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Analysis of micronutrients in *Syzygium zeylanicum* var. *Zeylanicum* fruits

Devi RC Bhanu, KK Sabu

Biotechnology and Bioinformatics Division, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram, Kerala, India

Abstract
The human body requires small quantities of vitamins and mineral elements for its proper functioning. Fruits and vegetables are the major sources of these micronutrients in our diet. Among them, vitamins are susceptible to degradation during storage. Hence, fresh locally harvested fruits and vegetables can be considered superior to exotic store bought ones in terms of micronutrient content. In the present study, fruits of *Syzygium zeylanicum* (L.) DC. var. *zeylanicum*, an underutilized tree, were analysed for the presence of various micronutrients. The fruits contained good amounts of vitamin A (78.445 ± 0.0123 IU/100g), vitamin K (0.291 ± 0.001µg/ 100g) and vitamin C (18.201 ± 0.003 mg/100g). The B vitamins, vitamin D and vitamin E were found in trace amounts. The fruits were particularly rich in iron; the iron content being 1.67 ± 0.03 mg/100g.Whereas the sodium content (16.9 ± 0.15 mg/100g) was unusually high.

Keywords: micronutrients, hidden hunger, HPLC, AAS, ICP-MS, water-soluble vitamins, fat-soluble vitamins

1. Introduction
Micronutrients, as the name indicates, are those nutrients that are required in small amounts. They include mineral elements and vitamins and are necessary for the proper functioning and well-being of the human body. The main sources of micronutrients are fresh fruits and vegetables. Although required only in small amounts deficiency of micronutrients can seriously affect the normal functioning of the body. Deficiency can result either from insufficient intake or malabsorption. The symptoms of micronutrient deficiency are not immediately obvious (with the exception of a few); hence micronutrient deficiency is also called hidden hunger. It is estimated that more than 2 billion people are victims of hidden hunger [1]. Hidden hunger is prevalent in the developing world and is not uncommon in the developed world. Sub-Saharan Africa and South East Asia are regarded as the hotspots of hidden hunger [2]. In the developing world, in recent times, there has been a shift in eating habits from minimally processed, locally available food to highly processed, calorie laden and micronutrient deficient foods. This has increased the incidence of micronutrient deficiencies [3]. In Latin America and the Caribbean, hidden hunger is relatively less prevalent owing to better eating habits (less dependence on single staples) and human intervention in form of nutrition education and distribution of supplements [4].

While staples provide macronutrients, the main sources of micronutrients are fruits and vegetables. Most micronutrients, especially the B vitamins and vitamin C decline on storage. Therefore, dieticians generally instruct people to include fresh fruits and vegetables in their diet. As most are aware, store-bought fruits and vegetables are far from fresh, having been in storage for considerable amount of time. A better alternative would be home grown or locally sourced produce.

Though the Western Ghats is home to a large number of fruit bearing trees, only a preferred few are harvested for fruits. Vast majority of such fruit bearing tress are underutilized, their fruits consumed only in a few tribal settlements. Most of these underutilized fruits are small in size, low in calories (since they have never been bred for size and sweetness) and rich in micronutrients. *Syzygium zeylanicum* (L.) DC. var. *zeylanicum* (Myrtaceae) is one such underutilized, fruit bearing tree. The fruits are edible white berries and are produced in clusters. The fruits are used by Paniya and Kattunaika tribes of Wayanad district, Kerala [5, 6]. In the present study, the fruits of *Syzygium zeylanicum* var. *zeylanicum* were investigated for minerals, fat-soluble and water-soluble vitamins.

2. Materials and methods
The fruits of *Syzygium zeylanicum* var. *zeylanicum* (*SZZ*) were collected from JNTBGRI, Palode, Thiruvananthapuram and University of Kerala, Karyavattom Campus, Thiruvananthapuram. The fruits were cleaned, with minimum use of water. The cleaned fruits were cored, and subjected to analyses.

Separation of vitamins was achieved on Shimadzu Class-VP V6.13 SP2 instrument with a variable wavelength UV detector (SPD 10 Avp). The column used was C18 (4.6 x 75mm, 3.5 µm). Injection volume was 5 µL. Mobile phases used were different for fat-soluble and water-soluble vitamins. For fat-soluble vitamins the mobile phase consisted of water and methanol at a flow rate of 1 ml/min. The mobile phase for the separation of water-soluble vitamins comprised of water and acetonitrile at a flow rate of 0.70 ml/min. The column temperature was held at 20 ºC and 40 ºC for fat-soluble and water-soluble vitamins respectively.

Vitamin C was estimated by volumetric analysis. Sample was extracted in 4% oxalic acid and titrated against the dye 2, 6-dichlorophenol indophenol. The end point was the appearance of light pink colour that persisted for 30 seconds. Ascorbic acid was used as the standard [7].

The elements Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg) and Phosphorous (P) were analysed by AAS (Pinnacle 900 H, Perkin Elmer). The samples were subjected to dry ashing at 500 ºC for 6 hours. The ash was dissolved in 1 N HCl, filtered and introduced into the AAS instrument.

Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Selenium (Se), Chromium (Cr), Cobalt (Co) were analysed by ICP-MS (Thermo Scientific ICAP Qc). For analysis of minerals using ICP-MS, the samples were subjected to microwave digestion (Anton Paar, Multiwave 3000) and introduced into the instrument.

The values are expressed as mean ± standard deviation on fresh weight basis.

3. Results

Table 1 shows the amount of various fat-soluble vitamins in *SZZ* fruits. The fat soluble vitamins tested were, vitamin A, vitamin D3, vitamin E and vitamin K. *SZZ* fruits contain 78.445 ± 0.0123 IU/100g vitamin A. The vitamin D3 (0.000041 ± 0.000002 mg/100g) and vitamin E (0.000052 ± 0.000001 mg/100g) content was found to be very low. The vitamin K content was 0.291 ± 0.001µg/ 100g.

Table 1: Fat-soluble vitamins in *SZZ*

Vitamin	*SZZ*
Vitamin A(Retinol acetate) (IU/100g)	78.445 ± 0.0123
Vitamin D3(Cholecalciferol) (mg/100g)	0.000041 ± 0.000002
Vitamin E(α-tocopherol) (mg/100g)	0.000052 ± 0.000001
Vitamin K (µg/100g)	0.291 ± 0.001

Values are mean ± standard deviation (n=3)

The water- soluble vitamins estimated were, B vitamins (B1, B2, B3, and B6) and vitamin C. Vitamin B6, Pyridoxine was absent could not be detected in the samples. The content of other B vitamins tested was low. In 100g (fresh weight) of *SZZ* sample, 0.009 ± 0.001mg Niacin (Vitamin B3), 0.0022± 0.0001 mg Thiamine (Vitamin B1), 0.0021± 0.00005 mg Riboflavin (Vitamin B2) could be detected. The Vitamin C content of *SZZ* was 18.201 ± 0.003 mg/100g. The results are summarized in Table 2.

Table 2**:** Water-soluble vitamins in *SZZ*

Vitamin	*SZZ*
Vitamin C (mg/100g)	18.201 ± 0.003
Niacin (Vitamin B3) (mg/ 100g)	0.009± 0.001
Pyridoxine (Vitamin B6) (mg/100g)	0
Thiamine (Vitamin B1) (mg/100g)	0.0022± 0.0001
Riboflavin (Vitamin B2) (mg/100g)	0.0021± 0.00005

Values are mean ± SD (n=3)

The major element in the fruit sample was potassium (K). The potassium content of fresh fruits was 45.8 ±0.23 mg/100g. In 100g of the sample, 16.9 ± 0.15 mg Na, 18.3±0.10 mg Ca, 14.9 ± 0.12 mg Mg and 30 ±0.05 mg P could be estimated. Considerable amount of Fe (1.67 ±0.03 mg/100g) was detected in the fruits. The Cu and Zn content was 0.16 ±0.01 and 0.054 ±0.003 mg/100g respectively. Selenium was not detected. The content of Mn and Cr were 0.356 ±0.014 and 0.24± 0.01 mg/100g respectively. The Co content was found to be 0.022 ±0.011 mg/100g. The results of mineral analysis are summarized in Table 3.

Table 3: Mineral elements (mg/100g) in fresh fruits of *SZZ*

Element	*SZZ*
Sodium (Na)	16.9 ±0.15
Potassium (K)	45.8 ±0.23
Calcium (Ca)	18.3±0.10
Magnesium (Mg)	14.9 ±0.12
Phosphorous (P)	30 ±0.05
Iron (Fe)	1.67 ±0.03
Copper (Cu)	0.16 ±0.01
Zinc (Zn)	0.054 ±0.003
Manganese (Mn)	0.356 ±0.014
Cobalt (Co)	0.022 ±0.011
Chromium (Cr)	0.24 ±0.01
Selenium (Se)	ND

Values are mean ± SD (n=3)

4. Discussion

Micronutrients (vitamins and minerals) are required in small doses for the proper functioning of the human body. Vitamins are organic compounds and are classified into two groups based on their solubility; the two classes being fat-soluble and water-soluble vitamins. Fat-soluble vitamins include, vitamin A, D, E and K. The water soluble-vitamins are vitamin C and the B vitamins [8]. A comparison of the vitamin content of *SZZ* fruits with a few commonly used fruits is given in Table 4.

The vitamin A content of *SZZ* fruits is 78.445 ± 0.0123IU per 100g. Chemically vitamin A is all-*trans*-retinol. Vitamin A deficiency mainly affects the eye. It causes xerophthlamia, a condition which affects the conjunctiva and cornea of the eye. If left untreated, it can lead to irreversible blindness. Vitamin A deficiency also causes skin lesions, loss of immune function and epithelial keratinisation [10]. Compared to banana (64 IU/100g) and apple (54 IU/100g) *SZZ* fruits have higher vitamin A content. However, there are fruits that supply greater amounts of vitamin A, like orange (225 IU/100g) and papaya (950 IU/100g). Vitamin D is a fat-soluble vitamin that is synthesized in the human body with the help of sunlight. When skin receives solar radiation, 7-dehydrocholesterol is converted to previtamin D3, which in turn is converted to vitamin D3. The absorption of essential minerals calcium and phosphorous depends on vitamin D. In the absence of vitamin D only 10-15 % of calcium and 60 % of phosphorous is absorbed. Vitamin D deficiency can cause rickets (weak skeletal system with soft bones and other deformities) and osteoporosis. It can also compromise your immunity. Vitamin D deficiency can also lead to chronic disorders like multiple sclerosis and colorectal cancer [11]. The vitamin D3 content of *SZZ* fruits (0.000041 ± 0.000002 mg/100g) is negligible. According to the USDA Nutrient Database, vitamin D is absent in several fruits like banana, apple, orange and papaya [12]. Vitamin D is absent in many of the foods that we eat. The major sources of vitamin D in our diet are, fish, egg yolks and milk [10].

Table 4: Vitamin content of *SZZ* and some selected fruits

	Fat-Soluble Vitamins				
Vitamins	***SZZ***	**Banana[9]**	**Apple[9]**	**Orange[9]**	**Papaya[9]**
Vitamin A (Retinol acetate) IU/100g	78.445 ± 0.0123	64	54	225	950
Vitamin D (Cholecalciferol) (mg/100g)	0.000041 ± 0.000002	0	0	0	0
Vitamin E (α-tocopherol) (mg/100g)	0.000052 ± 0.000001	0.10	0.18	0.18	0.30
Vitamin K (µg/100g)	0.291 ± 0.001	0.5	2.2	0	2.6
	Water-Soluble Vitamins				
Vitamins (mg/100g)	***SZZ***	Banana[9]	Apple[9]	Orange[9]	Papaya[9]
Vitamin C	18.201 ± 0.003	8.7	4.6	53.2	60.9
Niacin	0.009± 0.001	0.665	0.091	0.282	0.357
Pyridoxine	0	0.367	0.041	0.060	0.038
Thiamine	0.0022± 0.0001	0.031	0.017	0.087	0.023
Riboflavin	0.0021± 0.00005	0.073	0.026	0.040	0.027

Values are mean ± SD (n=3)

A major antioxidant in our cells is vitamin E. It prevents cellular damage due to free radicles [13]. *SZZ* fruits (0.000052 ± 0.000001mg/100g) have only trace amounts of vitamin E. Most fruits and vegetables have low vitamin E content. Vitamin E in our diets usually come from vegetable oils, nuts (peanuts, hazelnuts) and seeds (sunflower seeds) [14]. Clotting of blood requires a vitamin called vitamin K. Its deficiency can lead to excessive clotting. The intestinal bacteria produces vitamin K in animals and humans [15]. The vitamin K content of *SZZ* is 0.291 ± 0.001 µg/100g. Apples and papaya have nearly ten times as much vitamin K as *SZZ* fruits. However fruits like oranges do not contain any vitamin K. The major contributors of vitamin K in our diet are green leafy vegetables like spinach (493.6 µg/100g) and kale (817 µg/100g) [9].

Vitamin C is an essential water-soluble vitamin that the human body is unable to synthesise. It is an antioxidant and is also capable of generating other antioxidants like α-tocopherol. Vitamin C is known to boost immunity and increase the absorption of non-heme iron. Insufficient vitamin C intake can result in scurvy, the symptoms of which include, fatigue, connective tissue weakness and capillary fragility [16]. *SZZ* fruits contain 18.201 ± 0.003 mg/100g of vitamin C. Shilpa and Krishnakumar (2015) reported the vitamin C value of *Syzygium zeylanicum* fruits to be 0.06 % of dry weight [17]. This is lower than the value obtained in the present study (0.14 % dry weight or 18.201 mg/100g fresh weight). The vitamin C content of *SZZ* is greater than that of banana (8.7 mg/100g) and apple (4.6 mg/100g) [9]. However oranges (53.2 mg/100g) and papaya (60.9 mg/100g) have higher vitamin C content than *SZZ*. The B vitamins are another class of water-soluble vitamins that play important roles in cell metabolism. Eight B vitamins have been identified and together they constitute the B complex. Four B vitamins, niacin, pyridoxine, thiamine and riboflavin were analysed in the fruit samples of *SZZ*. The niacin (0.009± 0.001 mg/100g), thiamine (0.0022± 0.0001mg/100g) and riboflavin (0.0021± 0.00005 mg/100g) content of *SZZ* were negligible; pyridoxine was not detected. The conventional fruits, have greater vitamin B content and are better sources of B vitamins.

Minerals are inorganic micronutrients. Based on requirement, mineral elements can be of two types, macroelements and trace elements (microelements). For an adult, the requirement of macroelements is more than 100 mg/day. The macroelements are Na, K, Ca, Mg, P, S and Cl. When the requirement of an element is less than 100 mg/day, it is called trace element/ micro element. Some of the trace elements are Fe, Cu, Zn, Mn, Co, Cr and Se [18]. Table 5 lists the mineral composition of *SZZ* and some selected fruits.

The fruits of *SZZ* contain appreciable amounts of calcium (18.3 ±0.10 mg/100g), magnesium (14.9 ± 0.12 mg/100g) and phosphorous (30 ± 0.05 mg/100g). The phosphorous content of *SZZ* is higher than some of fruits that we eat daily (Table 5). Calcium and phosphorous are components of the bone. Calcium also functions as catalyst in blood clotting [21]. Magnesium is an important cation in the human body. Most of the magnesium in the body is intracellular, with less than 1 % in the extracellular space. A large number of biochemical reactions in the body require magnesium. Magnesium is also crucial to neuromuscular transmission and cardiovascular tone [22]. Magnesium content of *SZZ* is higher when compared to common fruits like apple and orange. Sodium and potassium act as electrolytes that influence the movement and distribution of fluids within the body.

Table 5: Mineral Composition (mg/100g) of *SZZ* and some selected fruits

	SZZ	**Banana[19]**	**Apple[19]**	**Orange[19]**	**Papaya[9]**
Sodium (Na)	16.9 ± 0.15	<0.5	1	1	8
Potassium (K)	45.8 ± 0.23	330	100	122	182
Calcium (Ca)	18.3 ±0.10	6	5	24	20
Magnesium (Mg)	14.9 ± 0.12	27	4	8	21
Phosphorous (P)	30 ± 0.05	23	8	16	10
Iron (Fe)	1.67 ± 0.03	0.27	0.09	0.11	0.25
Copper (Cu)	0.16 ± 0.01	0.10	0.03	0.03	0.06[20]
Zinc (Zn)	0.054 ± 0.003	0.18	< 0.06	< 0.06	0.08
Manganese (Mn)	0.356± 0.014	0.36	0.04	0.02	0.04[20]
Selenium (Se)	ND	< 0.5 µg/100g	< 0.5 µg/100g	< 0.5 µg/100g	NR
Chromium (Cr)	0.24± 0.01	NR	NR	NR	NR
Cobalt (Co)	0.022± 0.011	NR	NR	NR	NR

ND-not detected; NR-not reported. Values are mean ± SD (n=3)

Sodium is the chief cation outside of the cells, while potassium is the predominant cation inside of cells [23]. Sodium levels (16.9 ± 0.15 mg/100g) were high in *SZZ* compared to other fruits. Ideally the sodium to potassium ratio should be low i.e. it should favour potassium. High sodium to potassium ratio is implicated in high blood pressure (hypertension). In *SZZ* the potassium levels (45.8 ± 0.23 mg/100g) were almost three times higher than that of sodium. Iron is a trace element; the daily requirement being less than 100 mg. Iron has various functions in the body. It plays a central role in transport of oxygen, synthesis of DNA and in the electron transport chain. The iron content (1.67 ± 0.03 mg/100g) of *SZZ* fruits was found to be quite high. Mulberry (1.85 mg/100g) and dates (0.9-1.2 mg/100g) are fruits that are known to have high iron content. With an iron content that is comparable to mulberry and dates, *SZZ* fruits can be considered a good source of iron. Metabolism of iron in the body requires another trace element, copper. In the absence of copper, some tissues of the body experience iron deficiency, whereas others like intestine and liver accumulate the mineral in excess [24]. Copper is also necessary for bone health, immunity and cholesterol metabolism [25]. The copper content of *SZZ* (0.16 ± 0.01mg/100g) is higher than many of the fruits but is well within the permissible range. The recommended daily allowance (RDA) of copper is around 900 µg/day or 0.9 mg/day. The zinc requirement of an adult human ranges from 8-11 mg/day [26]. Zinc deficiency affects the skin, central nervous system, gastrointestinal tract, immune and reproductive systems [27]. Both animal and plant sources provide zinc. Some fruits like pomegranate (0.35 mg/100g) and avocado (0.64 mg/100g) are particularly rich in zinc [9]. Fruits like apple, orange and *SZZ* (0.054 ± 0.003 mg/100g) contain less than 0.06 mg/100g of zinc. Manganese is an element that is present in very small amounts in the body. It is a component of superoxide dismutase (SOD), an antioxidant enzyme. Thus magnesium is a vital part of the antioxidant machinery of the cell. The manganese requirement of the body ranges from 1.8-2.3 mg/day [26]. Typically vegetarian diets provide more manganese than non-vegetarian diets. Blueberries are a good source of manganese; 100g of fresh blueberries give 0.69 mg of manganese [19]. Banana (0.36 mg/100g) and *SZZ* fruits (0.356± 0.014 mg/100g) contain half the quantity of manganese in blueberries and can be considered reasonably good sources of manganese. Selenium is a trace element that is necessary for proper thyroid function and immunity. Fish and meat are rich sources of selenium. Vegan diets can also supply adequate amounts of selenium. The selenium requirement of the body is only 55µg/day. *SZZ* fruit samples did not contain selenium. In fruits like apple, orange and banana, selenium content is less than 0.5 µg/100g. Inadequate intake of chromium, a trace element can impair glucose and lipid metabolism. Studies have demonstrated that a diet rich in simple sugars can lead to excessive elimination of chromium through urine [28]. Brewer's yeast and broccoli are rich in chromium. *SZZ* fruits are good sources of chromium. Hundred grams of fresh *SZZ* fruits contain 0.24± 0.01 mg chromium. Trace element cobalt is a major component of vitamin B12 cynacobalamin. Thus it plays an important role in the formation of red blood cells (erythropoiesis). It is also needed for the proper functioning of pancreas. Green leafy vegetables are better sources of cobalt than meat. It is estimated that meat contains 7-25 µg/100g cobalt. *SZZ* fruits have 0.022± 0.011 mg/100g cobalt *i.e.* 22 µg /100g and can be considered a good source of cobalt.

Shilpa and Krishnakumar (2015) reported the mineral composition of *Syzygiumzeylanicum* fruits of Mangalore University Campus. The reported values for calcium (275.33 mg/100g), magnesium (458 mg/100g) and potassium (45.8 mg/100g) were exceptionally high. The copper (0.0019 mg/100g), iron (0.00532 mg/100g) and zinc (0.00204 mg/100g) content were very low and lower than the values reported in the present study. The difference in values may be attributed to the difference in soil composition.

5. Conclusion

The present study has established that *SZZ* fruits are reasonably good sources of vitamin A and vitamin K. Vitamin C is also present in appreciable amounts. *SZZ* fruits contain low levels of B vitamins (niacin, thiamine, riboflavin), vitamin D and vitamin E. Pyridoxine is completely absent. The sodium content is higher than in other fruits. The fruits of *SZZ* are also good sources of trace elements. The iron content is very high in *SZZ*. Along with mulberry and dates, the fruits of *SZZ* can be considered a good source of iron. The present study has revealed *SZZ* fruits to be an affordable source of micronutrients.

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7. References

1. The State of insecurity in the world, The multiple dimensions of food security, FAO, Rome, 2013.
2. Addressing the challenge of hidden hunger, Chapter 3, Global Hunger Index. The challenge of hidden hunger. International Food policy Research Institute. Welthungerhilfe. Concern Worldwide, 2014.
3. Pinstrup-Andersen P. Agricultural research and policy for better health and nutrition in developing countries: a food systems approach, Agricultural Economics. 2007; 37:187-198.
4. Weisstaub G, Araya M. Acute malnutrition in latin america: the challenge of ending avoidable deaths, J Pediatr Gastroenterol Nutr, 2008; l1:10-4.
5. Ratheesh Narayanan MK, Anilkumar N, Balakrishnan V, Sivadasan M, Alfarhan HA, Alatar AA. Wild edible plants used by the Kattunaika, Paniya and Kuruma tribes of Wayanad district, Kerala, India. Journal of medicinal plants research. 2011; 5(15):3520-3529.
6. Ratheesh Narayanan MK, Mithunlal S, Sujanapal P, Anil Kumar N, Sivadasan M, Alfarhan AH, *et al.* Ethnobotanically important trees and their uses by Kattunaika tribe in Wayanad Wildlife sanctuary, Kerala, India
7. Sadasivam S, Manickam R. Biochemical Methods. 3rdedition. New Age International Publications, New Delhi, India, 2010, 193-195.
8. Ball GFM, Vitamins in foods: Analysis, Bioavailability and Stability, Taylor and Francis group LLC, 2006.
9. U.S. Department of Agriculture, Agricultural Research Service. 20xx. USDA National Nutrient Database for Standard Reference, Release.

10. Nollet LML. Handbook of Food Analysis, Second edition, Volume I: Physical Characterisation and Nutrient Analysis, Marcel Dekker Inc., New York, 2004, 431-476.
11. Holick MF. Vitamin D deficiency, The New England Journal of Medicine, 2007, 357:266-281.
12. Factsheet Food sources of vitamin D, Dietitians of Canada, 2017.
13. Preedy VR, Watson RR. The encyclopedia of Vitamin E, CABI Publishing, 2007.
14. Packer L, Fuchs J. Vitamin E in Health and Disease: Biochemistry and Clinical Applications, Marcel Dekker, Inc., New York, 1993.
15. Sikorski ZE, Kolakowska A. Chemical and functional properties of food lipids, CRC Press LLC, FL, 2003.
16. Vitamin C - Health Professional Fact Sheet. https://ods.od.nih.gov/factsheets/VitaminC Health Professional/, 2017.
17. Shilpa KJ, Krishnakumar G. Nutritional, fermentation and pharmacological studies of *Syzygium caryophyllatum* (L.) Alston and *Syzygium zeylanicum* (L.) DC fruits, Cogent Food and Agriculture. 2015; 1:1018694.
18. Frieden E. The evolution of metals as essential elements [with special reference to iron and copper] In: Friedman M, editor. Protein-Metal Interactions. New York, NY, USA: Springer, Advances in Experimental Medicine and Biology, 1974; 48:1-31.
19. Nutrient analysis of fruit and vegetables Analytical Report, Department of Health, Government of UK, 2013.
20. Hardisson A, Rubio C, Baez A, Martin MM, Alvarez R. Mineral composition of the papaya (*Carica papaya* variety sunrise) from Tenerife Island. European Food Research and Technology. 2011; 212(2):175-81
21. Nabrzyski M. Functional Role of Some Minerals in Foods, In Szefer P, Nriagu J O, (Ed.), Mineral components in foods, CRC Press, 2007.
22. Spiegel DM. Normal and abnormal magnesium metabolism. In Schrier RW (Ed.) Renal and electrolyte disorder, 7th edition, LWW, 2010.
23. Boyle MA, Long S. Personal Nutrition. 8th edition. Wadsworth USA. 2013.
24. Kuo YM, Su T, Attieh Z, Syed BA, McKie AT, Ander-son GJ, *et al.* Mislocalisation of hephaestin, a multicopper ferroxidase involved in ba-solateral intestinal iron transport, in the sex linked anaemia mouse, Int. J. Gastroenterol. Hepatol. 2004; 53:201-206.
25. Araya M, Olivares M, Pizarro F. Copper in human health, Int. J. Environ. Health, 2007; 1:608-620.
26. Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, National academy of sciences.
27. Hambidge KM, Walravens PA. Disorders of mineral metabolism, Clin Gastroenterol. 1982; 11(1):87-117.
28. Kozlovsky AS, Moser PB, Reiser S. Anderson RA, Effects of diets high in simple sugars on urinary chromium losses, Metabolism, 1986; 35(6):515-518;