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Comparative study of bioavailability and forms of zinc in parts of watermelons from Nairobi and Kirinyaga counties, Kenya

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ABSTRACT

Chemical speciation of elements in foods sheds light on their bioavailability in organisms. Watermelon is a widely consumed fruit with substantial levels of essential elements. However, there is very little if any on studies dealing with the bioavailability of these elements in this fruit and therefore, the need for this study. This research aimed to identify forms of Zn element in watermelons, their bioavailability, and the effect of drying on bioavailability from the different parts of Crimson sweet (CS), Sugar baby (SB), and Charleston grey (CG) watermelons. The fruit samples were bought from Mwea and Ngara markets in Kenya, separated into seeds, peels, white-flesh and red-flesh parts. Total Zn levels were determined in the various parts of the fruit before sequential extraction with hexane to dichloromethane to ethanol to water, and the final residue digested with acid. Zn levels in the fractions were determined by AAS while plausible functional moieties chelating with Zn were identified by GC-MS. Data was subjected to ANOVA and t-test at 95% CL. There were significant variations ($p<0.05$) in Zn levels in (i) different parts, and varieties, (ii) various solvents used and, (iii) fresh and dry parts. Water and ethanol extracts (considered potentially bioavailable forms in the human body) had significantly higher levels of zinc compared to other solvent fractions. Total Zn levels in watermelon parts ranged from 4.74–9.71 mg/100 g, which were markedly higher than levels previously reported in many common fruits such as banana (0.11 mg/100 g), mango (0.13 mg/100 g), and tomato (0.15 mg/100 g), and vegetables such as cabbage (0.36 mg/100 g) and lettuce (0.27 mg/100 g). Fresh and dry SB watermelons bought from Ngara market had significantly higher levels of zinc than other varieties in either Ngara or Mwea markets. Drying of watermelon parts significantly ($p<0.05$) increased extraction in dichloromethane and water, and decreased in hexane and ethanol. The presence of organic acids and related complexing agents may have enhanced extraction of potentially bioavailable Zn fractions. These findings suggest that consumption of different edible watermelon parts may contribute substantially to dietary Zn intake.

Key words: watermelon, Zinc, sequential extraction, bioavailability

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is one of the widely grown crops globally (Huh *et al.*, 2008). Kenyan markets have three cultivars: the most popular sugar baby (SB), crimson sweet (CS) and charleston grey (CG) (Gichimu *et al.*, 2009). The fruit has sufficient levels of essential elements including citrulline (amino acid), different phenolic antioxidant compounds, trace elements (e.g. Cu, Zn and Fe) and lycopene (carotenoids). Their seeds have high levels of unsaturated fatty acids (e.g. oleic and linoleic acids), Fe, Mg, P, Zn and Cu (Nthiga *et al.*, 2013). Zn is a useful trace element which is a co-factor by >200 enzymes in the body (Grodner *et al.*, 2000) especially in protein, DNA and RNA syntheses, insulin formation, reduction of incidences of opportunistic infections in HIV and AIDS patients, and alkalinity balance in the body (Kabi, 2004).

Recommended Dietary Allowance (RDA) of Zn per day is 11mg (male adults and pregnant mothers), 8mg (female adult) and 12mg (lactating mothers) (Harlal *et al.*, 2005). Watermelon can be a very good source of Zn. Absorption of Zn mainly depends solubility influenced by the form it exists, and presence of factors affecting absorption (e.g. inhibitors or enhancers). Some foods which are highly rich in Zn, have it in a form which is not bioavailable due to presence of phytates e.g. in legumes and organ meet. Fruits and vegetables have low Zn levels but some like watermelon have relatively higher levels of Zn (Nthiga *et al.*, 2013) though its bioavailability and the distribution in various parts is unknown.

Watermelon was found to have significantly higher levels of Zn when compared to many fruits (Ismail *et al.*, 2011) and vegetables (Jean and Rachel, 2010). All minerals in a diet may not be absorbed and utilized by the body (Kulkarni *et al.*, 2006). The distribution of elements among the various chemical forms (chemical speciation) is of interest because their behavior in body systems is depicted by the form they exist, and their quantitative distribution (Sarret *et al.*, 2009). For example, ratios of phytate to zinc in a diet checks deficiency of zink (Akindahunsi and Oboh, 1999). Additionally, elements with equal configuration in water, and with whose coordination number are similar, compete for absorption pathways, where an excess of one element affects the metabolism of the other. This study focused on determination of (i) levels of zinc species and water content in the peels, white-flesh, red-flesh, and seeds of CG, SB and CS watermelon varieties in Ngara and Mwea of Nairobi and Kirinyaga county markets respectively, (ii) extent of bioavailable forms of Zn in the different parts of the watermelons by sequential extraction and gas chromatography linked with mass spectrophotometer (GC-MS) analysis and, (iii) effect of drying the different parts of watermelons on bioavailability of Zn. This study identified the levels of bioavailable form of Zn and, gave a recommendation of the parts of watermelon that need to be consumed.

METHODOLOGY

Sample collection

Watermelon samples were bought from Mwea and Ngara markets (Kenya) and identified by a Kenyatta University plant taxonomist. Three CG, CS and SB watermelon fruits were separately collected from each vendor, washed using distilled water, dissected and pooled into peels, white-flesh, seeds, and red-flesh and weighed separately. A portion of each part was separately dried in an oven at 105 °C for 24 hours, ground, sieved (600 µm mesh) to remove larger pieces and then re-weighed. Extraction of Zn species on the fresh and dry parts was carried out sequentially from hexane to dichloromethane to ethanol and lastly water solvents using the methods of Loiy *et al.* (2010). The residue was digested with a mixture of two acids (concentrated sulphuric and nitric acids), and hydrogen peroxide. Each fraction was analyzed for Zn levels using Atomic Absorption Spectrophotometer (model AA - 6300 Shimadzu). AAS operating condition was set as Zn as the operating parameter with 213.9nm wavelength, 0.5nm slit width, air/acetylene flame type, 0.9l/min oxidant flow rate, 0.01ppm sensitivity, 75 mA lamp current, and 2.0-8.0ppm as the optimum working range. Gas Chromatography (Model G3243A-2005) was used to determine functional groups that complex with Zn metal. In each experiment, three independent replicates were evaluated.

Reagents and solvents

Solvents and reagents used were of analytical grade (> 98 % pure); dichloromethane (Sarabhai M. chemicals-India), ethanol (Scharlau chemicals-Spain), nitric acid (Rankem Molychem-India), Hexane (Molychem-India), hydrogen peroxide (Merck chemicals-Germany) and zinc nitrate standards (Merck chemicals-Germany).

Data analysis

The mean levels of Zn species in the different parts of watermelons were analysed and separated by one-way ANOVA at 95 % confidence level using SPSS 22.0 software. The means were compared using SNK post-hoc analysis tests. Mean levels of Zn between the Mwea and Ngara markets, and fresh and dry samples were compared using student t – test.

RESULTS AND DISCUSSION

Results

Moisture content in parts of watermelon

There was no significant difference in the moisture levels ($p>0.05$) in the different parts of CG, CS and SB watermelons from Mwea and Ngara markets. In each watermelon variety from Kirinyaga and Nairobi counties, the moisture levels varied significantly ($p<0.05$) between i) seeds and the other parts, ii) peels and the other parts. All watermelon varieties from both Counties had water content ranging from 87.8-89.8 % in the peels, 94.7-96.0 % in the white flesh, 95.3-96.8 % in the red flesh and, 54.6-60.1 % in the seeds. The ranges were within the ones reported by Yau *et al.* (2010). The varying moisture content is due to environmental factors of the growing area, duration of storage, and physiological characteristics. The moisture contents in CG, CS and SB watermelon parts from Mwea and Ngara markets did not differ significantly ($p>0.05$).

Zn species in CG, CS and SB watermelon parts

All the parts of watermelon varieties had higher Zn levels (4.74–9.71 mg/100 g) compared to many commonly consumed fruits and vegetables. Previously reported Zn levels include tomato (0.15 mg/100 g), mango (0.13 mg/100 g), banana (0.11 mg/100 g), potato (0.48 mg/100 g), cabbage (0.36 mg/100 g), lettuce (0.27 mg/100 g), legumes (1.07 mg/100 g), and bulbs (0.46 mg/100 g) (Jean and Rachel, 2010; Ismail et al., 2011). These findings demonstrate that watermelon contains comparatively higher Zn concentrations.

In general, watermelon varieties from Ngara recorded higher zinc levels than Mwea (Table 1). Among all the watermelon varieties, SB from Ngara market recorded significantly higher ($p<0.05$) Zn levels (Table 1). CG white flesh from both markets had significantly higher Zn levels as compared to other parts. Moreover, CG from Mwea market had a higher level (1.24-folds) as compared to that of Ngara market (Table 1). Red flesh part and the peels of CS from Ngara and Mwea markets respectively, had higher Zn level as compared to other parts (Table 1). Peels and red flesh of SB from Ngara market had equally higher levels than the other parts, while its seeds from Mwea market had significantly lower levels than the other parts (Table 1).

Table 1: Levels of zinc (mg/100 g) in parts of watermelons from Ngara and Mwea markets

	Ngara			Mwea		
	CG	CS	SB	CG	CS	SB
Peels	7.59 ± 0.08^b	7.27 ± 0.03^b	9.48 ± 0.03^b	9.03 ± 0.03^c	7.27 ± 0.03^c	4.74 ± 0.02^a
White flesh	7.86 ± 0.03^c	7.50 ± 0.03^b	7.95 ± 0.01^a	9.71 ± 0.02^d	6.96 ± 0.03^b	5.56 ± 0.01^b
Red flesh	6.11 ± 0.02^a	9.11 ± 0.01^c	9.22 ± 0.08^b	6.55 ± 0.03^a	6.52 ± 0.02^{ab}	7.19 ± 0.03^c
Seeds	7.45 ± 0.01^b	6.13 ± 0.03^a	7.15 ± 0.03^a	7.35 ± 0.03^b	6.02 ± 0.01^a	7.56 ± 0.02^d

Means with the same superscript small letter in a column are not significantly different ($p>0.05$, ANOVA, Student–Newman–Keuls post-hoc test)

Levels of zinc in SB watermelon

Generally, fresh SB watermelon from Ngara market had significantly higher Zn extracted by all solvents in a sequential manner than those of Mwea market (Figure 1). Water>residual >ethanol solvents extracted the highest Zn in that order (Figure 1). The levels of Zn extracted by either solvent in the Ngara SB watermelons were significantly ($p<0.05$) different from those of Mwea markets, except white-flesh and red-flesh parts of the dichloromethane extracts, red flesh of the hexane extract, and the seeds of the residual (Figures 1 and 2).

The Zn levels on dry parts of SB watermelons from Ngara market had significantly ($p<0.05$) higher levels than those of Mwea market, except white flesh water extract, peels, and seeds of dichloromethane extract, and red flesh of the residual (Figure 2). These findings show that water extracted more Zn, followed by ethanol, and lastly hexane and dichloromethane extracted the same extent as shown in Figure 2.

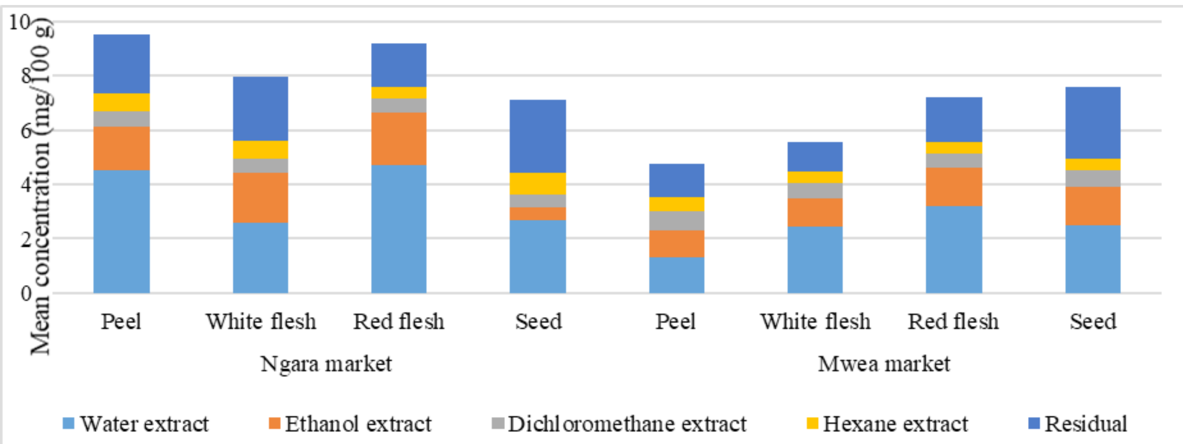

Figure 1: Sequential extraction profile of zinc (Zn) species in fresh peel, white flesh, red flesh, and seed parts of Sugar Baby (SB) watermelon from Ngara and Mwea markets. Values are expressed as mean Zn concentration (mg/100 g). Extraction solvents were applied sequentially (water, ethanol, dichloromethane, hexane), followed by residual fractions. Error bars represent standard deviations of triplicate measurements.

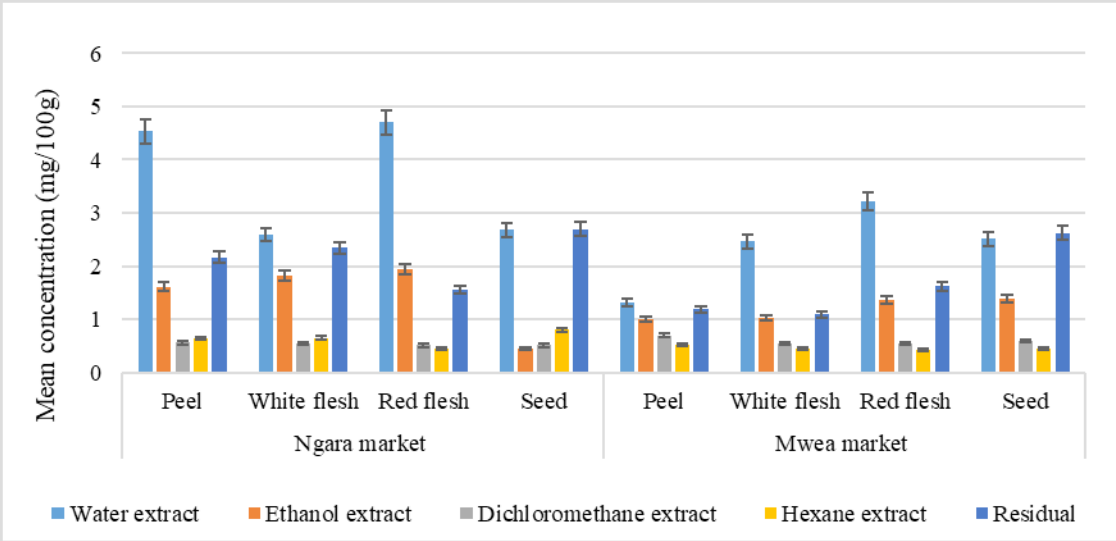

Figure 2: Sequential extraction profile of zinc (Zn) species in dried peel, white flesh, red flesh, and seed parts of Sugar Baby (SB) watermelon from Ngara and Mwea markets. Values are expressed as mean Zn concentration (mg/100 g). Solvent sequence included water, ethanol, dichloromethane, and hexane, with residual fractions analyzed. Error bars indicate standard deviations of triplicate measurements.

Levels of zinc species in CG watermelon

Mean levels of zinc species extracted from different parts of CG watermelon using different solvents between the two markets were significantly ($p<0.05$) different, except i) white flesh of dichloromethane extract and residual, and ii) peels of hexane extract and residual. Mwea market had significantly higher ($p<0.05$) levels in i) water extract in all parts, ii) ethanol in peels, and iii) residual in red flesh (Figure 3).

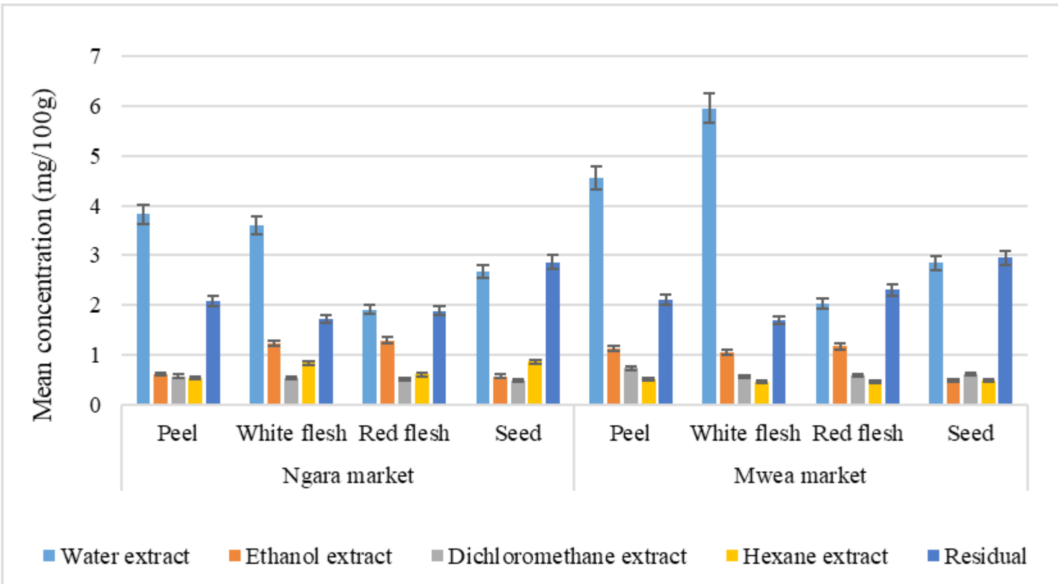

Figure 3: Levels of zinc (Zn) species extracted from dried peel, white flesh, red flesh, and seed parts of Charleston Grey (CG) watermelon from Ngara and Mwea markets. Sequential solvent extraction was performed using water, ethanol, dichloromethane, and hexane, followed by residual fractions. Values are presented as mean Zn concentration (mg/100 g) with standard deviations.

Levels of Zn species in CS watermelon

In general, mean levels of Zn extracted from CS watermelon with various solvents were significantly ($p<0.05$) different between the two markets except i) ethanol and dichloromethane extracts of the white flesh part and ii) the seeds of the residual. Ngara market had significantly higher ($p<0.05$) Zn levels than those of Mwea market except i) dichloromethane extracts of all the watermelon parts, ii) peels of ethanol and water extracts and, iii) seeds of water and residual extracts (Figure 4).

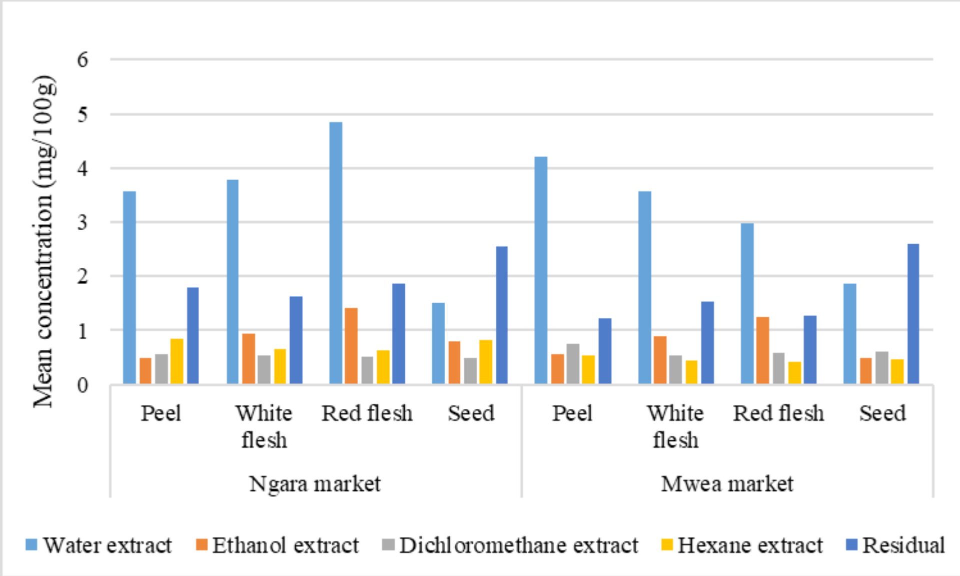

Figure 4: Sequential solvent extraction of zinc (Zn) species in dried peel, white flesh, red flesh, and seed parts of Crimson Sweet (CS) watermelon collected from Ngara and Mwea markets. Extraction solvents included water, ethanol, dichloromethane, and hexane, followed by residual fractions. Values represent mean Zn concentration (mg/100 g) with error bars showing standard deviations.

Levels of zinc species extracted with various solvents in dry and fresh parts of watermelons

There was no significant difference ($p>0.05$) in Zn levels extracted by water solvent between the dry and fresh watermelon parts, except the i) red-flesh of CS, CG, and SB, ii) seeds of CS and SB and iii) white flesh of SB (Figure 5). In addition, there were significantly higher levels of Zn species extracted with ethanol (Figure 6) and hexane (Figure 8) solvents in the fresh watermelon parts as compared to the dry parts. Finally, the Zn levels extracted by dichloromethane in the fresh watermelon parts were significantly higher than the dry parts, except in the i) seeds of CG and CS and ii) peels of CS and SB (Figure 7). Drying of watermelon parts significantly ($p<0.05$) increased extraction rates in dichloromethane and water, and decreased extraction rates in hexane and ethanol.

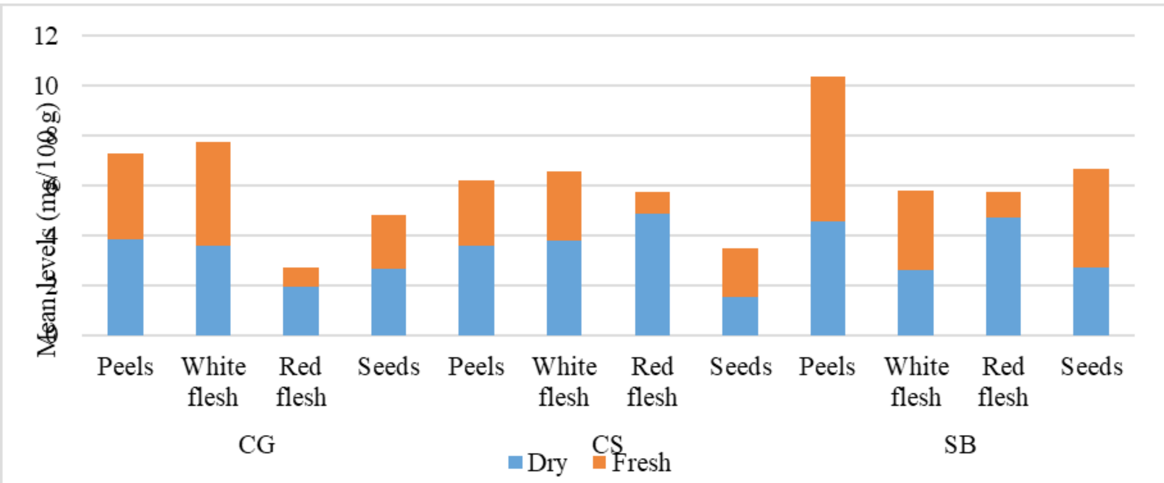

Figure 5: Comparison of zinc (Zn) species extracted using water solvent in fresh and dried peel, white flesh, red flesh, and seed parts of Charleston Grey (CG), Crimson Sweet (CS), and Sugar Baby (SB) watermelon varieties. Values are expressed as mean Zn concentration (mg/100 g). Statistical differences between fresh and dried samples are indicated.

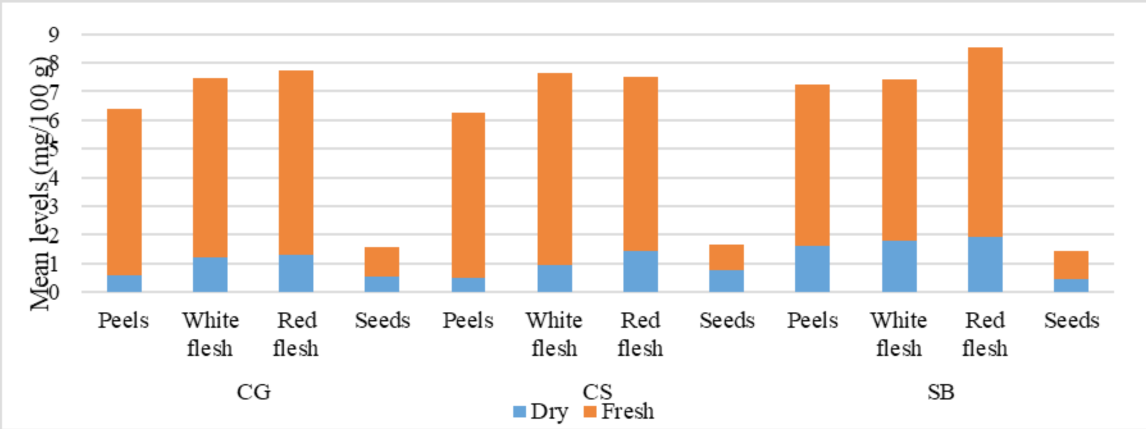

Figure 6: Comparison of zinc (Zn) species extracted using ethanol solvent in fresh and dried peel, white flesh, red flesh, and seed parts of Charleston Grey (CG), Crimson Sweet (CS), and Sugar Baby (SB) watermelon varieties. Values are expressed as mean Zn concentration (mg/100 g). Error bars represent standard deviations of triplicate analyses.

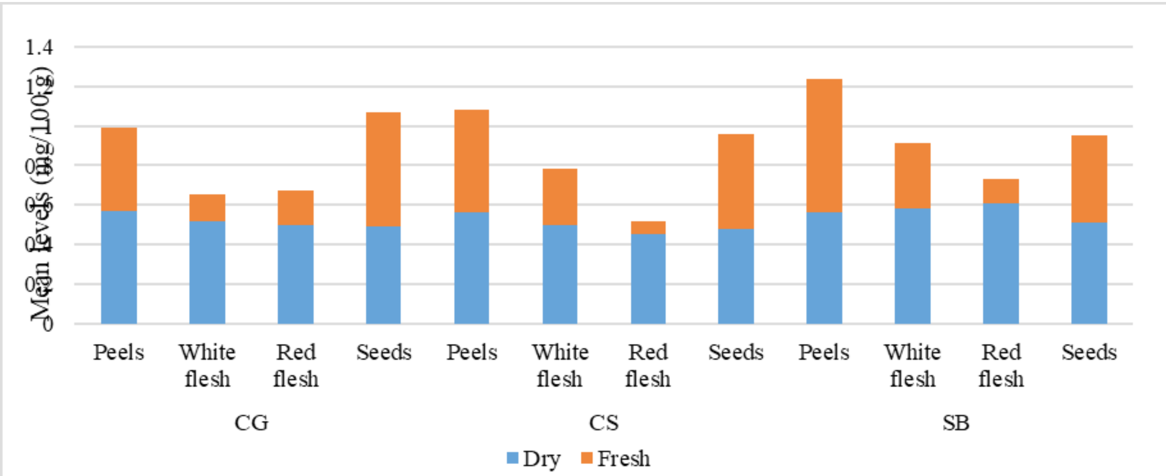

Figure 7: Comparison of zinc (Zn) species extracted using dichloromethane solvent in fresh and dried peel, white flesh, red flesh, and seed parts of Charleston Grey (CG), Crimson Sweet (CS), and Sugar Baby (SB) watermelon varieties. Values are expressed as mean Zn concentration (mg/100 g). Differences between fresh and dried samples highlight the effect of drying on non-polar solvent-extractable Zn fractions.

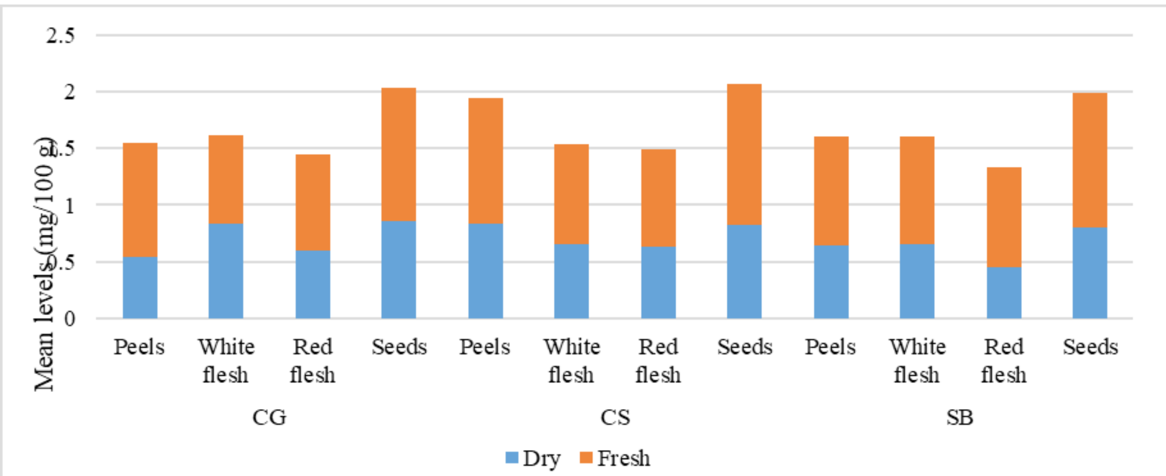

Figure 8: Comparison of zinc (Zn) species extracted using hexane solvent in fresh and dried peel, white flesh, red flesh, and seed parts of Charleston Grey (CG), Crimson Sweet (CS), and Sugar Baby (SB) watermelon varieties collected from Ngara and Mwea markets. Values are presented as mean Zn concentrations (mg/100 g), illustrating the effect of drying on hexane-extractable Zn fractions.

GC-MS analysis of water extracts from the peel of CS watermelon

Characterization of CS water extract by GC-MS identified n-hexadecanoic and n-octadecanoic acids, which enhance and foster absorption of Cu and Zn (Rosalind, 2007). SB red flesh water extracts had 4H-pyran-4-one, cyclohexanone, 2-Dodecanol and Octadec-9(z)-enol, which does not solubilize Cu and Zn (Antonia *et al*., 2009).

DISCUSSION

Mean levels of zinc in all watermelon parts reported in the study are higher than those of other studies. The variation of zinc levels in parts of a watermelon variety from different areas occurs due to seasonal variability, levels of soil minerals, soil pH, stage of harvesting the fruit, and physical conditions where the watermelon plant grew (Mathew *et al*., 2014; Matilda *et al*., 2014). Zinc mean levels in the same part of different watermelon variety grown under similar conditions occur due to the differences in stage of harvesting (Matilda *et al*., 2014). This study observed varying Zn levels in various extracts attributed to the ratio of Zn: phytate complex, anti-nutritional components, and protein in the plant matrix (Akindahunsi and Oboh, 1999).

Different watermelon parts had varying levels of Zn attributed to different plant constituents (phytates, oxalates, insoluble fibre tanins) and competing essential minerals, which reduce zinc absorption (Duo, 2011). Additionally, the levels also differ with species, as previously reported by Kiran *et al*. (2012). We also reported varying levels of Zn fro water extract in corresponding fresh and dry parts of SB, CG, and CS watermelons. Drying reduces phosphate groups in the sample to <5 in the phytate therefore, increasing zinc bioavailability (Kumar *et al*., 2010). Drying also reduces sites for zinc-nitrogen bond formation and free amino acids, leading to a decreased number of organo-zinc compounds with enhanced bioavailability (Rosalind, 2007). This is confirmed by: fresh watermelon is soluble in polar ethanol, but on drying, insoluble forms of Zn are obtained. On drying, the available forms of Zn in watermelon decreased suggesting zinc chelation with high molecular weight molecules (Antonia *et al.,* 2009). The zinc species in ethanol and water extracts are considered bioavailable (Chege 2019).

GC-MS characterization was limited to CS peel and SB red flesh because these parts showed distinct zinc extraction profiles in water fractions. The peel exhibited high levels of potentially bioavailable zinc, while the red flesh is the most commonly consumed portion. This targeted analysis provided contrasting insights into compounds that may enhance or inhibit zinc absorption. A limitation of this study is that GC-MS characterization was conducted only on CS peel and SB red flesh extracts. While these results provide valuable insights into potential zinc absorption enhancers and inhibitors, they may not represent the full spectrum of compounds present in other watermelon varieties and parts. Future work should expand GC-MS profiling to include additional cultivars and tissues to provide a more comprehensive understanding of zinc bioavailability in watermelon.

In conclusion, approximately 65–70% of the total zinc content in watermelon was recovered in water and ethanol extracts, which are considered bioavailable fractions. The remaining zinc was bound in the residue and less likely to be absorbed. People should be sensitized to eat all the watermelon parts in order to meet the recommended dietary intake of Zn species. White-flesh and peels should not be discarded since they have more bioavailable zinc than the commonly eaten red-flesh. We recommend speciation studies on the influence of concentration on the extraction of elements with similar configuration and coordination numbers in a given solvent. It will also be interesting to carry out bioavailability studies on different forms of other essential minerals in watermelon.

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