Distribution of minerals between orange juice and orange flesh in various cultivars

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Abstract: The aim of this study was to evaluate the distribution of essential and toxic elements between the orange juice and orange flesh of various orange cultivars. Different orange cultivars, such as Abousorah [*Citrus sinensis* (L.)], Aseear (*Citrus aurantium*), Afandi (*Citrus reticulata* Blanco), Helo (*Citrus sinensis*), and grapefruit (*Citrus paradisi*), were collected from local markets. Elemental analysis was carried out after microwave-assisted digestion using inductively coupled plasma mass spectrometry (ICP-MS) in 18 samples. Eleven elements (V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, and Pb) were analysed. Their average concentrations ranged from 0.006 μ g g⁻¹ to 7.13 μ g g⁻¹ in both orange juice and flesh (wet weight). There was a high increase in the average concentrations of all elements in the juice compared to the flesh of up to 40-fold.

Keywords: citrus; elements; essential; ICP-MS; orange extract; pulp; toxic

Fruits, mainly citruses, are very popular in many different parts of the world for their flavour and many other health benefits associated with them, such as the presence of bioactive compounds (Al-Juhaimi and Ghafoor 2013). There is a great need for the consumption of natural products, such as fruit juices, as they present a vital source of minerals and vitamins; in particular, orange juice is rich in vitamin C and has many beneficial health effects for the human body. For example, orange juice can increase the clearance of uric acid, and it possesses anti-diabetic effects as it contains antioxidant and anti-inflammatory properties (Huang et al. 2005; Aschoff et al. 2019).

Oranges are a source of both essential and toxic elements. The toxic elements in oranges are As, Cd, Pb, Al, U, and B, while the essential elements are Se,

Mn, Fe, Cu, Ni, Co, Cr, V, Li, P, Sr, Mg, K, Na, and Ca. The latter are important for human health, while toxic elements are harmful and/or undesirable in excessive amounts. In oranges, each of these toxic and essential elements is found in trace amounts (μ g L⁻¹) or major amounts (μ g L⁻¹) (Foster et al. 2006).

Essential elements are crucial components of oranges. Some studies have investigated the presence of essential elements in oranges using different methods. For example, in a study conducted in Malaysia on the most edible species of orange, using an inductively coupled plasma mass spectrometry (ICP-MS) method, the results revealed that the maximum levels of heavy metals in orange tissue were 4.81 $\mu g \, g^{-1}$ for Cr, 0.49 $\mu g \, g^{-1}$ for Mn, 8.3 $\mu g \, g^{-1}$ for Fe, 0.33 $\mu g \, g^{-1}$ for Ni, 66.07 $\mu g \, g^{-1}$ for Zn, 2.55 $\mu g \, g^{-1}$ for As, and 0.77 $\mu g \, g^{-1}$

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for Pb. The study showed that the levels of some elements were slightly higher than the maximum permissible limits set by the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) and by Malaysian food regulation. The maximum permissible levels (MPL) ($\mu g g^{-1}$) for the elements Cr (1.2), Mn (10), Fe (0.8), Ni (0.3), Zn (100), As (1), Cd (1), and Pb (2) were reported in the study of Ateshan et al. (2019).

A recent study (Dehelean and Dana 2013) was conducted on orange juice in Romania, finding that the levels of heavy metal mineral content in orange juice - the concentrations of Co, Cu, Zn, As, and Cd - were below the acceptable limits, except for the values of Ni and Pb, which exceeded the limits (Ni = $100 \mu g L^{-1}$ and $70 \,\mu g \, L^{-1}$, Pb = 15 $\mu g \, L^{-1}$ and 10 $\mu g \, L^{-1}$) imposed by the EPA (2009) and WHO Guidelines for Drinking Water Quality (2008), respectively. Also, another study in Jeddah, Saudi Arabia, was conducted to assess the levels of trace elements in oranges, finding that the mean concentration values of Zn, Fe, Cu, Mn, Co, Cr, and Ni in orange juice were 0.895, 0.361, 0.500, 0.021, 0.008, 0.006, and $0.006 \,\mu g \, g^{-1}$, respectively. The study concluded that the levels of toxic elements corresponded with the recommended dietary allowance (RDA) for different countries, as available in the literature (Farid and Enani 2010). The RDA per day was reported in their study for the following elements: Zn (8 mg and 11 mg for females and males, respectively), Fe (8 mg), Cu (2-3 mg), Mn $(50-200 \mu g)$, Co $(40 \mu g)$, Cr (2.5-5 mg), and Ni (100-300 μg).

Furthermore, oranges can be exposed to contaminated trace metals that accumulate in soil and polluted water. Polluted irrigation water and soil are considered as prevailing sources of metal that can pose a risk to human health in regard to the consumption of oranges grown in polluted areas. Orange contamination by metals poses both non-carcinogenic and carcinogenic risks to the public (Islam et al. 2018). Therefore, it is important to determine the level of toxic elements in orange juice in comparison with international levels to protect people's health. In Saudi Arabia, there is a wide range of oranges that are imported from abroad due to the harsh climatic conditions and lack of freshwater for the production of crops (Chowdhury et al. 2019). As a result, the country depends on importing agricultural products from abroad, where agricultural imports reached an estimated quantity of 16.46 million t in 2012 (Chowdhury et al. 2019). In this setting, it is crucial to analyse essential elements such as K and Na, or toxic elements such as Cd and Pb, in fresh orange juice and compare these values with the global and local levels to ensure the quality of orange juice.

The aim of this study was to evaluate the distribution of elements between the juice and flesh of different orange cultivars. The second purpose was to investigate which part of an orange has a higher level of elements and to determine which cultivars are good sources of specific elements.

MATERIAL AND METHODS

Chemicals and reagents. Calibration standards of 5, 10, 20, 50, and 100 $\mu g \ L^{-1}$ were prepared by dilution with 1% HNO₃ from a single-stock solution mixture of 29 elements at a concentration of 10.0 \pm 0.05 $\mu g \ m L^{-1}$, obtained from ULTRA Scientific, USA. The internal standards were Sc (100 $\mu g \ L^{-1}$), Rh (20 $\mu g \ L^{-1}$), and Ge (20 $\mu g \ L^{-1}$), as prepared from the stock solution, using 1 000 $\mu g \ m L^{-1}$ (ULTRA Scientific, USA), 1 g L^{-1} (AppliChem, Germany), and 1 g L^{-1} (AppliChem, Germany), respectively.

Sample collection and pre-treatment. Five types of oranges, across nine samples, were collected from the cities of Khamis Mushait and Abha (Saudi Arabia), where oranges are commonly consumed. The types of orange samples used were the Abousorah or Navel orange [Citrus sinensis (L.)], Aseear (Citrus aurantium), Afandi or mandarin (Citrus reticulata Blanco), Helo (Citrus sinensis), and grapefruit (Citrus paradisi). The sources of these oranges were different. The Aseear, Helo, and Abousorah cultivars were obtained from Ethiopia, grapefruit was obtained from Turkey, and Afandi was obtained from Morocco. All samples were squeezed, and each orange was separated into juice and flesh. In total, 18 samples were pre-treated for measurement.

Digestion of all samples. All orange juice and flesh samples were digested with a microwave (Multiwave 3000 Microwave Sample Preparation System; Anton-Paar, Austria). Weights of about 0.5 g were taken for digestion for both, the orange juice and orange flesh. Then, 4 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. The conditions of the microwave were the use of power (1 000 W) for 5 min (increased in steps to reach 1 000 W) and then a further 40 min (held at 1 000 W). The temperature set during the microwave digestion was 125 °C, then cooling proceeded for 15 min. The digested solution was made up to 25 mL with deionised water.

Element analysis using inductively coupled plasma mass spectrometry (ICP-MS). In total, 11 elements

were measured in all of the collected orange samples in both, the flesh and juice. The concentrations of the 11 elements - namely, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Cd, and Pb – were measured in all 18 samples, with each sample divided into two parts, the juice and flesh $(2 \times 2 \text{ Afandi}, 2 \times 2 \text{ grapefruit}, 2 \times 2 \text{ Aseear}, 2 \times 2 \text{ Helo},$ and 1 × 2 Abousorah). These samples were measured by ICP-MS (iCAP Q; Thermo Fisher Scientific, USA) and analysed in triplicate (n = 3). The iCAP Q ICP--MS Thermo Scientific operating conditions were the same as reported in Brima (2017). The conditions were as follows: the power, cool gas flow, nebulizer gas flow, and cool gas flow were set to 1 550 W, 14.1 L min⁻¹, 0.94 L min⁻¹, and 0.79 L min⁻¹, respectively. The dwell time for each element was 0.01 s. The total time taken for each sample measurement was 3 min. The Q cell condition was He mode, and the peristaltic pump speed 40 rpm. The measured isotopes (m/z) for each element were: V (51), Cr (52), Mn (55), Co (59), Ni (60), Cu (63), Zn (66), As (75), Se (82), Cd (111), and Pb (208).

Quality control. The sensitivity of the ICP-MS was checked daily using tuning B solution (iCAP), which had a concentration of 1 μ g L⁻¹ each of Li, Co, and U, i.e. the same as reported in Brima (2017). For all measured elements, the limits of detection (LODs) and limits of quantification (LOQs) were calculated. A blank of 1% HNO₃ was measured ten times, and standard deviation (SD) was used for the calculations (LODs = 3 × SD and LOQs = 10 × SD). The LODs and LOQs, respectively, were calculated in μ g L⁻¹ as follows: V (0.032 and 0.106), Cr (0.024 and 0.080), Mn (0.087 and 0.289), Co (0.015 and 0.051), Ni (0.282 and 0.939), Cu (0.145 and 0.482), Zn (1.103 and 3.675), As (0.087 and 0.290), Se (0.776 and 2.586), Cd (0.047 and 0.157), and Pb (0.636 and 2.120).

The blank was treated the same as the samples $[(\mu g\ L^{-1} \times 0.025L) \times (0.5\ g)^{-1}]$. The LODs and LOQs, expressed in $\mu g\ g^{-1}$, for the real samples (flesh and juice) were calculated as follows: V (0.0016 and 0.0053), Cr (0.0012 and 0.004), Mn (0.0044 and 0.0145), Co (0.0008 and 0.0026), Ni (0.014 and 0.047), Cu (0.007 and 0.024), Zn (0.055 and 0.184), As (0.0044 and 0.015), Se (0.039 and 0.13), Cd (0.0024 and 0.0079), and Pb (0.032 and 0.11).

A quality control check was performed by measuring 50 μ g L⁻¹ of mixed elements after each set of five samples, which is known as continuous calibration verification (CCV). The recoveries (%) of the triplicate measurement of each element were as follows: V (95.9), Cr (93.1), Mn (99.0), Co (95.5), Ni (91.4), Cu (92.8), Zn (105), As (107), Se (117), Cd (103), and Pb (100).

Quality assurance. NIES CRM 10a Rice Flour-Unpolished, Low-Level Cadmium was analysed for quality assurance. The certified values ($\mu g g^{-1}$) and measured values ($\mu g g^{-1}$), respectively, were as follows: Mn (34.7 \pm 1.8 and 27.1 \pm 0.6), Cu (3.5 \pm 0.3 and 3.5 \pm 0.2), Zn (25.2 \pm 0.8 and 24.0 \pm 0.9), Ni (0.19 \pm 0.03 and 0.25 \pm 0.01), and Cd (0.023 \pm 0.003 and 0.029 \pm 0.026).

SRM 1570a trace elements in spinach leaves were also analysed, and the certified values (μg g⁻¹) and measured values (μg g⁻¹), respectively, were as follows: V (0.57 \pm 0.03 and 0.5 \pm 0.01), Mn (75.9 \pm 1.9 and 61.0 \pm 0.2), Co (0.39 \pm 0.05 and 0.36 \pm 0.02), Ni (2.14 \pm \pm 0.10 and 1.5 \pm 0.04), Cu (12.2 \pm 0.6 and 10.3 \pm 0.5), Zn (82.0 \pm 3.0 and 69.0 \pm 0.11), As (0.068 \pm 0.012 and 0.095 \pm 0.02), and Cd (2.89 \pm 0.07 and 2.64 \pm 0.1).

A 50 μ g L⁻¹ mixed standard was spiked in a sample (grapefruit) and the obtained recoveries (%) were as follows: V (101.2 \pm 3.8), Cr (98.1 \pm 5.0), Mn (97.6 \pm 6.6), Co (100 \pm 3.5), Ni (100 \pm 5.1), Cu (98.6 \pm 1.4), Zn (88.4 \pm 4.2), As (113.9 \pm 13.0), Se (114.7 \pm 13.0), Cd (98 \pm 6.0), and Pb (75.1 \pm 4.7).

Statistical analysis. ANOVA (IBM SPSS Statistics 28.0.0.0) was carried out, finding a 95% confidence level when evaluating the differences in the concentrations of elements between the orange juice and flesh. The correlation was also assessed for all measured elements in all orange parts.

RESULTS AND DISCUSSION

Results. The concentrations of all elements in the juice were found to be higher than those in the flesh for all of the considered orange cultivars (Table 1).

The ratios of concentration between the juice and flesh ranged from 9-fold to 40-fold for elements (Cr, Mn, Co, and Cu) detected in both the juice and flesh. The Aseear cultivar had the highest concentrations compared to the other orange types for the following elements: Cu, Co, and Mn in the flesh and Cr, Mn, Co, Ni, and Zn in the juice. The Helo cultivar had the lowest concentrations in the flesh as compared to the other orange types, which was the case with Cr, Mn, Co, and Cu. However, Afandi in juice had the lowest concentrations compared to the other orange types for V, Cr, Mn, Co, Ni, and Zn. In general, the increasing order of the element concentrations can be expressed as follows in the flesh: Co < Cu < Cr < Mn. Abousorah flesh was the highest in Cr and Co and lowest in Cu. The seven other elements (As, Se, Cd, Pb, Zn, Ni, V) presented levels lower than the LOD and were therefore not used in further evaluation. The increasing or-

Table 1. Average concentrations (µg g⁻¹) of 11 measured elements in the flesh and juice of five different types of oranges [each sample was analysed in triplicate and a sample duplicate was used for digestion (mean \pm SD, n=6)]

< LDQ	Flesh	Afandi	Grapefruit	Aseear	Helo	Abousorah*	Mean	SD	P-value
0.0576 ± 0.04 0.0574 ± 0.03 0.0549 ± 0.0004 0.0424 ± 0.03 0.0683 ± 0.001 - LOQ - LOQ - CLOQ - CLO	>	< LOQ	<	< LOQ	< LOQ	< LOQ	ı	ı	1
0.0725±0.02 0.0610±0.01 0.1114±0.09 0.0386±0.02 0.0500±0.0001 < LOQ	Cr	0.0576 ± 0.04	0.0574 ± 0.03	0.0549 ± 0.0004	0.0424 ± 0.03	0.0683 ± 0.001	0.0561	0.0093	0.023
<ldq< td=""> <ldq< td=""> <ldq< td=""> 0.0046 ± 0.004 0.0034 ± 0.003 0.0094 ± 0.000 <ldq< td=""> <ldq< td=""></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<></ldq<>	Mn	0.0725 ± 0.02	0.0610 ± 0.01	0.1114 ± 0.09	0.0386 ± 0.02	0.0500 ± 0.0001	0.0667	0.0280	0.030
<loq< td=""> <td< td=""><td>°</td><td>< TOQ</td><td>< TOQ</td><td>0.0046 ± 0.004</td><td>0.0034 ± 0.003</td><td>0.0094 ± 0.0004</td><td>0.0058</td><td>0.0032</td><td>0.002</td></td<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<></loq<>	°	< TOQ	< TOQ	0.0046 ± 0.004	0.0034 ± 0.003	0.0094 ± 0.0004	0.0058	0.0032	0.002
0.0596 ± 0.01 0.0502 ± 0.002 0.0629 ± 0.01 0.0440 ± 0.02 0.0200 ± 0.001 < LOQ	ï	< TOQ	< LOQ	< TOQ	< TOQ	< TOQ	ı	I	I
< LOQ	Cu	0.0596 ± 0.01	0.0502 ± 0.002	0.0629 ± 0.01	0.0440 ± 0.02	0.0200 ± 0.001	0.0513	0.0098	0.034
< LOQ	Zn	< TOQ	< LOQ	< TOQ	< TOQ	< TOQ	I	I	I
< LOQ < LOQ < LOQ < LOQ < LOQ < LOQ	As	< TOQ	< LOQ	< TOQ	< TOQ	< TOQ	I	ı	I
< LOQ < LOQ <th< td=""><td>Se</td><td>< TOQ</td><td>< LOQ</td><td>< TOQ</td><td>< TOQ</td><td>< TOQ</td><td>I</td><td>ı</td><td>I</td></th<>	Se	< TOQ	< LOQ	< TOQ	< TOQ	< TOQ	I	ı	I
< LOQ < LOQ <th< td=""><td>Сд</td><td>< LOQ</td><td>< LOQ</td><td>< TOQ</td><td>< LOQ</td><td>< TOQ</td><td>I</td><td>I</td><td>I</td></th<>	Сд	< LOQ	< LOQ	< TOQ	< LOQ	< TOQ	I	I	I
Afandi Grapefruit Aseear Helo Abousorah* 0.01 ± 0.001 0.06 ± 0.02 0.05 ± 0.04 0.01 ± 0.01 0.05 ± 0.0002 0.20 ± 0.08 0.77 ± 0.05 1.39 ± 1.0 0.23 ± 0.18 0.75 ± 0.002 0.33 ± 0.09 4.57 ± 0.6 4.95 ± 4.47 0.40 ± 0.01 3.21 ± 0.01 0.02 ± 0.02 0.04 ± 0.005 0.05 ± 0.02 0.03 ± 0.02 0.02 ± 0.002 0.08 ± 0.02 0.87 ± 0.26 0.95 ± 0.71 0.12 ± 0.06 0.50 ± 0.0002 0.28 ± 0.02 1.80 ± 0.22 2.72 ± 0.84 0.27 ± 0.06 0.50 ± 0.002 0.78 ± 0.36 7.17 ± 2.93 14.88 ± 12.95 1.43 ± 1.01 11.37 ± 0.06 < LOQ	Pb	< LOQ	< TOQ	< LOQ	< LOQ	< LOQ	I	I	I
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Juice	Afandi	Grapefruit	Aseear	Helo	Abousorah*	Mean	SD	Concentrations of elements in juice/flesh (ratio)
$0.20 \pm 0.08 \qquad 0.77 \pm 0.05 \qquad 1.39 \pm 1.0 \qquad 0.23 \pm 0.18 \qquad 0.75 \pm 0.002$ $0.33 \pm 0.09 \qquad 4.57 \pm 0.6 \qquad 4.95 \pm 4.47 \qquad 0.40 \pm 0.18 \qquad 3.21 \pm 0.01$ $0.02 \pm 0.02 \qquad 0.04 \pm 0.005 \qquad 0.05 \pm 0.02 \qquad 0.03 \pm 0.02 \qquad 0.02 \pm 0.002$ $0.08 \pm 0.02 \qquad 0.87 \pm 0.26 \qquad 0.95 \pm 0.71 \qquad 0.12 \pm 0.06 \qquad 0.50 \pm 0.0002$ $0.28 \pm 0.02 \qquad 1.80 \pm 0.22 \qquad 2.72 \pm 0.84 \qquad 0.27 \pm 0.06 \qquad 3.56 \pm 0.03$ $0.78 \pm 0.36 \qquad 7.17 \pm 2.93 \qquad 14.88 \pm 12.95 \qquad 1.43 \pm 1.01 \qquad 11.37 \pm 0.06$ $< LOQ \qquad < LOQ \qquad 0.01 \pm 0.0002 \qquad 0.01 \pm 0.0004$	>	0.01 ± 0.001	0.06 ± 0.02	0.05 ± 0.04	0.01 ± 0.01	0.05 ± 0.0002	0.04	0.02	I
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C_{Γ}	0.20 ± 0.08	0.77 ± 0.05	1.39 ± 1.0	0.23 ± 0.18	0.75 ± 0.002	29.0	0.49	12
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Mn	0.33 ± 0.09	4.57 ± 0.6	4.95 ± 4.47	0.40 ± 0.01	3.21 ± 0.01	2.69	2.22	40
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Co	0.02 ± 0.02	0.04 ± 0.005	0.05 ± 0.02	0.03 ± 0.02	0.02 ± 0.0002	0.03	0.01	6
$0.28 \pm 0.02 \qquad 1.80 \pm 0.22 \qquad 2.72 \pm 0.84 \qquad 0.27 \pm 0.02 \qquad 3.56 \pm 0.03$ $0.78 \pm 0.36 \qquad 7.17 \pm 2.93 \qquad 14.88 \pm 12.95 \qquad 1.43 \pm 1.01 \qquad 11.37 \pm 0.06$ $< LOQ \qquad < LOQ \qquad 0.29 \pm 0.01$ $0.06 \pm 0.07 \qquad 0.01 \pm 0.0002 \qquad 0.01 \pm 0.004 \qquad 0.02 \pm 0.02 \qquad 0.01 \pm 0.0004$	ï	0.08 ± 0.02	0.87 ± 0.26	0.95 ± 0.71	0.12 ± 0.06	0.50 ± 0.0002	0.50	0.41	I
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Cu	0.28 ± 0.02	1.80 ± 0.22	2.72 ± 0.84	0.27 ± 0.02	3.56 ± 0.03	1.72	1.46	34
< LOQ $<$ LOQ <	Zn	0.78 ± 0.36	7.17 ± 2.93	14.88 ± 12.95	1.43 ± 1.01	11.37 ± 0.06	7.13	6.14	I
< LOQ < LOQ < LOQ < LOQ 0.29 \pm 0.01 0.06 \pm 0.07 0.01 \pm 0.0002 0.01 \pm 0.004 0.02 \pm 0.02 0.01 \pm 0.0004	As	< LOQ	< TOQ	< LOQ	< TOQ	< LOQ	1	1	I
0.01 ± 0.0002 0.01 ± 0.004 0.02 ± 0.02 0.01 ± 0.0004	Se	< LOQ	< FOQ	< TOQ	< LOQ	0.29 ± 0.01	ı	ı	I
1000 · 1000 · 1000 · 1000 · 1000	Сд	0.06 ± 0.07	0.01 ± 0.0002	0.01 ± 0.004	0.02 ± 0.02	0.01 ± 0.0004	0.02	0.022	I
0.10 ± 0.004 0.25 ± 0.004 0.28 ± 0.01 0.21 ± 0.001	Pb	0.29 ± 0.16	0.10 ± 0.004	0.25 ± 0.004	0.28 ± 0.01	0.21 ± 0.001	0.23	0.077	ı

*Only one sample was used; SD – standard deviation; LOQ – limits of quantification

der of the element concentrations in the juice was Cd < Co < V < Pb < Ni < Cr < Cu < Mn < Zn. A similar trend was found in the flesh and juice for the elements' concentrations, with Mn at the top of the list and Co at the bottom of the list. Aseear juice had the highest concentrations of five essential elements: Cr, Mn, Co, Ni, and Zn. Abousorah juice, meanwhile, reported only two of the highest elements (Cu and Se), and grapefruit juice only one, with the highest level of V. Afandi juice had the highest levels of two toxic elements (Cd and Pb). It was noteworthy that As was not detected in all cultivars in both the flesh and juice and that Abousorah juice was the only cultivar that reported Se among all of the studied cultivars.

Table 1 shows that, in juice, Abousorah (Cu and Se) and Aseear (Cr, Mn, and Zn) were the best sources of essential elements, and Afandi was a considerable source of toxic elements (Cd and Pb).

The concentration ratios (juice/flesh) of the elements, in decreasing order, were as follows: Mn > Cu > Cr > Co. The ratios range from 2.13 to 89, with the highest ratios for the measured elements in the different orange types as follows: grapefruit (Mn), Aseear (Co and Cr), and Abousorah (Cu), as shown in Figure 1. There were significant differences (P < 0.05) between the concentrations of V, Co, As, and Pb in the flesh and juice. However, no significant differences ($P \ge 0.05$) were reported for Cr, Mn, and Cu.

Significant (P < 0.05) positive correlations were reported among the measured elements in the flesh and juice of five different types of oranges. A significant (P < 0.05) positive correlation was reported for the five different types of oranges, relating to the concentra-

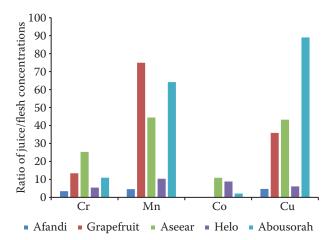


Figure 1. The juice/flesh concentration ratios of four elements (detected in both flesh and juice) in five cultivars of orange (Afandi, grapefruit, Aseear, Helo, and Abousorah)

tions of detected and measured elements in both, the flesh and juice (Table 2).

Discussion. Different studies have investigated the concentrations of trace elements in oranges. A recent study (Ateshan et al. 2019) showed high levels of heavy metals in orange tissues, of 4.81 μ g g⁻¹ for Cr, 0.49 μ g g⁻¹ for Mn, 8.3 μ g g⁻¹ for Fe, 0.33 μ g g⁻¹ for Ni, 66.07 μ g g⁻¹ for Zn, 2.55 μ g g⁻¹ for As, and 0.77 μ g g⁻¹ for Pb. Their results were higher than our results for Cr and As, while their Mn results showed values lower than those in our results. However, the same study showed that the mean concentrations of Cr, Fe, and As were 1.52 \pm 2.26, 2.09 \pm 4.14, and 1.68 \pm 0.95 μ g g⁻¹, slightly higher than the maximum permissible limits set by JECFA and Malaysian food regulation (Cr, Fe, and As measured 1.2, 0.8, and 1.0 μ g g⁻¹, respectively).

Another study conducted in Egypt on orange juice revealed that the level of Pb $(0.05 \,\mu g \,g^{-1})$ was lower than the MPL of 0.066 $\mu g g^{-1}$ (Hassan et al. 2014), indicating that most Pb levels in orange juice are lower than the allowed limits. Their study also showed that the concentration ranges for Cu and Zn in orange juices were $1.24-4.31 \text{ } \mu \text{g } \text{g}^{-1} \text{ and } 0.32-1.62 \text{ } \mu \text{g } \text{g}^{-1}, \text{ respectively.}$ The MPLs were reported in their study for Co, Pb, Fe, Cu, and Zn to be 0.05, 0.05, 5, 5, and 5, respectively. The findings of their study agree with our study results, as we found low levels of Pb, Cu, and Zn in our orange juice samples. Beyond this, a study in Jeddah, Saudi Arabia was conducted to assess the levels of trace elements in oranges, finding that the mean concentration values of Zn, Fe, Cu, Mn, Co, Cr, and Ni in orange juice were 0.895, 0.361, 0.500, 0.021, 0.008, 0.006, and $0.006 \mu g g^{-1}$, respectively. The study concluded that the levels of toxic elements corresponded with RDA levels for different countries, as specified in the available literature (Islam et al. 2018). Food safety guideline (FSG) limits were reported by Ahmed et al. (2016), for Zn and Mn, to be 30 μg g $^{-1}$ and 1 μg g $^{-1}$ (FAO 1983), then 30 µg g⁻¹ for Cu [WHO Environmental Health Criteria No. 165: Inorganic Lead (1995)], and were $12-13 \,\mu g \, g^{-1}$ and $80 \,\mu g \, g^{-1}$ for Cr and Ni [U.S. Food and Drug Administration (USFDA): Guidance Document for Arsenic in Shellfish (1993)]. The concentrations of different elements, Cu (0.8 μ g g⁻¹), Mn (0.5 μ g g⁻¹), Cs (0.09 $\mu g g^{-1}$), Fe (27.4 $\mu g g^{-1}$), Mg (25 $\mu g g^{-1}$), and Si $(8 \mu g g^{-1})$, were reported in a study (Turra et al. 2017) carried out in Sao Paulo, Brazil, on orange juice. Their results for Cu and Mn were lower than our results. The variations of the results between our study and theirs could be due to different environmental and soil effects. Also, a study in Kaani, Bori, a river state in Ni-

Table 2. Correlations between elements (Cr, Mn, Co, and Cu)^a within different orange cultivars (Afandi, grapefruit, Aseear, Helo and Abousorah)

	Afandi	Grape	Aseear	Helo	Abousorah
Juice					
Afandi	1.000	0.899**	0.936**	0.972**	0.937**
Grapefruit	0.899**	1.000	0.960**	0.913**	0.938**
Aseear	0.936**	0.960**	1.000	0.980**	0.988**
Helo	0.972**	0.913**	0.980**	1.000	0.969**
Abousorah	0.937**	0.938**	0.988**	0.969**	1.000
Flesh					
Afandi	1.000	0.988*	0.920	0.950*	0.833
Grapefruit	0.988*	1.000	0.871	0.964*	0.908
Aseear	0.920	0.871	1.000	0.754	0.642
Helo	0.950*	0.964*	0.754	1.000	0.867
Abousorah	0.833	0.908	0.642	0.867	1.000

^{*, **}Correlation is significant at the 0.05 and 0.01 level, respectively (2-tailed); ^aonly four elements detected and measured in both juice and flesh

geria, revealed that the concentrations of Fe, Pb, Ni, and Mn in oranges exceeded the permissible limits of 0.8, 0.20, 0.14, and 0.30 μ g g⁻¹, respectively (Ihesinachi and Eresiva 2014). The study showed that all metal concentrations in the oranges were low, except for Co and Pb, which had the highest concentrations. The high values found may be attributable to the prevalent environmental pollution from refineries and gas flares in the area (Ihesinachi and Eresiva 2014).

A recent study (Czech et al. 2020) was performed to compare the mineral content of peel and pulp from various cultivars. The reported range concentrations of Zn, Mn, and Cu in the pulp of eight different citrus cultivars (orange, pomelo, mandarin, lemon, key lime, red grapefruit, green grapefruit, and white grapefruit) were 1.7–2.4, 0.1–0.2, and 0.3–0.6 $\mu g g^{-1}$, respectively. Our study shows a similar trend in the flesh for the concentrations of Mn (0.07 $\mu g g^{-1}$) and Cu (0.05 $\mu g g^{-1}$) for all orange types. This suggests that different orange cultivars from different countries have a similar elemental distribution. A previous study (Simpkins et al. 2000) reported measurements of trace elements in different orange juices and peels. The differences found in elements' levels in both, the juice and peel extracts of oranges from Australia and Brazil, were attributed to soil and rootstock. Our study showed higher concentrations compared to their study [Mn (0.19 μ g g⁻¹), Cu (0.36 μ g g⁻¹), and Zn (0.34 μ g g⁻¹)], which could be due to the differences in the geographical regions. The different cultivars investigated in our study seem to be better sources of antioxidants compared to the Australian varieties. Our study may greatly contribute toward advice on healthy orange juices from a nutritional point of view, such that consumers may obtain an optimum intake of beneficial elements.

CONCLUSION

This is the first study to have evaluated the distribution of minerals between the juice and flesh of various orange cultivars imported from different countries and consumed in the Asir region, Saudi Arabia. The average concentrations of the measured elements in the different orange types were higher in the juice than in the flesh by up to a 40-fold increase. In general, Aseear showed the highest concentrations for the majority of elements in both the juice and flesh when compared to other cultivars, whereas Helo and Afandi showed the lowest levels of essential elements in the flesh and juice, respectively.

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