Punjab, Pakistan were evaluated for their physico-chemical characteristics. The experiment was conducted in five locations (Multan, Sahiwal, Faisalabad, Sargodha and Chakwal), at a distance of 100–150 km of each other. Fruit quality assessment included physical and biochemical attributes.** *Results and discussion* – **The physico-chemical characteristics of fruits presented significant differences between the two cultivars. Both mandarin cultivars gave the maximum average fruit weight, diameter, peel thickness, number of seeds per fruit, seed and rag contents at Chakwal, whereas fruits harvested at Sahiwal showed higher peel thickness as compared with those from the other locations. The maximum juice content was obtained in the fruit from Faisalabad and Sargodha. 'Kinnow' fruit attained the maximum total soluble solids (TSS) at Sargodha, followed by those from Faisalabad and Chakwal. Moreover, less acidity was recorded in 'Kinnow' fruit harvested from Sargodha. The maximum sugar content was recorded in 'Kinnow' fruit harvested from Faisalabad, followed by those from Chakwal. 'Feutrell's Early' mandarin juice from Chakwal showed higher vitamin C content. Total phenolic content (TPC) varied among cultivars, the maximum TPC of 'Kinnow' and 'Feutrell's Early' were found in the fruits from Sahiwal and Multan, respectively.** *Conclusion* – **The mandarin cultivars produced in the agro-climatic conditions from Faisalabad to Sargodha expressed a high fruit quality, so that this area can be recommended for a commercial production.**

Keywords

Pakistan, Punjab, mandarin, *Citrus* spp., fruit quality, physico-chemical characteristics, phenolics

Résumé

Les conditions agro-climatiques affectent la qualité des fruits du mandarinier (*Citrus reticulata* Blanco).

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Significance of this study

What is already known on this subject?

- Agro-climatic conditions influence the different quality parameters of fruit. Mandarin (*Citrus reticulata* Blanco) cultivars have major commercial importance in the citrus industry of Pakistan. Different citrus cultivars are grown on limited/specific areas of country as a commercial fruit crop.

What are the new findings?

- In terms of fruits physico-chemical characteristics, the mandarin cultivars performed better in the agro-climatic conditions from Faisalabad to Sargodha, so that this area is recommended for their commercial production.

What is the expected impact on horticulture?

- A bigger window of better quality fruit production for fresh and processed citrus industry is expected in Pakistan from the plantation of commercial citrus groves in explored potential areas in the future. Overall, potential citrus growing areas provide a base for expansion of citrus industry in national and international fruit markets.

Introduction – **Les agrumes occupent une place de choix dans l'industrie mondiale du fruit. La qualité des agrumes est affectée par divers facteurs biotiques et abiotiques. Parmi ces derniers, les facteurs climatiques doivent avoir une influence notable que cette étude a permis d'apprécier.** *Matériel et méthodes* – **Les fruits de deux cultivars de mandarinier ('Kinnow' et 'Feutrell's Early') cultivés sous différentes conditions agro-climatiques de la province du Penjab, au Pakistan, ont été évalués pour leurs caractéristiques physico-chimiques. L'expérimentation a été menée dans cinq sites (Multan, Sahiwal, Faisalabad, Sargodha et Chakwal), à une distance de 100 à 150 km les uns des autres. L'évaluation de la qualité des fruits comprenait des attributs physiques et biochimiques.** *Résultats et discussion* – **Les caractéristiques physico-chimiques des fruits ont présenté des différences significatives entre les deux cultivars. Les deux cultivars ont présenté des moyennes maximales du poids d'un fruit, du diamètre, de l'épaisseur de la peau, du nombre de graines par fruit, et de teneur en pépin et en mésocarpe dans les fruits produits sur le site de Chakwal,**

alors que ceux récoltés à Sahiwal ont présenté une épaisseur de la peau plus élevée par rapport aux fruits des autres sites. La teneur maximale en jus a été obtenue pour les fruits de Faisalabad et Sargodha. Les fruits du cv. Kinnow ont été les plus riches en matières solubles totales (TSS) en provenance de Sargodha, suivi de ceux issus de Faisalabad et de Chakwal. Une moindre acidité a été enregistrée dans les fruits de 'Kinnow' récoltés à Sargodha. La teneur maximale en sucre a été enregistrée pour les fruits de 'Kinnow' récoltés à Faisalabad, suivis par ceux cultivés à Chakwal. Le jus des mandarines 'Feutrell's Early' cultivées à Chakwal avait une teneur en vitamine C élevée. La teneur en composés phénoliques totaux (TPC) variait entre les cultivars, le maximum pour 'Kinnow' et 'Feutrell's Early' ayant été trouvé dans les fruits de Sahiwal et Multan, respectivement. *Conclusion* **– Les cultivars de mandarinier produits dans les conditions agro-climatiques entre Faisalabad et Sargodha ont exprimé une qualité élevée des fruits, de sorte que cette zone peut être recommandée pour une production commerciale.**

Mots-clés
Pakistan, Penjab, mandarinier, agrumes, qualité du fruit, caractères physico-chimiques, composés phénoliques

Introduction

The genus *Citrus* belongs to the family Rutaceae, which encompasses several commercially important species including mandarin (*C. reticulata*), sweet orange (*C. sinensis*), lemon (*C. limonia*), grape fruit (*C. paradisi*), tangors (*C. reticulata* × *C. sinensis*) and tangelos (*C. reticulata* × *C. paradisi*) (Gmitter *et al*., 2008). Citrus fruits are one of the important horticultural crops, grown all over the world in more than 140 countries (Liu *et al*., 2012). In Pakistan, citrus fruit ranks first in area (193,669 ha) and production (2.17 Mt year^{-1}) among all the fruits produced. These are grown in all the four provinces and Punjab alone produces approximately 97% of the crop due to more suitable agro-climatic conditions (Anonymous, 2015). Citrus fruit is famous for its refreshing smell, pleasant flavor and consumed either as fresh or processed (Nagy and Attaway, 1980). It is well established that citrus and their byproducts are rich source of vitamin C, folic acid, minerals and dietary fiber (Gorinstein *et al*., 2001; Bermejo *et al*., 2011). Citrus fruit contains phytochemicals such as phenolics, limonoids, flavonoids, carotenoids and volatile compounds (Dhuique-Mayer *et al*., 2009; Hashempour *et al*., 2013). Moreover, carbohydrates, organic acids and sugars are among the major compounds which determine fruit organoleptic quality characteristics (Tucker, 1993). These biological compounds have beneficial role in maintaining human health due to their antioxidant properties, help in blood regulation, and act as anti-allergic and anti-carcinogenic agents (Liu *et al*., 2012). Citrus leaf, flower and fruit during developmental stages exhibit distinctive flavor and volatile compounds in which monoterpene hydrocarbons (70 to 95%) are the major class (Ahmad *et al*., 2006; Azam *et al*., 2013a, 2013b).

It has been previously studied that soil, climatic, cultural and environmental factors affect the performances of different cultivars in term of plant growth, yield, and physico-chemical characteristic of citrus fruits (Hafiz *et al*., 1997; Ishfaq *et al*., 1999, 2007). Several factors influence fruit quality, such as rootstock (Ramin and Alirezanezhad, 2005; Hussain *et al*., 2013), cultivar (Cano and Bermejo, 2011), fruit maturity stage (Rekha *et al*., 2012), genetic factors (Dhuique-Mayer *et al*., 2009), climatic factors (Clements, 1964; Marsh *et al*., 1999) and cultural practices (Lee and Kader, 2000). Among climatic factors temperature is the crucial one that contributes mostly to growth, yield, internal and external fruit quality in profitable citrus producing areas (Richardson *et al*., 1997; Hutton and Landsberg, 2000).

Few studies reported on the physico-chemical characters of different cultivars of sweet orange and grape fruit in only one location in Pakistan (Ishfaq *et al*., 1999, 2007; Rehman *et al*., 1983). There is a lack of knowledge regarding the evaluation of citrus cultivars in the different agro-climatic conditions of the country. We recently began a study for the potential exploitability of the mandarin (*Citrus reticulata* Blanco) cvs. Kinnow and Feutrell's Early, which are of major commercial importance in the citrus industry of Pakistan (Hussain *et al*., 2013). The objective of the present study was to explore an area/region having more favorable climatic conditions for potential yield and quality of promising mandarin cultivars rather than the traditional growing area.

Materials and methods

Collection of fruit samples

To study the effect of different agro-climatic conditions on physico-chemical characteristics of two mandarin (*Citrus reticulata* Blanco) cvs. Kinnow and Feutrell's Early, five locations were selected. Starting from Multan, the locations were chosen every 100–150 km distance, toward an increasing altitude from sea level (Table 1). These included Multan, Sahiwal, Faisalabad, Sargodha and Chakwal in Punjab, Pakistan.

The fruits samples were collected from healthy citrus trees growing in the orchards of research institutes at the selected locations for two successive cropping seasons during 2012–2013 and 2013–2014. Twelve year-old trees, set apart (7 × 7 m), grafted on rough lemon (*C. jambheri* L.) and grown under standard cultural practices were selected. The plants were fertilized with their recommended dose (NPK at 1500:750:500 g plant^{-1} along with farm yard manure 50 kg plant^{-1}) during the study period of 2012 to 2014. Soil samples were collected randomly from selected orchards of studied locations at depth of 30 cm. Samples were labeled location-wise and sent to the crop nutrition diagnostic laboratory, Engro Fertilizers Ltd., Multan, for analysis. Soil sample analyses were performed according to the method described by Ryan *et al.* (2001). Soil status of citrus groves in each studied location is presented in Table 2. The experiment was laid out in randomized complete block design (RCBD) with four replications. Meteorological data of the selected locations were collected from Pakistan Meteorological Department (Figure 1). Fruits of each cultivar were randomly harvested at the ripe stage on the basis of peel color development. 'Feutrell's Early' was harvested on 15th December, whereas, 'Kinnow' was harvested on 15th January during both study years. Quality analysis was carried out at the Postgraduate Laboratory, Department of Horticulture, Bahauddin Zakariya University, Multan (Pakistan).

Table 1. Eco-meteorological distribution data of five locations over the study period (2012–2014). Source: Pakistan Meteorological Department.

Locations	Altitudes (m)	Latitudes and longitudes	Mean maximum temperatures (°C)	Mean minimum temperatures (°C)	Annual rainfalls (mm)	Average humidity (%)
Multan	125	30°15'N; 71°36'E	32.09	20.06	278.45	55.85
Sahiwal	150	30°45'N; 73°80'E	31.57	19.31	382.71	58.13
Faisalabad	184	31°30'N; 73°05'E	31.04	18.56	486.97	60.40
Sargodha	190	32°10'N; 72°40'E	30.64	18.88	829.38	60.04
Chakwal	498	32°56'N; 72°53'E	28.14	14.75	664.00	57.80

Table 2. Soil status of citrus groves at the five study locations in Punjab, Pakistan. SAR: Sodium Adsorption Ratio. Source: Crop Nutrition Diagnostic Laboratory, Engro Fertilizers Ltd., Multan (Pakistan).

Locations	Soil depth (cm)	Texture	Saturation (%)	pH	SAR	Electrical conductivity (ds m^{-1})	Total N (%)	Available P (ppm)	Available K (ppm)
Multan	30	Loam	42	7.9	0.6	1.82	0.048	8	153
Sahiwal	30	Loam	35	8.2	6.0	1.67	0.038	9	250
Faisalabad	30	Loam	35	7.8	1.2	2.52	0.050	23	288
Sargodha	30	Loam	35	7.8	1.1	1.60	0.028	8	288
Chakwal	30	Loam	35	8.1	4.8	1.33	0.067	16	325

Physical parameters

Sixty fruits of each cultivar were randomly collected from selected trees of each selected orchard at studied locations, and were divided in four replications, each consisted of 15 fruits. The total fruit, peel, seed, juice and rag weight from each fruit per cultivar were measured by using an electronic weighing balance (WT6002D, WANT Balance Instrument Co. Ltd., China) and their averages were calculated. The total fruit weight was recorded in g, while peel, seed, juice and rag contents were evaluated after cutting the fruit in half without damaging the seeds and extracting the juice with a hand extractor. These were expressed as percentage of total fruit weight. After juice extraction, it was divided into two halves, one for quality parameters [total soluble solids (TSS), titratable acidity (TA) and total sugars (TS)], and the other was immediately stored at -80 °C for the remaining biochemical analysis (vitamin C, antioxidant activity and total phenolic content).

Fruit diameter and peel thickness

The fruit diameter and peel thickness (in mm) of each fruit for both cultivars were measured separately by using a digital vernier caliper.

Number of seeds per fruit

The total number of seeds extracted from fruits of each cultivar was counted and then average number of seeds per fruit was calculated by dividing the total number of seeds by total number of fruits.

Total soluble solids

Total soluble solids (TSS, in °Brix) of the juice samples was determined with a hand refractometer (MASTER-53S, Atago, Japan) at 20 °C by taking the translucent part of the juice after decantation as described in the Official Methods of Analysis, AOAC (AOAC, 2000). Five observations were made in each replication separately and their mean was computed.

Titratable acidity

Juice samples were subjected to the determination of titratable acid content (TA, in %), following the 0.1 N NaOH-based titration method (Hortwitz, 1960), using a citric acid coefficient of 0.064. Values were expressed in percentage citric acid content of juice. Five individual samples of each replication were analyzed and average was calculated.

Ripening index

The ripening index (RI) was calculated by dividing the values of TSS (°Brix) with TA (%) of citrus juice samples as described by Hardy and Sanderson (2010):

$$RI = \frac{TSS}{TA}$$

Total sugars

The total sugar contents in juice samples were determined by the titration based Fehling's solution method, previously reported by Hortwitz (1960). The amount of the total sugars was expressed as percentage of the juice.

Biochemical parameters

Stored juice samples were defreezed at room temperature and immediately used for following biochemical analysis.

Vitamin C

For the measurement of vitamin C content of the fruit juice, the indophenol's titration method was used as described by Ruck (1963). A homogenized extracted juice sample of 10 mL was taken in 100 mL volumetric flask and final volume was made by adding 0.4% oxalic acid solution. A filtered aliquot of 5 mL was taken, titrated against freshly prepared 2,6-dichlorophenolindophenol dye to light pink color end point (persisted at least for 15 s). Vitamin C (ascorbic acid) content was computed as mg 100 mL^{-1} juice.

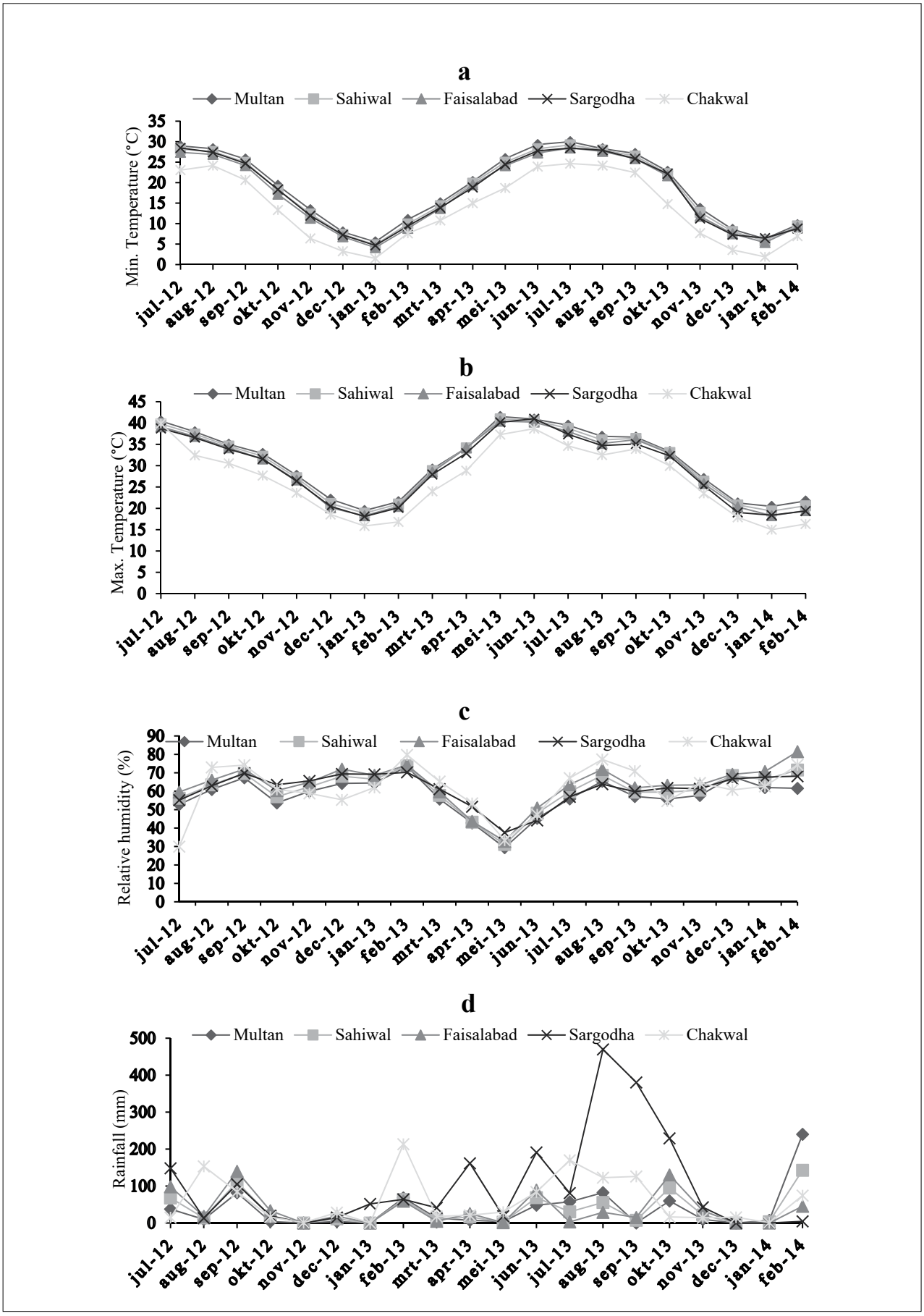

FIGURE 1. Climatic data at the five study locations from July 2012 to February 2014: (a) Minimum temperatures; (b) Maximum temperatures; (c) Relative humidity; (d) Rainfall (Source: Pakistan Meteorological Department).

Antioxidant profile of the fruit juice

1. Sample extraction. The DPPH radicals scavenging activity was measured by making slight changes in the method of Shimada *et al.* (1992). Antioxidant components in citrus juice were extracted with methanol. Homogenized extracted juice samples of 20 and 50 mL methanol were taken in a conical flask and shaken in a water bath with an automatic shaker at room temperature for 2 h. The methanol extracts were concentrated on a rotary evaporator, adjusted to a volume of 10 mL with methanol and then analyzed for antioxidant components and activity.

2. Antioxidant activity through DPPH radicals scavenging activity. Methanolic extract and 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution, 1 mL each, were mixed. The absorbance was measured in a UV-VIS spectrophotometer (UV-3000, ORI Germany) after 4 min of initial mixing at 571 nm and the percentage of DPPH scavenging activity was determined as follows:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{(Ao - A1)}{Ao} \times 100$$

where A_o = Absorbance of control sample and A_1 = Absorbance of juice sample. Pure methanol was used as blank while control sample contained pure methanol + 0.2 mM DPPH solution.

3. Antioxidant capacity. Antioxidant capacity was measured by Trolox assay as described by Arts *et al.* (2004). Trolox standard graph was plotted against different concentrations (5–30 µM). Then samples absorbance readings were compared with trolox standard series absorbance values by drawing standard curve of Trolox. The Trolox equivalent antioxidant capacity (TEAC) of the samples was expressed as millimolar Trolox equivalent per 100 mL of fruit juice (mM Trolox 100 mL^{-1}).

Total phenolic content

The total phenolic content was measured by using Folin-Ciocalteu reagent with the slight modification in the protocol of Gorinstein *et al.* (2001). Juice samples were centrifuged for 15 min at 4 °C at 10,000 rpm. The supernatant was separated and stored at -20 °C till further analysis.

The centrifuged juice sample of 0.2 mL was added into a test tube, then 1 mL of 5-fold diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3 solution were added and mixed with vortex. The solution was diluted to a total volume of 10 mL by adding distilled water, followed by thorough mixing and left for incubation at room temperature for 30 min. Then, the absorbance was read at 765 nm in a UV-VIS spectrophotometer (UV-3000, ORI Germany); distilled water was used as a blank and gallic acid as the standard. The total phenolic content in the samples were expressed as microgram gallic acid equivalent per milliliter of fruit juice (µg GAE mL^{-1}).

Statistical analysis

Experiment was conducted for two consecutive years however, no significant effect of years was observed for studied parameters. Therefore, the data for both years were pooled and analyzed statistically, based on four replications using Statistix 8.1 analytical software (Tallahassee, Fl., USA). Means were compared by using Least Significant Difference (LSD) test at 5% probability level. The treatment means showing significant differences at $P \leq 0.05$ were separated by using small alphabets.

Results and discussion

Physical and biochemical characters are very important quality parameters in citrus fruits that directly affect the fruits qualitatively and quantitatively (Guardiola and García-Luis, 2000). Variations were observed in physical and biochemical parameters between fruits of two studied cultivars ('Kinnow' and 'Feutrell's Early'). These variations might be due to genetic factors (Dhuique-Mayer *et al.*, 2009; Arts *et al.*, 2004). Besides genetic variations, environmental factors like temperature, rainfall and sunlight also affect physico-chemical attributes of citrus fruits (Ishfaq *et al.*, 1999; Chelong and Sdoodee, 2013).

Physical characteristics

'Kinnow' had greater fruit weight in all the locations as compared to 'Feutrell's Early', probably due to genetic factors (Table 1). The maximum fruit weight was recorded for 'Kinnow' from Multan, followed by 'Kinnow' from Chakwal and Sahiwal. These three treatment combinations were statistically at par. The minimum fruit weight was obtained in 'Feutrell's Early' from Faisalabad that significantly differed from all other treatment combinations. These results are in line with the findings of several researchers who reported that 'Kinnow' is best suited to a broader range of ecological regions in Pakistan (Ahmed *et al.*, 2007; Altaf *et al.*, 2008), so giving higher fruit weight in comparison with 'Feutrell's Early' (Nawaz *et al.*, 2012). 'Kinnow' gains more fruit weight in moderately warm environment (Chaudhary, 2009) what explains that it reached highest fruit weight in Multan, where the climate is relatively hot. According to the data of studied locations obtained from Pakistan Meteorological Department, Multan exhibits higher mean temperatures throughout the studied period as compared to other locations (Figure 1a,b).

Table 3. Physical analysis of the fruit of two mandarin cultivars (KN: 'Kinnow'; FE: 'Feutrell's Early') grown in five locations in Punjab, Pakistan. Data are mean values* over 2 years ($n = 4$).

Locations	Fruit weight (g)			Fruit diameter (mm)			Fruit peel thickness (mm)			Number of seeds per fruit		
	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*
Multan	186.05 a	106.64 e	*146.35 a*	74.67 a	63.13 d	*68.90 a*	3.25 b	3.52 a	*3.39 c*	20.49	13.33	*16.91 bc*
Sahiwal	170.57 ab	110.83 de	*140.70 ab*	73.44 a	63.96 cd	*68.70 a*	3.61 a	3.55 a	*3.58 a*	24.82	14.29	*19.55 b*
Faisalabad	143.47 c	76.48 f	*109.98 c*	67.42 b	53.58 e	*60.50 c*	3.28 b	3.61 a	*3.45 bc*	19.54	13.00	*16.33 c*
Sargodha	154.69 bc	111.22 de	*133.34 b*	64.86 cd	63.29 d	*64.07 b*	3.31 b	3.61 a	*3.46 bc*	23.54	15.71	*19.44 b*
Chakwal	179.39 a	123.65 d	*151.23 a*	73.97 a	65.90 bc	*69.94 a*	3.52 a	3.58 a	*3.55 ab*	27.08	20.60	*23.78 a*
Means	*166.83 a*	*105.77 b*		*70.87 a*	*61.97 b*		*3.40 b*	*3.58 a*		*23.09 a*	*15.31 b*	

* Means sharing similar letter(s) in a group are statistically non-significant at $P \leq 0.05$ (LSD test).

Table 4. Fruit component analysis of two mandarin cultivars (KN: 'Kinnow'; FE: 'Feutrell's Early') grown in five locations in Punjab, Pakistan. Data are mean values* over 2 years ($n = 4$).

Locations	Peel content (%)			Seed content (%)			Juice content (%)			Rag content (%)		
	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*
Multan	31.54	30.47	*31.00 c*	1.39 f	1.59 ef	*1.49 d*	40.81 c	35.92 d	*38.37 ab*	26.23 def	32.34 b	*29.29 b*
Sahiwal	34.61	36.05	*35.33 a*	1.46 ef	1.76 de	*1.61 d*	37.71 d	33.38 ef	*35.54 c*	26.15 ef	28.85 cd	*27.50 b*
Faisalabad	30.22	32.15	*31.26 c*	1.72 ef	2.05 cd	*1.89 c*	43.56 ab	36.07 d	*39.82 a*	24.50 f	24.09 f	*24.30 c*
Sargodha	32.57	34.28	*33.25 b*	2.30 c	2.10 c	*2.20 b*	43.89 a	35.43 de	*39.66 a*	21.26 g	28.52 cde	*24.89 c*
Chakwal	26.87	28.51	*27.79 d*	2.72 b	3.68 a	*3.20 a*	41.31 bc	32.04 f	*36.67 bc*	29.14 c	35.58 a	*32.36 a*
Means	*31.16 b*	*32.29 a*		*1.92 b*	*2.24 a*		*41.46 a*	*34.57 b*		*25.46 b*	*29.88 a*	

* Mean sharing similar letter(s) in a group are statistically non-significant at $P \leq 0.05$ (LSD test).

'Kinnow' had also greater fruit diameter than 'Feutrell's Early' (Table 3). This distinction was probably due to some genetic factors and their interaction with prevailing climatic conditions. 'Kinnow' fruit attained the maximum diameter in Multan, then in Chakwal and Sahiwal and the minimum was observed for 'Feutrell's Early' in Faisalabad. Goldschmidt (1997) reported a positive relationship of temperature with the fruit expansion and concluded that under high temperatures, prevailing in the tropics fruit development is fast and the fruit gets very large. In subtropics, fruit growth stops during cool winter months and resumes again at a low rate during spring, but the final size of fruit is considerably smaller than that attained in the tropics.

The fruit of both mandarin cultivars significantly differed in their peel thickness (Table 3). 'Feutrell's Early' had relatively thicker fruit peel than 'Kinnow' over the two experimental years. The 'Feutrell's Early' fruit harvested from all five locations attained the maximum peel thickness which was statistically at par with that of 'Kinnow' from Sahiwal and Chakwal. In general, fruit peel is affected by the rootstock used, plant nutrition especially P and K and prevailing climatic factors (Khan *et al.*, 2011). Cohen *et al.* (1972) reported that low average minimum winter temperatures resulted in thick-peeled grape fruit. In the present study, mandarin cultivars attained the maximum peel thickness and peel weight in Chakwal and Sahiwal locations, probably due to the prevailing low winter temperatures. According to the meteorological data, Sahiwal and Chakwal have a relatively extended period of low winter temperatures (Figure 1a,b).

The number of seeds per fruit was significantly higher in 'Kinnow' than in 'Feutrell's Early'. The highest number of seeds (23.78) was counted in the fruits from Chakwal and the lowest in those from Faisalabad (16.33) and Multan (16.91). The number of seeds per mandarin fruit has been reported to be in direct correlation with fruit size, fruit weight and peel thickness: the more seeds per fruit, the greater size, weight and peel thickness (Ketsa, 1988). However, such a correlation was not drawn in the present study, what might be due to the cultivar genetic specific character (Goldenberg *et al.*, 2014). The formation of healthy and mature seeds depends upon growing environment. Variations among cultivars occur possibly due to any difference in their genetic makeup as seed weight per fruit varies due to genotypic differences and is also influenced by prevailing climate. A cool and humid climate favors the formation of healthy and thick seeds. Therefore, Chakwal and Sargodha, having relatively cool and humid climate at the time of seed formation, exhibited more seeds in the mandarin fruits.

The peel content was significantly higher in the 'Feutrell's Early' fruits than in the 'Kinnow' (Table 4). 'Feutrell's Early' fruits may have a relatively higher peel content due to smaller size. The maximum peel content was recorded in fruits harvested from Sahiwal, followed by those from Sargodha. The minimum peel content was gained in fruits harvested from Chakwal, followed by those from Multan. However, no significant interaction between genotype and location was observed.

Average juice content of 'Kinnow' and 'Feutrell's Early' fruits significantly differed according to the cropping site (Table 4). 'Kinnow' fruits had more juice content as compared to 'Feutrell's Early' ones, this character being highly sensitive to the genetic makeup. Hafiz *et al.* (1997) also found a higher juice content in 'Kinnow' than in 'Feutrell's Early' fruits. 'Kinnow' contained the highest fruit juice content when produced in Sargodha, followed by Faisalabad and Chakwal production sites. However, 'Feutrell's Early' presented the least fruit juice content when harvested from Chakwal, preceded by Sahiwal. Our results correlate with previous findings (Ishfaq *et al.*, 1999, 2007) concluding that mandarin fruit produced in a cooler agro-climate yields more juice when compared with fruit produced in a relatively warmer agro-climate. 'Feutrell's Early' and 'Kinnow' fruits yielded the

Table 5. Quality analysis of the fruit of two mandarin cultivars (KN: 'Kinnow'; FE: 'Feutrell's Early') grown in five locations in Punjab, Pakistan. Data are mean values* over 2 years ($n = 4$).

Locations	TSS (°Brix)			TA (%)			Ripening Index			Total Sugar (%)		
	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Means*
Multan	11.09 b	9.89 d	*10.49 b*	1.36 c	0.76 f	*1.06 a*	8.42 ef	13.25 d	*10.83 c*	7.73 c	6.86 e	7.29 d
Sahiwal	10.36 c	9.31 e	*9.84 c*	1.47 b	0.47 h	*0.97 b*	7.67 f	19.63 b	*13.65 b*	7.59 cd	7.23 de	7.41 cd
Faisalabad	11.43 ab	9.78 d	*10.60 ab*	1.62 a	0.51 h	*1.06 a*	7.24 f	19.82 b	*13.53 b*	8.80 a	6.92 e	7.86 b
Sargodha	11.51 a	9.27 e	*10.39 b*	0.86 e	0.42 i	*0.64 d*	13.73 d	22.51 a	*18.12 a*	7.81 c	7.50 cd	7.66 bc
Chakwal	11.19 ab	10.40 c	*10.80 a*	1.27 d	0.58 g	*0.92 c*	9.42 e	18.24 c	*13.83 b*	8.70 ab	8.29 b	8.50 a
Means	*11.12 a*	*9.73 b*		*1.31 a*	*0.55 b*		*9.30 b*	*18.69 a*		8.13 a	7.36 b	

* Means sharing similar letter(s) in a group are statistically non-significant at $P \leq 0.05$ (LSD test).

maximum rag content when cropped in Chakwal (Table 4). The maximum rag content was recorded in 'Feutrell's Early' fruits from Chakwal while the lowest rag content was recorded in 'Feutrell's Early' fruits from Faisalabad and in 'Kinnow' fruits from Faisalabad and Sahiwal. These three treatment combinations stood statistically at par with each other.

Fruit composition, especially TSS and TA, is influenced by prevailing environmental factors such as temperature and rainfall at different locations. TSS and TA contents were significantly higher in the 'Kinnow' fruits than in the 'Feutrell's Early' ones (Table 5). 'Kinnow' fruits have the maximum TSS at Sargodha, followed by Faisalabad and Chakwal. However, the minimum TSS was recorded at Sahiwal, preceded by Sargodha which were statistically similar. As for concern to TA, the maximum acidity was recorded in 'Kinnow' fruits harvested from Faisalabad while 'Feutrell's Early' fruits exhibited low acidity at Sargodha. Davies and Albrigo (1994) reported that high average temperatures during the maturation phase results in a decrease in TSS and increase in acids. According to the meteorological data, during the studied period low average temperature prevailed at Chakwal and Sargodha comparative to remaining locations (Table 1; Figure 1) which favoured the synthesis of more total soluble solids and low accumulation of acid content in fruits. In the present study, 'Kinnow' fruits had the highest TSS and titratable acidity which correlates with the findings of Nawaz *et al.* (2012) who recorded the maximum amount in 'Kinnow' as compared to 'Feutrell's Early' fruits.

Location had a significant effect on the ripening index (RI) of both the mandarin cultivars (Table 5). 'Feutrell's Early' fruits produced in Sargodha exhibited greater RI, whereas the lowest RI was expressed by 'Kinnow' fruits from Faisalabad, preceded by those from Sahiwal and Multan. According to the climatic data, Chakwal and Sargodha have low temperature ranges as compared to the other studied locations. These observations are in agreement with the finding of Ishfaq *et al.* (1999), who observed that a cooler climate favored high ripening index.

Sugar content is an important component of the mandarin fruit, that correlates with sweetness, and is a basic ingredient of fruit quality (aroma, texture and flavor). Significant differences between the cultivars were observed in the total sugars which ranged from 6.86% to 8.80% of their fruit juice (Table 5). 'Kinnow' remained at the top in term of total sugar content of the fruit juice in comparison with 'Feutrell's early'. 'Kinnow' fruits from Faisalabad, followed by those from Chakwal, had the highest sugar contents, while 'Feutrell's Early' fruits produced in Multan, preceded by those from Faisalabad and Sahiwal, yielded the least sugar contents. The prevailing temperature and rainfall distribution over the growing areas probably affects the growth and composition of mandarin fruits especially during the later stage of the fruit development. A restricted supply of water during the latest stage of fruit growth enhances soluble solid as well as sugar accumulation. Similar observations have been reported on apple (Kilili *et al.*, 1996) and cactus-pear (Felker *et al.*, 2002).

Biochemical attributes of the fruit juice

'Feutrell's Early' fruits had higher vitamin C content compared with those of 'Kinnow' in all production sites (Table 6). Among the locations, Chakwal produced the 'Feutrell's Early' fruits with the highest vitamin C content. The lowest vitamin C contents were recorded in the fruits of 'Feutrell's Early' from Faisalabad, followed by those of 'Kinnow' from Sargodha. The vitamin C content in fruit juice is influenced by various factors such as genotype, pre-harvest climatic factors and maturity stage (Lee and Kader, 2000). Fluctuation in day temperature in the different localities might be responsible for the observed variability in vitamin C content. Fruits grown in low temperature areas are reported to have higher vitamin C content than those from warm localities (Ishfaq *et al.*, 2007). According to our data, 'Feutrell's Early' had greater vitamin C content in the fruit juice compared with 'Kinnow' when grown under the cool agro-climate of Chakwal. The variability in vitamin C content found in the present study could also be due to genetic makeup of the cultivars. Genotypic variations in vitamin content of different fruits were already reported (Stevens, 1974; Harris, 1975).

'Feutrell's Early' expressed a higher DPPH radical scavenging activity (RSA) of the fruit juice than 'Kinnow'. Among the locations, Faisalabad produced fruit with significantly lower RSA of the juice than any other location. However, no significant cultivar × location interaction was observed (Table 6). Vitamin C has a direct correlation with the antioxidant potential (Rekha *et al.*, 2012; Gardner *et al.*, 2000; Tsai *et al.*, 2007). The high vitamin C content in 'Feutrell's Early' fruit juice should be responsible for the high RSA measured. The total phenolics should have also contributed to the antioxidant potential of mandarin fruits (Hashempour *et al.*, 2013; Ghafar *et al.*, 2010). The antioxidant capacity (TEAC) of the fruit juice was affected by the cultivar or the location; however, it was not significantly affected by their interaction (Table 6). 'Feutrell's Early' showed higher TEAC than 'Kinnow', whereas fruits from Faisalabad had the least TEAC.

'Kinnow' had significantly greater total phenolic content of the fruit juice than 'Feutrell's Early' (Table 6). The variation in antioxidant activities and total phenolic contents may be due to genotypic variations as previously reported in citrus (Bocco *et al.*, 1998). 'Feutrell's Early' expressed a max-

Table 6. Biochemical analysis of the fruit of two mandarin cultivars (KN: 'Kinnow'; FE: 'Feutrell's Early') grown in five locations in Punjab, Pakistan. Data are mean values* over 2 years ($n = 4$).

Locations	Vitamin C content (mg 100 mL^{-1})			DPPH Radical scavenging activity (%)			Antioxidant capacity (mM Trolox 100 mL^{-1})			Total phenolic content (µg GAE mL^{-1})		
	KN	FE	*Means*	KN	FE	*Means*	KN	FE	*Mean*	KN	FE	*Means*
Multan	38.73 ef	55.13 b	46.93 b	74.13	78.45	76.29 a	15.34	16.35	15.84 a	336.35 a-d	344.81 ab	340.58
Sahiwal	42.31 cde	43.77 cd	43.04 cd	71.54	79.18	75.36 a	14.91	16.46	15.69 a	368.19 a	304.71 de	336.45
Faisalabad	45.02 c	36.72 ef	40.87 d	68.19	69.35	68.77 b	14.19	14.52	14.36 b	338.65 abc	307.41 cde	323.03
Sargodha	39.52 def	51.82 b	45.67 bc	75.22	74.47	74.85 a	15.64	15.54	15.59 a	357.98 a	283.25 e	320.61
Chakwal	43.00 cde	60.86 a	51.93 a	72.97	81.91	77.44 a	15.14	17.02	16.08 a	349.72 ab	321.23 bcd	337.00
Means	*41.72 b*	*49.66 a*		*72.41 b*	*76.67 a*		*15.05 b*	*15.98 a*		*350.18 a*	*312.28 b*	

* Means sharing similar letter(s) in a group are statistically non-significant at $P \leq 0.05$ (LSD test).

imum total phenolic content when grown in Multan, and a minimum in Sargodha. The highest content of phenolic compounds was recorded in 'Kinnow' fruits from Sahiwal, followed by those from Chakwal; the lowest content was found in 'Feutrell's Early' fruits from Sargodha, preceded by those from Sahiwal and Faisalabad.

Large variations occur among citrus cultivars in their response to climate especially regarding fruit growth and quality indicating a significant effect of the environment on these fruit characteristics (Davies and Albrigo, 1994). Climatic factors were mainly responsible for the year to year variation reported in the orange yield (Du Plessis, 1983). Various researchers have studied the effects of different production sites with variable climate on citrus fruits (Turnell *et al*., 1964; Sgroppo *et al*., 2012). According to Turnell *et al.* (1964), citrus species (oranges, lemons and grapefruits) are not equally adoptable to the climatic conditions of southern California. Washington Navel orange has been reported to produce poor fruit quality in coastal and desert areas because of peel thickness (in relation to fruit size) decreasing from coastal to desert areas (Davies and Albrigo, 1994). According to Zekri (2011), Navel oranges develop best external and internal fruit quality in the Mediterranean climate (cool and wet winters; hot and dry summers). In wet tropical climates Navel orange fruit contains low TSS and TA in the juice. However, Valencia orange performs well in a wide range of climates, producing acceptable to excellent fruit quality in most of the citrus growing areas in the world.

Conclusion

Our results clearly indicated the effects of agro-climate on physical and biochemical attributes of the mandarin fruit. Statistically significant differences were observed between the cultivars 'Kinnow' and 'Feutrell's Early', probably due to genotypic variations. Both mandarin cultivars gave the highest average values of fruit weight, diameter, peel thickness, rag content and seed number when produced in Chakwal, while more juicy fruits were produced in Faisalabad and Sargodha. 'Kinnow' fruits with the maximum total soluble solids and the minimum acidity were from Sargodha. Among biochemical properties, 'Feutrell's Early' showed the maximum vitamin C content when grown in Chakwal. The area between Faisalabad and Sargodha presents a suitable climate for high quality mandarins in term of physico-chemical traits, suggesting to explore it as a potential area for commercial production.

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