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Determination of ascorbic acid and its influence on the bioavailability of iron, zinc and calcium in Fijian food samples

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ABSTRACT

The total content of ascorbic acid (AA) determined in selected Fijian foods from different food groups of cereals [long grain rice, brown rice, white bread, wholemeal bread, weet-Bix, oats and flours], pulses [yellow split, black eye beans, chickpea, green gram/moong whole, bengal gram/chana dal, spilt red gram/toor dal, blue peas, red kidney beans], poultry/meat products [chicken breast, lamb chops, fish and egg] and dairy products [milk powders and cheese] was in the range 24.54 ± 1.37 to 1.87 ± 0.69 mg/100 g. Further, the influence of AA on the bioavailability of Fe, Zn and Ca was determined using *in vitro* gastrointestinal digestion method by addition of (5 or 10 mg) AA in 2 g food sample. Pulses showed low content of AA whereas green leafy vegetables showed high content but AA was not detected in cereals, poultry/meat and dairy products. Our study confirmed that AA in chickpea reduced Fe bioavailability from 38.69 to 22.77% but increased in red kidney beans from 1.04 to 11.57%, in green gram (whole moong) 1.79 to 6.48% and wholemeal bread 2.44 to 8.76% whereas bioavailability of Zn and Ca was increased.

1. Introduction

Iron (Fe), zinc (Zn) and calcium (Ca) are the most important minerals for the human body and play vital role in body regulatory functions such as development, disease prevention, wellbeing, *etc.* Low consumption of micronutrients has been reported to be the main cause of minerals deficiency in the world's population [1]. Fe has a vital role as an oxygen carrier in the blood and is also involved in DNA (Deoxyribonucleic acid) synthesis, energy production and metabolic processes. Zn is an essential trace mineral required by the human body with its significant role in cell growth and replication, bone formation, skin integrity, immune system function and sexual maturation [2,3]. Ca is the fifth most essential mineral that plays an important role in bone health, muscle contraction, blood clotting, nerve conduction, enzyme regulation, weight loss, *etc.* [4,5]. The bioavailability of these minerals is influenced by many factors which may enhance or inhibit their absorption from the human diet [6–10]. Generally, polyphenols and dietary fibers are the inhibitors while organic acids act as enhancers of the absorption [11]. Organic acids have significant influence on the bioavailability of polyphenols and dietary fibers in the grains [12].

Ascorbic acid (AA), also known as Vitamin C, is water soluble and easily oxidized by air, heat and light. The deficiencies of AA cause anemia, scurvy, muscle degeneration, poor wound healing, capillary hemorrhaging, and neurotic disturbances and can weaken the immune system [13,14]. Humans are not able to synthesize AA and thus its intake is completely dependent on dietary sources [14]. Good dietary sources of vitamin C are citrus fruits, green leafy vegetables (GLVs), fruits, peppers, strawberries, tomatoes, broccoli, sprouts and turnip. It also plays an important role in the metabolic function, bone formation, maintenance of healthy gums and wound healing [13]. In human body it acts as a cofactor for many enzymatic reactions as well as an antioxidant. Vitamin C rich diets have significant effect to prevent cardiovascular diseases and different types of cancers [13]. It also facilitates fat metabolism, which controls fatty plaques deposition on blood vessel walls. About 80–90% of AA is absorbed in gastrointestinal tract and circulates freely in plasma, red blood cell, and leukocytes and enters in tissues [13,14].

Enhancing effect of AA on mineral absorption has been observed by a few researchers [7,12,15,16]. It also has a good relationship with other nutrients where it increases Fe absorption, converts inactive forms of folic acid to active forms. It has a significant enhancing effect on dietary non-heme Fe but inhibits the dietary absorption of Cu [8]. However, no demonstrable effect on Zn absorption has been reported [7]. It has been reported that a small additional dose of AA increased 25% of heme Fe absorption in Fe deficient women in few weeks and also improved hemoglobin, erythrocyte, protoporphyrins and serum [17].

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Despite the intervention of mineral deficiency program, a problem of mineral deficiency still remains there for developing countries like Fiji and other Pacific Island Countries. It has also been reported that Fiji has been facing anemia problem since 1940. The report showed that 33–40% children and women were anemic out of which about 37% had multiple micronutrients deficiency [18]. Thus, we have been interested in enhancing intake for optimal nutrition and food security in the South Pacific [19]. We have reported the determination of the nitrate contents in commonly consumed fresh, cooked and frozen leafy, root and fruit vegetables marketed in Fiji [20–22]. In addition, we have also reported the retention of folate contents in vegetables upon different methods of cooking [23]. Therefore, in continuation of our studies on Fiji's foods with aim of enhancing the food security in the South Pacific [19] and given the fact that the bioavailability is an important factor as most recently reported by us [24], this study was initiated with the aim to assess the total content of AA in selected food samples consumed in Fiji and to find out whether addition of AA in food samples could increase Fe, Zn and Ca bioavailability.

2. Material and method

A total of 28 food samples were selected from different food groups consisting of cereals [long grain rice, brown rice (*Oryza sativa*), white bread, wholemeal bread, weet-Bix, oats and flours], pulses [yellow split, black eye beans (*Vigna unguiculata* subsp. *unguiculata*), chickpea (*Cicer arietinum*), green gram/moong whole (*Vigna radiata*), bengal gram/chana dal (*Cicer arietinum*), spilt red gram/toor dal (*Cajanus cajan*), blue peas (*Pisum sativum*), red kidney beans (*Phaseolus vulgaris*)], poultry/meat products [chicken breast (*Gallus gallus domesticus*), lamb chops (*Ovis aries*), fish (*Thunnini*) and egg], dairy products [milk powders and cheese]. These food samples were collected from different supermarkets in Suva, Fiji. Where required, the food samples were cooked. Spectrophotometric method for the determination of total AA in fruits and vegetables was utilized in this study with minor modifications [25].

2.1. Reagents and standards

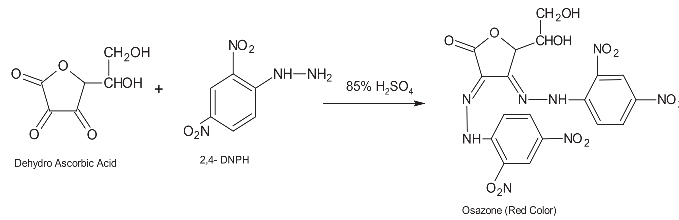
All reagents used in this study were of analytical reagent (AR) grade. All aqueous solutions were prepared in distilled de-ionized water (DDW) (Millipore 18 MΩ cm). Acetic acid (10%) was prepared using 50 mL in 500 DDW. Metaphospheric acid (5%) was prepared by dissolving 25 g into of 10% acetic acid. The stock solution of AA (500 mg/L) was prepared daily just before sample analysis. The accurately weighed 0.05 g of L-ascorbic acid (Sigma-Aldrich, USA) was taken in a 100 mL amber volumetric flask and dissolved in combined metaphosphoric acid (5%) and acetic acid (10%) solution already prepared. The working solution of AA was prepared in the range of 0 to 12 (mg/L) by diluting the stock solution with metaphosphoric acid and acetic acid (5% and 10%). Then 2,4-dinitrophenylhydrazine (2,4-DNPH) solution (2%) was prepared by dissolving 2 g in 100 mL of 4.5 M sulfuric acid (85%). The thiourea solution (4%) was prepared by dissolving 4 g in 4.5 M 100 mL H_2SO_4, filtered and kept in the refrigerator.

2.2. Sample preparation

Dry food samples (5 g) were homogenized with 25 mL solution of metaphosphoric and acetic acid (5% and 10%) and transferred into 50 mL volumetric flask. The solution prepared was gently shaken to homogenize and then diluted to 50 mL with metaphosphoric and acetic acid solution. It was than centrifuged for 15 min at 4000 rpm and the supernatant was filtered and collected to determine the total content of AA.

2.3. Determination of ascorbic acid

Addition of 3% bromine water oxidized AA to dehydroascorbic acid, which on reaction with 2,4-DNPH forms red color osazone in chilled 85% H_2SO_4. The reaction involved in the determination of AA is shown below.



Bromine water (0.23 mL) was added to 4 mL of the extracted sample and working standards to oxidize AA to dehydroascorbic acid. After 1 min, a few drops (0.13 mL) of 4% thiourea solution were added to remove the excess bromine from the extracted as well as the standard samples and a clear solution was obtained for the determination of AA. 2,4-DNPH solution (1 mL) was added to all the samples including standard solutions and the reagent blank to form osazone. All standard solutions, samples and the reagent blank were kept in thermostatic water bath for 3 h at 37 °C. Thereafter, all samples were kept in an ice bath for 30 min and then treated with 5 mL chilled 85% H_2SO_4 with twirling. It led to the formation of a red colored solution of osazone which absorbs at λ_{max} 521 nm. Thus, the absorbance of the osazone was measured at 521 nm using UV–visible spectrophotometer (Lambda 25, Perkin Elmer).

2.4. Effect of AA on bioavailability of Fe, Zn and Ca

The effect of AA on the bioavailability of Fe, Zn and Ca was studied in the selected food samples such as chickpea, green gram (whole moong), red kidney beans and wholemeal bread by adding AA to these food samples. To 2 g of each sample of the same food, 5 mg and 10 mg of AA was added separately. The food samples were then digested following simulated *in vitro* gastrointestinal digestion technique [24]. The homogenized raw and cooked each type of food samples (2 g) were taken in triplicate in centrifuge tubes and 25 mL of 0.5% pepsin-HCl acid solution was added to each. The content in each centrifuge tube was adjusted to pH 1.35 using 1% HCl solution and incubated at 37 °C in a shaker water bath for 90 min. After incubation, the pH of the samples was slowly adjusted to 7.5 using 20% NaOH solution. This was followed by the addition of trypsin (5 mg/g of sample) to each centrifuge tube content and further incubated for 90 min under the same conditions as done with pepsin *i.e.*, 37 °C. The contents were then centrifuged at 6000 rpm for 30 min. The supernatants were collected for *in vitro* determination of the bioavailability of Fe and Zn and Ca using flame atomic absorption spectrophotometer (AAS). The bioavailability (%) of Fe, Zn and Ca in the food samples was calculated using equation as shown below [24,26].

$$\text{Bioavailability}\ (\%) = \frac{\text{The fraction of the element } \textit{in vitro} \text{ digested sample} \left(\frac{\text{mg}}{100\text{g}}\right)}{\text{The total content of mineral} \left(\frac{\text{mg}}{100\text{g}}\right)} \times 100$$

3. Results and discussion

3.1. Total content of AA

The total contents of AA in the food samples of different food groups were determined by the UV–visible spectrophotometer [25]. The total contents of AA determined in different raw or cooked food samples are given in Table 1. Pulses/legumes showed a small concentration of AA whereas cereals, dairy and meat products did not show any AA concentration but the GLV's had relatively high content of AA. Yellow split peas and green gram (moong whole) showed 1.87 ± 0.69 mg/100 g and 11.88 ± 0.38 mg/100 g, respectively. Dalo leaves (boiled) and spinach (boiled) showed AA concentration as 24.54 ± 1.37 and 3.46 ± 1.27 mg/100 g, respectively. Black eye beans, chickpea, bengal gram (chana dal), split red gram (toor dal) and blue peas showed AA concentration as 4.31 ± 0.38, 4.05 ± 2.04, 8.06 ± 0.39, 3.39 ± 1.10 and 2.48 ± 0.02 mg/100 g, respectively in raw food samples.

The comparison of the present results with those given in literature is shown in Table 1 [27]. The concentration of AA in dalo leaves was found to be 24.54 mg/100 g while previous study showed 20.0 mg/100 g, which is close to our results. However, in case of spinach it showed low as 3.46 mg/100 g whereas previous study reported 16 mg/100 g [27]. Significant reduction in AA concentration may be due to different storage conditions, different temperatures and under different cooking methods [28]. Information of AA contents in some fruits and GLVs is available, however it lacks in cereals, pulses and legumes which is part of our everyday diet.

3.2. Cluster analysis

The cluster analysis of the studied food samples that showed the presence of AA was carried out using SPSS 21, dendrogram Ward linkage method. The results are presented in the form of the dendrogram. The dendrogram in Fig. 1 shows that the food samples made only one clade except one single leaf (simplicifolious). The split red gram (toor dal), spinach (boiled), black eye bean, chickpea and blue peas are on the first leaf of the clade and bengal gram, red kidney beans and green gram with closer content of AA are on the second leaf of clade. Dalo leaves (boiled) made simplicifolious with high content of AA as 24.54 ± 1.37 mg/100 g. Clade (leaf) 1 is from toor dal (split red gram) to blue peas have AA content 1.87 ± 0.69 to 4.31 ± 0.38 mg/100 g. Clade (leaf) 2 is from 7.40 ± 0.47 to 11.88 ± 0.38 mg/100 g having bengal gram to green gram (moong whole).

3.3. Effect of ascorbic acid on bioavailability Fe, Zn and Ca

The influence of AA on Fe, Zn and Ca was determined by adding 5 mg and 10 mg of AA in some selected food samples such as chickpea, red kidney beans, green gram (whole moong) and wholemeal bread while in each case the food sample taken was 2 g. The present study showed a wide difference in mineral absorption in the presence and absence of AA in studied food samples (Table 2). The data presented clearly showed that AA is a good enhancer of mineral bioavailability. The influence of AA on Fe, Zn and Ca from chickpea, red kidney beans, green gram (whole moong) and wholemeal bread is shown in Fig. 2 (A, B and C). The presence of AA in food samples showed a positive influence in mineral content absorption. Fig. 2A shows that the presence of AA increased Fe absorption in red kidney beans, green gram (whole moong) and wholemeal bread. Fe absorption increased on adding AA in food samples whereas chickpea showed negative influence. The negative influence of food acidulants on the bioaccessibility of Fe and Zn from selected food grains including chickpea has also been reported in

Table 1
Comparison of current and literature values of total ascorbic acid in selected raw food samples (mg/100 g).

Food samples	Scientific name	Ascorbic acid (mg/100 g)	Literature value (mg/100 g)	Reference
Cereals				
Long grain rice	*Oryza sativa*	ND	0.0	[27]
Brown rice	*Oryza sativa*	ND	0.0	[27]
Wholemeal bread	*Triticum*	ND	NR	
White bread	*Triticum*	ND	NR	
Weet-Bix		ND	0.0	[27]
Oats (rolled)	*Avena sativa*	ND	0.0	[27]
Normal flour (Fmf)	*Triticum*	ND	NR	
Normal flour	*Triticum*	ND	0.0	[27]
Roti atta (Fmf)	*Triticum*	ND	NR	
Wholemeal flour (Fmf)	*Triticum*	ND	NR	
Pulses/legumes				
Yellow split peas (decorticated)	*Pisum sativum*	1.87 ± 0.69	NR	
Black eye bean	*Vigna unguiculata*	4.31 ± 0.38	NR	
Chickpea	*Cicer arietinum*	4.05 ± 2.04	NR	
Green gram (whole moong)	*Vigna radiata*	11.88 ± 0.38	1.0	[27]
Bengal gram (decorticated)	*Cicer arietinum*	8.06 ± 0.39	NR	
Split red gram (decorticated) Toor dal	*Cajanus cajan*	3.39 ± 1.10	1.0	[27]
Blue peas	*Pisum sativum* var. *saccharatum*	2.48 ± 0.02	NR	
Red kidney beans	*Phaseolus vulgaris*	7.40 ± 0.47	NR	
Dairy products				
Milk powder (Red cow)		ND	NR	
Milk powder (Punjas)		ND	NR	
Milk powder (Rewa)		ND	NR	
Cheese pizza (Rewa)		ND	0.0	[27]
Poultry/meat products				
Chicken breast (Crest)	*Gallus gallus domesticus*	ND	0.0	[27]
Lamb chops	*Ovis aries*	ND	0.0	[27]
Fish (Tin Tuna)	*Thunnini*	ND	NR	
Egg		ND	0.0	[27]
Green leafy vegetable				
Dalo leaves (boiled)	*Colocasia esculenta*	24.54 ± 1.37	20.0	[27]
Spinach (boiled)	*Spinacia oleracea*	3.46 ± 1.27	16.00	[27]

Results are presented as mean ± SD of triplicate determinations.
ND: not detected.
NR: not reported.

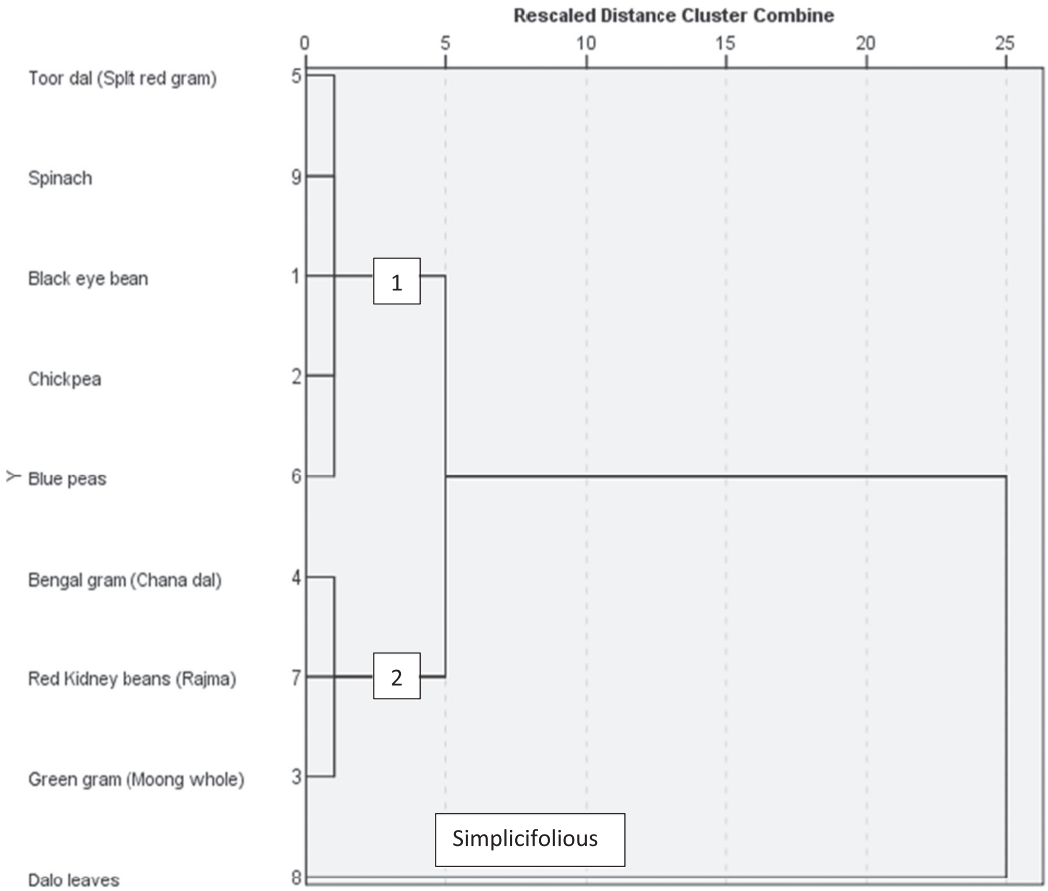


Fig. 1. Dendrogram of cluster analysis (ward linkage method) in selected raw food samples (mg/100 g).

literature by Hemalatha et al. [15]. However, a positive increase in Fe bioavailability was found after adding 10 mg of AA in presently studied food samples with an exception of chickpea [15].

The influence of AA on Ca absorption is presented in Fig. 2B. The addition of AA in selected food samples chickpea, red kidney beans, green gram (moong whole) and wholemeal bread showed an increase in Ca absorption. The total content of Ca in chickpea was 67.14 mg/100 g and after adding 5 mg and 10 mg AA, the content of Ca increased to 74.67 and 95.25 mg/100 g, respectively while in red kidney beans the Ca content increased from 106.31 to 151.18 mg/100 g. Fig. 2C shows the influence of AA on Zn absorption. The addition of AA (5 mg or 10 mg) considerably increased Zn in chickpea from 6.54 to 7.94 mg/100 g while in red kidney beans increased from 9.19 to 11.00 mg/100 g and in green gram (whole moong) from 9.69 to 11.71 mg/100 g. The Zn content in wholemeal bread showed a minor increase in the presence of AA. A small dose addition of AA in high fiber

Table 2
Effect of ascorbic acid on the bioavailability (%) of Fe, Zn and Ca in selected food samples and comparison.

Food samples	Bioavailability (%)			Bioavailability (mg/100 g)			Comparison			
							Literature value (%)			Reference
	Fe	Zn	Ca	Fe	Zn	Ca	Fe	Zn	Ca	
Chickpea	38.69	104.00	31.34	1.32	6.54	67.14	60.5	78.1	29	[29]
							6.9	NR	NR	[30]
							22.0	NR	NR	[26]
							36.9	NR	NR	[31]
Chickpea + 5 mg AA	14.89	126.00	34.86	0.51	7.94	74.64	NR	NR	NR	
Chickpea + 10 mg AA	22.77	122.97	44.46	0.77	7.73	95.25	NR	NR	NR	
Red kidney beans	1.04	119.00	65.65	0.05	9.19	106.31	NR	NR	NR	
Red kidney beans + 5 mg AA	11.57	127.99	93.36	0.60	9.82	152.18	NR	NR	NR	
Red kidney beans + 10 mg AA	0.78	143.37	54.78	0.04	11.00	88.71	NR	NR	NR	
Green gram (Whole moong)	1.79	463.71	31.73	0.04	9.69	46.74	20.9	NR	NR	[26]
							8.2	NR	NR	[31]
							2.3	NR	NR	[30]
Green gram + 5 mg AA	1.79	494.60	32.15	0.04	10.34	47.37	NR	NR	NR	
Green gram + 10 mg AA	6.48	560.19	42.02	0.14	11.71	61.90	NR	NR	NR	
Wholemeal bread	2.44	99.85	24.86	0.04	8.35	32.85	NR	NR	NR	
Wholemeal bread + 5 mg AA	2.44	99.68	32.57	0.04	8.33	43.04	NR	NR	NR	
Wholemeal bread + 10 mg AA	8.76	102.51	56.28	0.14	8.57	74.36	NR	NR	NR	

NR: not reported.

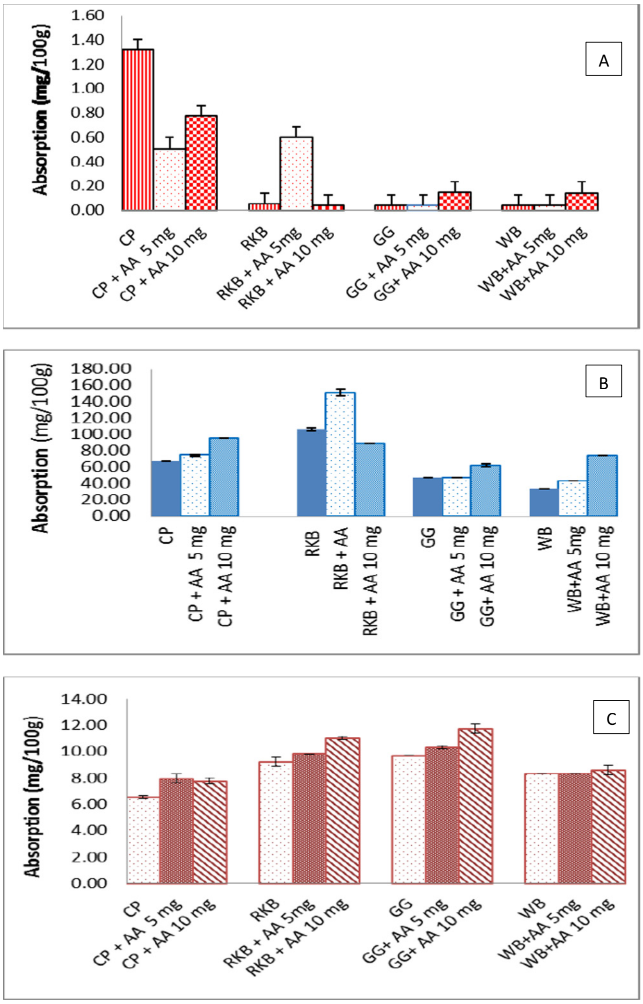


Fig. 2. Effect of ascorbic acid on the bioavailability of (A) iron, (B) calcium and (C) zinc (CP = chickpea, RKB = red kidney beans, GG = green gram (whole moong bean), WB = wholemeal bread and AA = ascorbic acid).

diet like wholemeal bread had no influence on Zn absorption [6]. Table 2 also shows a comparison of Fe, Zn and Ca bioavailability (%) after adding ascorbic acid. Present Fe and Ca bioavailability (mg/100 g) is quite similar to the literature reported values [25,29–31]. The comparison of the bioavailability of Fe, Zn and Ca with other reported results shows different from the present data. This may be due to different food structure and matrix, type of processing, geographical conditions, used chemicals, and different methods [32].

The ascorbic acid has high potential to improve Fe absorption whereas not much information is available for Zn and Ca [33,34]. It is a potent enhancer of non heme Fe, where absorption is directly proportional to the quantity of AA present in foods [35]. AA in amla (*Phyllanthus emblica*) is known to increase intestinal absorption of Fe [9] but addition of AA in normal diet did not affect the absorption of Zn and Ca [6]. It has also been reported that 0.5, 1.0 and 2.0 g dosages of AA had no effect on the inorganic Zn absorption [7]. However, high phytic acid content in breakfast cereals inhibited Fe absorption [36] but the molar ratio of AA to Fe as 4:1 strongly enhanced Fe absorption [37]. Fortified Fe compound absorption has also been reported to increase in the presence of AA [38]. This happens due to chelation of Fe in intestinal lumen which efficiently solubilizes and thus prevents binding with phytates and tannins [5]. On the addition of organic acid such as citric acid in raw and cooked grains foods the bioaccessibility of Fe increased from 30 to 86% and Zn bioaccessibility increased up to 40% in chickpea and rice [15].

Phytic acid and tannin are strong inhibitors of Fe, Zn and Ca absorption [15,30]. Whole grain foods are rich in phytate and tannin [15]. However, Abebe et al. [39] have reported that Southern Ethiopia people eat indigenous cereals with the combination of AA rich foods like tomatoes and kale which increase the solubility and absorption of minerals. Foods with high tannin and phytate showed negative effect on Fe and Zn absorption in the presence of promoters [16]. However, the inhibitory effect of tannic acid (polyphenol) and phytate on non-heme Fe absorption is reduced by AA [40]. Thus, meals in combination with organic acids improve Fe absorption. Fe fortified breakfast cereal with carotenoid rich meal improved the Fe status in women [41]. Vegetables with moderate or good bioavailability of Fe contained one or more organic acids such as malic, citric and AA [11]. Most recently, Jimenez-Aguilar and Grusak [42] has reported that commonly used amaranth leaves, rich in minerals and an excellent source of AA, help increase the bioavailability of Fe, Zn. Thus, the above discussion confirms that degradation of food phytase, tannic acid, improvement in daily diets and supplementation of minerals with AA would enhance essential minerals in human body [43].

4. Conclusions

The present study showed high content of AA in GLV's and low content in pulses and legumes but not detected in cereals, dairy and meat products. The influence of AA on the bioavailability of Fe and Ca in the studied foods showed the significant increase whereas relatively small increase was observed in case of Zn. Overall, the addition of AA in different amounts (5 mg or 10 mg) in selected food samples increased Fe, Zn and Ca bioavailability. The present data is expected to be very helpful for minerals fortification in Fiji foods in controlling the mineral deficiencies. This is the first study on the influence of AA on the bioavailability of Fe, Zn and Ca from the selected foods consumed in Fiji.

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