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Comparative analysis of citrus fruits for nutraceutical properties

Shams Ur REHMAN[1,2], Kashif Sarfraz ABBASI[2], Abdul QAYYUM[3*], Muhammad JAHANGIR[1], Asma SOHAIL[2], Sobia Nisa[4], Muhammad Naveed TAREEN[5], Muhammad Javed TAREEN[5], Peter SOPADE[6]

Abstract

Fruits and vegetables (F and V) are valuable for their micronutrients, but many F and V in diverse growth areas have not been scientifically validated for these components. The nutraceutical characteristics of the pulp of eight citrus fruits grown in Pakistan were, therefore, investigated. Expectedly, the fruits differed in moisture (86.9-88.9%), ash (0.37-0.53%), pH (2.9-5.8), total soluble solids (8.1-13.7 °Brix), titratable acidity (7.9-13.1%), total sugars (5.1-8.8%), reducing sugars (2.8-4.3%), and non-reducing sugars (0.3-0.5%) contents. Potassium, sodium and magnesium were the major minerals, while iron, zinc and manganese were present in minor fractions in the pulps. Phytochemical analysis (total phenolic content, 132-243 µg/g GAE; total flavonoids content, 4.2-12.1 µg/g QE; vitamin C, 36.3-62.3 mg/100 g) revealed antioxidant potential that was confirmed by a high 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical scavenging activity, showing a highly positive correlation ($r^2 > 0.921, 0.917, 0.807$ $p > 0.05$) with the measured antioxidant components (Total Phenolics, Total Flavonoids and vitamin C). Principal component analysis shows a significant relationship between the citrus varieties and their quality parameters to guide their industrial potential as sources of nutraceuticals.

Keywords: antioxidant potential; *Citrus* spp.; major minerals; principal component analysis.

Practical Application: Physiochemical attributes of citrus varieties.

1 Introduction

Citrus fruits (*Rutaceae*) are popularly grown all over the world and sub-divided into 78 species, with their distinctive and varied flavors (Texeira et al., 2005; Okwi & Emenike, 2006). Fruit characteristics are one of the important parameters used for the selection of best genotypes for further propagations (Paudyal & Haq, 2008), and total soluble solid is an important economic index, especially with frozen concentrates (Rouse, 2000). Citrus fruits are mostly considered as acid fruits, since their soluble solids are composed mainly of organic acids and sugars (Kelebek et al., 2009). The acid content of juices is an important quality and maturity index (Song et al., 2016), and as with other fruit characteristics, it depends on fruit varieties, cultural practices and climate, amongst others (Burdurlu et al., 2006). Citrus fruits are also important because of their constituents with antioxidant potential, which have been investigated with different *in-vitro* assays such as DPPH radical scavenging activity (Zou et al., 2016). There are also anticancer (e.g. taxol), chemotherapic, antiviral, and anti-inflammatory components, and other bioactive constituents, which make citrus fruits valuable ingredients in functional foods (Ismail et al., 2004; Xu et al., 2008).

The Rawalpindi region (Taxila valley) of Pakistan is well known for its citrus fruits with a viable local industry based on the produce (Siddique & Garnevska, 2017). The region produces many citrus varieties, and with environmental factors exercising well-known effects on quality and characteristics of agricultural produce, a study on the citrus varieties from the region is important to understand how the unique citrus varieties in the region, and how the well-known varieties compare with other from other regions or places. We are not aware of any detailed study along these lines, and the present study reports quality attributes of eight citrus varieties from the region that were chosen because of their perceived unique characteristics.

2 Materials and methods

2.1 Materials

Eight citrus varieties, *Citrus sinensis* cv (Hamlin, Red blood, Succuri), *Citrus limetta* (Mosambi), *Citrus reticulate* (Tangerine), *Citrus paradise* macfed (Grape fruit), *Citrus aurantium* and *Citrus jambhiri* lush, were collected from the Rawalpindi Region of Pakistan. The fruits were washed, sorted, graded, and stored at room temperature till further analysis. The fruits were pulped prior to analysis.

2.2 Chemical analysis

Total soluble solid (TSS as °Brix) was determined (Association of Official Analytical Chemists, 2005) using a digital refractometer PAL-3 (ATAGO, Japan) at 29 ± 1 °C with temperature corrections.

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[1] *Department of Food Science and Technology, The University of Haripur, Haripur, Pakistan*
[2] *Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan*
[3] *Department of Agronomy, The University of Haripur, Haripur, Pakistan*
[4] *Department of Microbiology, The University of Haripur, Haripur, Pakistan*
[5] *Agriculture Research Institute, Sariab, Quetta, Pakistan*
[6] *Department of Food Science and Engineering, Xichang University, Xichang, China*
*Corresponding author: aqayyum@uoh.edu.pk

pH (pH-meter, Inolab. WTW Series, Germany), titratable acidity (TA), total sugars (TS) and reducing sugars (RS) (Lane and Eynon titration method, Fehling's solutions), moisture content (Model: 605, Precision Oven, Thermo Fisher Scientific; 105 °C till constant weight), ash content (muffle furnace, lef-2055-0, Daihan Labtech, Korea, 550 °C), crude fiber (gravimetric enzymatic digestion procedure) were measured (Association of Official Analytical Chemists, 2005). The mineral content was determined (Association of Official Analytical Chemists, 2005) by digesting 5 g of the pulp with 10 mL of a nitric acid:perchloric acid (7:3) mixture at 180-200 °C till completion. The digest was made up to 100 mL with distilled water, and Mg, Fe, Zn, and Mn contents of the pulp were determined in an Atomic Absorption Spectrophotometer (GBC-932, Scientific Equipment Limited, Australia), whereas Na and K were measured by Flame Photometer (Model PFP 7, Jenway, England). Vitamin C was also determined (Association of Official Analytical Chemists, 2005).

2.3 Phytochemical analysis

Sample preparation

During the extraction of antioxidants from the citrus pulp samples, methanol was used to assess their extraction efficiency. The samples were subjected to orbital shaker for 7 hr followed by centrifugation (model: 800 electronic centrifuge, RENONLAB) 1342 g for 10 min. The supernatant was decanted, and the residue was re-dissolved in 10 mL of the methanol and centrifuged for 5 min., before combining both supernatants (extracts) for the following analyses (Rusak et al., 2008):

Total Phenolic Content (TPC)

One mL of the extract was oxidized with 2.5 mL of Folin-Ciocalteau's reagent (10%), followed by neutralization with 2 mL sodium carbonate (7.5%). The mixture was kept in the dark for 45 min., and the absorbance was measured at 765 nm wavelength using a spectrophotometer (UV-9200, Biotech Engineering Management Co., UK). Gallic acid (ug/g) was used as the standard (Anagnostopoulou et al., 2006).

Total Flavonoids Content (TFC)

One mL of the extract was mixed with 0.3 mL of sodium nitrite (5%). After an interval of 5 min., 0.6 mL of aluminum chloride (10%) was added and mixed. This was followed by adding 2 mL of 1 M sodium hydroxide after an interval of 5 min., and the absorbance was measured at 510 nm wavelength with the spectrophotometer. The total flavonoid was calculated using quercetin (ug/g) as a standard (Toh et al., 2013).

1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical scavenging assay

An equal volume of the extract was added to methanolic solution of DPPH (0.7 mM) and held for 30 min. at room temperature before the absorbance was measured at 517 nm using the spectrophotometer. The percent of radical scavenging activity was calculated as the ratio of the absorbance of the sample, A_{sample}, relative to the control, $A_{control}$ (Mishra et al., 2012). The control was the DPPH solution without the fruit extract, and the radical scavenging activity (%) was calculated as Equation 1:

$$\text{Radical scavenging activity } (\%) = 100 \times \left(A_{control} - A_{sample}\right) / A_{control} \quad (1)$$

2.4 Statistical analysis

Results were subjected to one-way analysis of variance (ANOVA). Statistical differences with P-values less than 0.05 were considered significant and means were compared by LSD test according to Steel et al. (1997). Principal component analysis was performed by using SIMCA-P software. All analyses on the pulps were triplicated.

3 Results and discussion

Knowledge about biochemical composition of citrus pulp in different varieties is very important for various purposes including measuring maturity indices (Rouse, 2000), chemical profiling of citrus fruits (Álvarez et al., 2014), its antioxidant potential (Ortuño et al., 1997), mineral profiling (Barros et al., 2012), conversions of citrus fruit to different products and its taste characterization.

3.1 Chemical constituents

Table 1 shows the results for the moisture, pH, TSS, TA, TS, RS, NRS, and crude fiber, and there were varietal differences. The moisture content of the pulps is within the range (85.8-87.9%)

Table 1. Chemical components of the citrus varieties#.

Variety	Moisture %	pH	TSS°	TA %	TS %	RS %	NRS %	CF %
CSH	87.7 ± 0.6bc	3.79 ± 0.06d	11.4 ± 0.2c	1.01 ± 0.05d	11.1 ± 0.4c	7.33 ± 0.2bc	3.71 ± 0.3bc	0.31 ± 0.005e
CSR	88.8 ± 0.4a	4.17 ± 0.03c	11.1 ± 0.3c	0.91 ± 0.03d	10.59 ± 0.4c	6.90 ± 0.2d	3.69 ± 0.2bc	0.31 ± 0.007e
CSS	86.1 ± 0.4e	5.85 ± 0.07a	13.6 ± 0.3a	0.23 ± 0.02f	13.1 ± 0.3a	8.76 ± 0.1a	4.31 ± 0.2a	0.41 ± 0.011b
CLM	87.5 ± 0.3cd	4.43 ± 0.05b	12.2 ± 0.4b	0.58 ± 0.02e	11.8 ± 0.2b	7.63 ± 0.2b	4.21 ± 0.1ab	0.41 ± 0.009b
CRT	88.4 ± 0.5ab	4.41 ± 0.04b	11.2 ± 0.4c	0.54 ± 0.02e	10.8 ± 0.6c	7.23 ± 0.1cd	3.57 ± 0.5c	0.33 ± 0.004d
CPM	87.1 ± 0.2cd	3.40 ± 0.05e	11.2 ± 0.3c	1.82 ± 0.07c	11.2 ± 0.2bc	7.16 ± 0.1cd	4.11 ± 0.1ab	0.37 ± 0.009c
CAT	86.9 ± 0.1d	2.96 ± 0.05g	8.1 ± 0.1d	4.46 ± 0.08a	7.85 ± 0.12d	5.06 ± 0.2e	2.78 ± 0.2d	0.26 ± 0.006f
CJL	87.1 ± 0.2cd	3.15 ± 0.03f	8.5 ± 0.2d	4.08 ± 0.12b	8.19 ± 0.17d	5.26 ± 0.1e	2.93 ± 0.2d	0.54 ± 0.007a

#Dissimilar letters within a column indicate significant differences (p≤0.05). Values are means of three measurements ± standard errors. TSS = total soluble solids; TA = titratable acidity; TS = total sugars; RS = reducing sugars; NRS = non-reducing sugars; CF = crude fibre; CSH = *Citrus sinensis* cv hamlin; CSR = *Citrus sinensis* cv red blood; CSS = *Citrus sinensis* cv succuri; CLM = *Citrus limetta* mosambi; CRT = *Citrus raticulata* tangerine; CPM = *Citrus paradise* macfed; CAT = *Citrus aurantium* L; CJL = *Citrus jambhiri* lush. These apply to all tables and figure where they appear.

reported by Barros et al. (2012) for citrus varieties, and moisture content of citrus fruits determines their freshness and keeping quality (Chien et al., 2007).The results on the other parameters of the pulps also agree with findings from previous studies (Álvarez et al., 2014; Barros et al., 2012), and genotype and environmental differences are not uncommon between and within agricultural produce (Petropoulos et al., 2018). The *Citrus sinensis* cv succuri had the highest TSS (13.6 °Brix), TS, (13.1%), RS (8.76%), NRS (4.31%) and pH (5.85), and it is generally the sweetest amongst the eight citrus varieties. On the other hand, the CAT (*Citrus aurantium* L) is generally the most bitter of the varieties, and this could be due to it having the lowest values in the parameters studied (TSS, 8.1 °Brix; TS, 7.85%; RS, 5.06%; NRS, 2.78%; pH, 2.96). The solid and sugar parameters are positively correlated ($r^2 > 0.88$; $p > 0.05$), and the pH and the TA are negatively correlated ($r^2 > -0.85$; $p > 0.05$), the pH is positively ($r^2 > 0.05$ 0.65; $p > 0.05$) and the TA is negatively ($r^2 > -0.82$; $p > 0.05$) correlated with the total soluble solids and total sugar parameters. Seymour et al. (2012) observed that high pH and TSS are indications of sweetness, while more tartness in citrus fruits manifests in high TA.

3.2 Antioxidant properties

Table 2 summarizes the antioxidant parameters of the citrus pulps that are variety dependent and are within published ranges (Burdurlu et al., 2006; Barros et al., 2012). The *Citrus sinensis* succuri had the highest values, while the *Citrus jambhiri* lush was the least in nearly all the parameters. Table 3 shows the correlations between these antioxidant parameters, and the highly significant correlations obtained, similar to those reported elsewhere (Xu et al., 2008), indicate how the antioxidant components are good predictors of antioxidant capacities, as expected.

3.3 Mineral components

Citrus fruits are highly nutritious owing to its significant mineral potential as Table 4 shows the major and trace minerals in the pulps, which are also variety dependent, and within the ranges of Barros et al. (2012). Potassium is the principal mineral in the pulps (103.9-172.9 mg/100 g), while sodium is relatively much lower (1.6-2.8 mg/100 g). The balance between these minerals in citrus plays an important role in balancing electrolytes in human cells (Ladaniya, 2008). The pulps contained more

Table 2. Antioxidant properties of the citrus varieties#.

Variety	DPPH %	TPC ug/g	TFC ug/g	Vit C mg/100 mL
CSH	64.8 ± 1.8ab	222.3 ± 3.6c	9.93 ± 0.46c	57.4 ± 1.8b
CSR	63.6 ± 1.7b	207.0 ± 4.8d	9.04 ± 0.17d	53.2 ± 1.3c
CSS	66.4 ± 1.2a	243.3 ± 1.8a	12.1 ± 0.56a	61.6 ± 1.5a
CLM	65.3 ± 2.6ab	234.6 ± 2.5b	10.9 ± 0.40b	62.3 ± 1.6a
CRT	60.1 ± 1.2c	180.6 ± 3.1e	7.67 ± 0.32e	49.6 ± 1.3d
CPM	58.6 ± 1.2c	165.6 ± 4.3f	6.15 ± 0.21f	38.9 ± 1.5f
CAT	58.6 ± 1.3c	158.9 ± 3.1g	5.10 ± 0.14g	36.3 ± 1.1f
CJL	55.3 ± 2.1d	132.6 ± 2.9h	4.18 ± 0.18h	43.9 ± 1.3e

#Dissimilar letters within a column indicate significant differences (p≤0.05). Values are means of three measurements ± standard errors. DPPH = 1, 1-diphenyl 1-2-picrylhydrazyl; TPC = total phenolic content; TFC = total flavonoids content; Vit C = vitamin C; CSH = *Citrus sinensis* cv hamlin; CSR = *Citrus sinensis* cv red blood; CSS = *Citrus sinensis* cv succuri; CLM = *Citrus limetta* mosambi; CRT = *Citrus raticulata* tangerine; CPM = *Citrus paradise* macfed; CAT = *Citrus aurantium* L; CJL = *Citrus jambhiri* lush.

Table 3. Correlation between the antioxidant properties of the citrus varieties.

	DPPH	TPC	TFC
TPC	0.921*		
TFC	0.917*	0.984**	
Vit C	0.807*	0.916*	0.890*

Significant at p < 0.05*; Significant at p < 0.001**. DPPH = 1, 1-diphenyl 1-2-picrylhydrazyl; TPC = total phenolic content; TFC = total flavonoids content; Vit C = vitamin C.

Table 4. Mineral components of the citrus varieties#.

Variety	Ash %	Na mg/100 g	K mg/100 g	Mg mg/100 g	Fe mg/100 g	Zn mg/100 g	Mn mg/100 g
CSH	0.42 ± 0.01bc	2.17 ± 0.05d	146 ± 7.6bc	9.96 ± 0.4bc	0.17 ± 0.005d	0.094 ± 0.003b	0.047 ± 0.003c
CSR	0.37 ± 0.01d	2.55 ± 0.09b	141 ± 3.2c	10.3 ± 0.1b	0.25 ± 0.005b	0.066 ± 0.003de	0.055 ± 0.002b
CSS	0.52 ± 0.01a	2.87 ± 0.04a	172 ± 4.2a	8.56 ± 0.5d	0.17 ± 0.003d	0.093 ± 0.004b	0.039 ± 0.003de
CLM	0.44 ± 0.02bc	1.69 ± 0.05f	103 ± 7.5e	4.93 ± 0.1f	0.11 ± 0.004g	0.066 ± 0.005de	0.035 ± 0.002ef
CRT	0.53 ± 0.02a	2.84 ± 0.06a	146 ± 6.1bc	9.66 ± 0.3c	0.15 ± 0.004e	0.069 ± 0.003cd	0.046 ± 0.002c
CPM	0.40 ± 0.02cd	2.41 ± 0.04c	139 ± 4.1c	7.78 ± 0.4e	0.18 ± 0.005c	0.073 ± 0.003c	0.041 ± 0.004d
CAT	0.43 ± 0.02bc	1.98 ± 0.04e	117 ± 4.4d	5.32 ± 0.1f	0.12 ± 0.004f	0.061 ± 0.003e	0.032 ± 0.003f
CJL	0.45 ± 0.03b	2.77 ± 0.06a	153 ± 4.3b	11.7 ± 0.2a	0.27 ± 0.005a	0.101 ± 0.004a	0.068 ± 0.006a

#Dissimilar letters within a column indicate significant differences (p≤0.05). Values are means of three measurements ± standard errors. Na = Sodium; K = Potassium; Mg = Magnesium; Fe = Iron; Zn = Zinc; Mn = Manganese; CSH = *Citrus sinensis* cv hamlin; CSR = *Citrus sinensis* cv red blood; CSS = *Citrus sinensis* cv succuri; CLM = *Citrus limetta* mosambi; CRT = *Citrus raticulata* tangerine; CPM = *Citrus paradise* macfed; CAT = *Citrus aurantium* L; CJL = *Citrus jambhiri* lush.

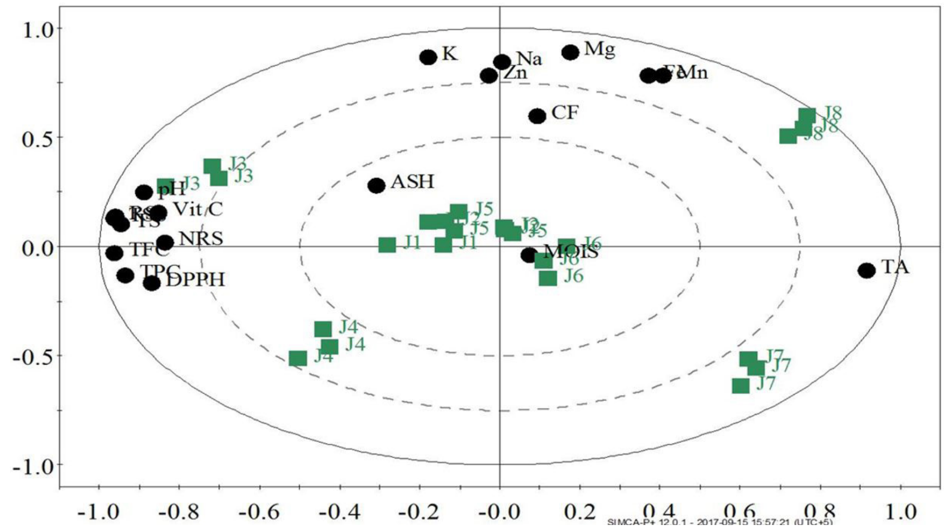

Figure 1. J1 = (CSH) *Citrus sinensis* Hamlin; J2 = (CSR) *Citrus sinensis* Red Blood; J3 = (CSS) *Citrus sinensis* Succuri; J4 = (CLM) *Citrus limetta* mosambi; J5 = (CRT) *Citrus reticulate* Tangerine; J6 = (CPM) *Citrus paradise* Macfed; J7 = (CAT) *Citrus aurantium*; J8 = (CJL) *Citrus jambhiri* Lush; DPPH = 1, 1-diphenyl 1-2-picrylhydrazyl; TPC = total phenolic content; TFC = total flavonoids content; Vit C = vitamin C; TS = total sugars; CF = crude fibre; MOIS = moisture; TA = titratable acidity; NRS = non-reducing sugars; Na = Sodium; K = Potassium; Mg = Magnesium; Fe = Iron; Zn = Zinc; Mn = Manganese.

magnesium (4.9-11.7 mg/100 g) than sodium. Antioxidant abilities depend on micronutrients, and some minerals are components of antioxidants enzymes, for example, superoxide dismutase depends on Mn and Zn, and catalase depends on Fe (Evans & Halliwell, 2001). Hence, quantifying these minerals present a holistic view of the antioxidant potential of these citrus varieties.

3.4 Multivariate analysis

Data obtained from citrus varieties based on chemical, elemental and antioxidant components were analyzed by the principal component analysis (Figure 1). Multivariate data analysis brings the data together and distinguishes the varieties and treatments into groups. The PCA showed the *Citrus sinensis* cv succuri (J3) with high amounts of the antioxidant and chemical components, except its low TA, while the *Citrus aurantium* L (J7) and *Citrus jambhiri* lush (J8) showed the opposite trends. The *Citrus paradise* macfed (J6) gave the most dilute pulps (high moisture), and the crude fibre and mineral values were segregated with slightly close association with the *Citrus jambhiri* lush (J8).

4 Conclusions

From the eight citrus varieties of the Rawalpindi district of Pakistan, differences were measured in the chemical, antioxidant and mineral components. The *Citrus sinensis* cv succuri was effectively the best in the tested components. The *Citrus jambhiri* lush had the highest contents of magnesium, iron, zinc, and manganese. The study revealed functional potential of citrus fruits to be used in different nutraceutical product development. Varietal characterization would also be helpful in the development of targeted commercial products. In addition, correlations in the studied quality attributes will offer better understanding of post-harvest physiology of citrus fruits.

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