QUANTIFICATION, CORRELATION AND HIERARCHICAL CLUSTER ANALYSIS OF THE MINERAL CONTENT DATA OF CITRUS FRUIT VARIETIES

Salau, R. B., Nwakife, C. N., Bisiriyu, M. T., Jibril, M. J. and Oyewunmi, A. H.
Department of Chemistry, Federal University of Technology, P.M.B. 65, Minna, Nigeria
*Corresponding Author: rasaqsalau@futminna.edu.ng,
Tel: +2347032637182

ABSTRACT
Citrus fruits are widely grown and consumed all over the world, their health benefits are highly cherished as well. However, how varied and similar are the varieties of citrus fruits remain a contentious issue in quality control of processed citrus fruits drinks. Triplicates of twelve samples containing six citrus fruit varieties were collected. The varieties were peeled and prepared as both juice and whole edible portion. The samples were digested with nitric acid and hydrogen peroxide. Flame Emission Photometer and Atomic Absorption Spectrometer were used to determine the mineral elements. Physicochemical studies including sensory were also conducted. The result shows that all the varieties recorded the presence of the mineral elements in the following ranges: Na (1.09-15.30); K (9.87-37.28); Ca (1.83-3.65); Mg (2.14-4.41); Fe (4.98-13.60); Mn (1.84-3.72); Cu (1.82-3.70); Zn (2.06-4.63) mg/100g. The dendrogram of the hierarchical cluster analysis derived from two principal components with cumulative variance of 92.36%, classifies citrus into three groups based on elements signatures. The grapefruit pair of Duncan (edible portion) and Pumelo (juice) is found to be closest substitute. There are prominent mutual correlations between density and colourimetry as well as sensory taste and conductivity. The hierarchical cluster analysis characterized the parameters that are peculiar to citrus species. This characterization is invaluable in quality control and authenticity monitor in processed fruit drinks.

Key words: citrus; mineral; dendrogram; correlation; authenticity

INTRODUCTION
Fruit and fruit juices are taken as refreshing, healthy foods. This has encouraged the rise in number of juice processing both home-made and industry. Public health organisations too are not left out in the promotion of health benefits inherent in the consumption of fruits and fruit juices [1]. Citrus fruits in particular are highly appreciated for their pleasant juices and for the myriad of health benefits attributed to them. Therapeutic and health management potency of citrus fruits have been widely reported in mineral deficiency, antiviral, anticancer and anti-inflammatory activities [2]. Citrus contains a large number of bioactive chemical and nutrient constituents which are responsible for the sensory, nutritional and medicinal qualities of citrus fruits [3, 4, 5]. The employment of the citrus fruits in the management of mineral deficiency diseases is traceable to the substantial values of their mineral contents [6, 2, 7]. Chemometrics as analytic method for foods was borne out of its versatility in the extraction of relevant information [8, 9]. Reports of some fraudulent practices are on increase. These include orange labeled juice products which are mischievously containing traces of grapefruit juice [1, 3]. In this study, FES and AAS with HCA and multivariate correlation analysis are employed. This is to extract information in order to explore the discriminating and unique properties of pure citrus fruit varieties.

MATERIALS AND METHOD

Triplicate samples of 12 citrus fruits plant parts and varieties were obtained from Minna metropolis in Niger State. Samples of orange, tangerine, lemon, lime, grapefruit and pomelo were collected. The citrus food samples were prepared both in juice and both edible portion forms. The six physicochemical properties measured include the pH, Electrical Conductivity, Density, Colour and Sensory. Taste response was the only sensory property determined. Taste response was scaled as: 7- sweet, 6-slightly sweet, 5 slightly sour, 4-sour, 3- slightly bitter, 2-bitter, 1- bland. The average of six volunteers was recorded.

Two gram (2 g) of each of the samples was digested with acid [6]. FES was used to determine the concentration of Na and K while Fe, Zn, Cd, Mg, Cu and Mn were determined by AAS. Descriptive statistics and the multivariate correlation analysis were carried out on Microsoft Excel spread sheet for Windows.The HCA was performed using MATLAB with PLS toolbox. Accuracy of the results obtained was validated with the use of SRM 1570a: a certified reference material of trace elements in spinach leaves as well as quality control in the HCA model. Only the good-fit calibration plots with R^2 above 0.980 were used.

RESULTS AND DISCUSSIONS

Physico-chemical parameters

The citrus fruit varieties show wide range of physicochemical properties. Table 1 presents the values of physicochemical parameters. The result shows that all the citrus fruits are acidic.This is attributed to the remarkable presence of some acids like citric, ascorbic and hydroxcynamic acid [3]. Bitterness in citrus fruits is sometimes borne out of substituted saccharide naringin [5]. Generally, acids taste sour while bases taste bitter. The moderate taste response in citrus fruits could be as a result of blends of acidic and basic phyto-chemical contents [10]. They all share fair conductivity, implying that they have ionisable molecular content. They are close in terms of density but higher than water. Their colours are also of close range of intensity.

Table 1: Physicochemical Properties of Citrus Varieties

	pH	Conductivity x10^2 (Sm^{-1})	Colourimetry	Density(g/cm^3)	Sensory
Lemon	2.19	53	0.86	1.08	4
Tangerine	3.83	41	0.90	1.08	6
Lime	1.77	22	0.81	1.08	3
Duncan	2.31	41	0.72	1.08	3
Orange	3.27	39	0.79	1.09	4
Pomelo	3.06	55	0.79	1.09	4
Water	7.01	0.5	0.00	1.05	1

Descriptive Statistics of the Mineral Contents

The average elements distribution in citrus fruits was considered statistically. Table 2 gives the summary.

Table 2: Descriptive Statistics of the Mineral Contents of Citrus Varieties

	Na	K	Fe	Mg	Mn	Cu	Zn	Ca	
Mean	4.33	25.22	6.44	2.34	2.00	1.98	2.43	1.98	
Mode	2.19	37.28	5.53	2.16	1.86	1.83	2.37	1.84	
Standard Deviation	4.95	9.07	2.23	0.62	0.52	0.52	0.67	0.50	
Kurtosis	2.84	-0.66	10.84	12.97	12.99	12.99	11.77	13.00	
Skewness	2.04	-0.28	3.18	3.60	3.60	3.60	3.37	3.60	
Minimum	1.09	9.87	4.98	2.14	1.84	1.82	2.06	1.83	
Maximum	15.30	37.28	13.60	4.41	3.72	3.70	4.63	3.65	
Confidence Level(95.0%)	2.99	5.48	1.35	0.38	0.31	0.31	0.41	0.30	

The elements distributions, in Table 2 at 95% confidence interval showed positive skewness values except K. The concentrations of the elements are unique to specific specie of citrus fruit. The kurtosis values suggest peaked clustered distribution relative to the normal distribution. The negative kurtosis value obtained in the case of potassium distribution is an evidence of exceptional high values of it in the citrus contents which contributes to the flatness of the distributions. These varied contents of the citrus varieties lend support to the uniqueness of each of them despite similar major contents.

The mean and the ranges of concentrations of elements showed the citrus fruits can play supplementary role in mineral health management. The food quality index of Na/K molar ratio of the citrus fruits 0.29 which is within the less than 1 value recommended for blood pressure and cellular fluid regulations in healthy individual [11, 12].

Pair wise Correlation Matrix

The relevance of the correlation matrix analysis is to give insight into element interdependence in the studied citrus fruits [10, 13]. Information about the interdependence of the physico-chemical properties is needed to know if increasing consumption of the fruits for certain desirable properties might carry along the correlated undesirable property. Similarly, the correlation analysis information also helps in characterization of the general profile of the citrus fruits. [13] . Table 3 and 4 give the correlation matrix of the physico-property and element content of citrus fruits.

Table 3: Correlation Matrix for Citrus Fruits Physico-chemical Properties

	pH	Colourimetry	Conductivity	Density	Sensory
pH	1				
Colourimetry	-0.873	1			
Conductivity	-0.677	0.818	1		
Density	-0.798	0.904	0.838	1	
Sensory	-0.822	0.831	0.945	0.783	1

Negative correlation exists between pH and both sensory and Colourimetry. High positive correlation exist between conductivity and sensory as well as density and Colourimetry.

Table 4: Correlation Matrix for Citrus Fruits Element Content

	Na	K	Fe	Mg	Mn	Cu	Zn	Ca
Na	1.0000							
K	0.1290	1.0000						
Fe	0.5975	0.1306	1.0000					
Mg	0.6637	0.1913	0.9655	1.0000				
Mn	0.6606	0.1844	0.9664	0.9998	1.0000			
Cu	0.6661	0.1897	0.9627	0.9995	0.9998	1.0000		
Zn	0.6339	0.1864	0.9689	0.9853	0.9829	0.9807	1.0000	
Ca	0.6651	0.1860	0.9650	0.9995	0.9998	0.9998	0.9807	1.0000

Strong correlations are also observed in Mg and Fe, Mn and Fe, Cu with Fe and Mg, Zn with Fe, Mg, Mn and Cu, Ca with Fe, Mg and Zn. The correlations of K, Na and Fe with other elements were below 0.9. This means that Mg, Mn, Cu and Ca are always present in all citrus fruits. In food quality control analysis, these elements represent the signature of citrus fruits. The integrity of any of the citrus fruits without these elements as major content is questionable. The high correlation values between these elements imply that they go along together in the fruits.

Hierarchical Cluster Analysis

The HCA is employed to give further information about interrelationship, similarity and closeness of the Citrus fruit samples. Figure 1 shows the dendrogram of the HCA. Three major clusters were observable: [2,8, 10 (Duncan edible portion, orange edible portion, Pumelo Juice)], [5,7,10 (Duncan Juice, Lime Juice, Tangerine Juice)] and [1,3,4,6,9,11(orange Juice, Orange edible portion, Lemon edible portion, Lime edible portion, Tangerine edible portion, Tangerine Juice)]. Each group have samples of similar mineral quantity and quality. The dendrogram further shows pairs of fruit varieties that have close substitutability. This means any of these varieties consumed have equivalent elements content. Consumer can make choice without any element content prejudice.

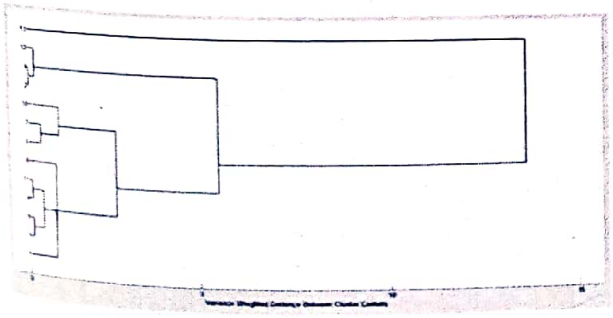

Figure 1: Dendrogram HCA of the mineral Content of the Citrus Fruits

CONCLUSION

All the studied mineral elements were observed to have significant presence in citrus fruits. The citrus foods have propensity as source for mineral health application. From the correlation analysis, K was found to be higly prominent. Other element signatures of the citrus fruits are Mn, Mg, Cu and Ca.The pH is inversely related to other physicochemical parameters, the values were majorly below 7 and hence acidic. The Hierarchical Cluster

Analysis (HCA) also reveals citrus fruit substitutability. The consumption of Duncan grapefruit edible portion is equivalent to Pumelo grapefruit juice because the values of the mineral content in them are very close. With the obvious signature properties revealed, the correlation and HCA can be employed in identifying authenticity, origin and fraudulent labelled citrus fruits product.

REFERENCES

1. M. V. Cuny, G. C. Le Gall, M. Lees and D.N. Rutledge (2008)., *Fruit juice authentication by 1H NMR spectroscopy in combination with different chemometrics tools*. Analytical and Bioanalytical Chemistry, 2008. **390**(1): p. 419-427.
2. R. B. Salau (2012). Comparative Evaluation of Analytical Techniques for the Quantification of Potassium in Fruit Juices and corresponding Beverages. *Journal of Science, Technology, Mathematics and Education (JOSTMED), 8*(3), 8-13.
3. F. Zielinski, C. W. I. Haminiuk, C. A. Nunes, E. R. Schnitzler, Saskia M, andD. Granato (2014). Chemical composition, sensory properties, provenance, and bioactivity of fruit juices as assessed by chemometrics: a critical review and guideline. *Comprehensive reviews in food science and food safety, 13*(3), 300-316.
4. Rekha, G. Poornima, M. Manasa, V. Abhipsa, P. J. Devi, V. H. T. Kumar and P. T. R. Kekuda (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transactions, 1*(2), 303-310.
5. M. Puri, S. S. Marwaha, R. M. Kothari, andJ. F. Kennedy (1996). Biochemical basis of bitterness in citrus fruit juices and biotech approaches for debittering. *Critical reviews in biotechnology, 16*(2), 145-155.
6. R. B. Salau and M. N. Hasan (2014). Evaluation and Analysis of Dietary Essential Mineral Micronutrients in Selected Malaysian Foods Using FAAS and ICP-MS. *Modern Applied Science, 8*(6), 103-111.

   A. K. Paul andR. K. Shaha (2004). Nutrients, vitamins and minerals content in common citrus fruits in the northern region of Bangladesh. *Pakistan Journal ofBiological Sciences 7*(2), 238.
7. R. Brereton (2009). *Chemometrics for pattern recognition*. Toronto: John Wiley & Sons.
8. K. R. Beebe, R. J. Pell andM. B. Seasholtz (1998). *Chemometrics:A Practical Guide*. Toronto, Canada: John Wiley & Sons, Inc.
9. R. B. Salau (2015). *Profiling of the Mineral Content of Typical Malaysian Muslim Foods*. (PhD), Department of Chemistry, Universiti Teknologi Malaysia, UTM.
10. M. O. Aremu, A. Olonisakin, O. D. Opaluwa, Y. Mohammed and R. B. Salau (2007). Nutritional qualities assessment of tilapia fish (Tilapia quineensis). *Indian Journal of Multidisciplinary Research, 3*(3), 443-456.
11. J. Horacio, M. D. Adrogué and E. Nicolaos and M. D. Madias (2007). Sodium and Potassium in the Pathogenesis of Hypertension. *New England Journal of Medicine, 356*, 1966-1978. doi: DOI: 10.1056/NEJMra064486
12. Sola-Larrañaga andI. Navarro-Blasco (2006). Preliminary chemometric study of minerals and trace elements in Spanish infant formulae. *Analytica Chimica Acta, 555*(2), 354-363. doi: 10.1016/j.aca.2005.09.015