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In vitro bioavailability of calcium in products incorporated with green leafy vegetables

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Abstract
Plant foods high in calcium were collected from representative markets in Allahabad. The purpose of this research was to study the bioavailability of calcium in products prepared from green leafy vegetables. Products were prepared from green leafy vegetables (fenugreek and cauliflower leaves) respectively and analyzed for proximate composition, mineral, total carotene and *in vitro* calcium bioavailability (by equilibrium dialysis after simulated gastric digestion method). Prepared product were found rich in protein, calcium, iron, and total carotene content. *In vitro* high level of dialysable calcium was found 29.5 percent in paneer fenugreek pakoda and 15.7 percent in paneer cauliflower stuffed paratha. Thus Calcium bioavailability was found more in fenugreek leaves as compared to cauliflower leaves.

Keywords: *In Vitro*, Bioavailability, Calcium, Green Leafy Vegetables, Fenugreek Leaves, Cauliflower Leaves

1. Introduction
Calcium status is determined by both the total amount of calcium consumed and the bioavailability of calcium (i.e., absorption and/or utilization by the body) (Institute of Medicine, 2011) [4]. Both non dietary and dietary factors influence the bioavailability of calcium. Bioavailability is preferably determined by *in vivo* techniques, but these experiments are often unethical, rather complicated to perform, and yield variable results. *In vitro* methods are simple and rapid, and minor variations in food composition can be evaluated promptly and thoroughly. Therefore, these methods are useful to predict the bioavailability of nutrients (Bosscher, 2001) [3]. Leafy green vegetables are an important component of the human diet, providing fibre, minerals and vitamins and are low in calories. They are also a very good source of antioxidants (Acikgoz, 2011) [1]. Green leafy vegetables are good alternative sources of calcium, in addition to cow milk and fish with bones. The green leafy vegetables are commonly consumed by people but seeds and legumes are generally consumed by vegetarians. Cauliflower leaves, which are generally thrown away as waste, are a rich source of iron and beta carotene and can contribute these nutrients to the diet. These are cheap and within reach of common man. The leaves of cauliflower are available only for a short period but these can be dried or stored for use during lean season (Singh *et al*., 2005) [12]. Fenugreek leaves are known to be very high in iron as well as having significant levels of potassium, fibre, vitamin K and calcium. Fenugreek has good medicinal properties. Fenugreek leaves *(Trigonella foenum graceum*) are leguminous herb used as a diabetic folk medicine and anti-inflammatory agents (Vaidya *et al.*, 2008) [14]. Therefore, the objective of this research was to study the bioavailability of calcium from prepared products with green leafy vegetables.

2. Methodology
2.1 Materials
2.1(a) Experimental Site: The present investigation was carried out in the Nutrition Research Laboratory, Foods and Nutrition, Ethelind School of Home Science.

2.1(b) Sample collection: Fresh leaves of Fenugreek, Cauliflower and other ingredients required for the experiment were collected from the local market of Allahabad district. The leaves were carefully clean, sort to remove defective one of the lots graded according to the size and colour.

2.1(c) Development of Value Added Food Products: The fenugreek and cauliflower leaves were utilized in the preparations of two products with 6 replications of each product. For each product the basic recipes (control T_0) was three variations T_1, T_2, T_3 respectively where the amount of fenugreek and cauliflower leaves was varied. Table 1. Shows the details of control and treatment combinations.

Table 1: Details of control and treatment combinations

Sl No.	Ingredients	Treatments			
		T_0	T_1	T_2	T_3
1.	**Paneer pakoda (Fenugreek leaves)**				
	Besan	40%	30%	30%	30%
	Paneer	30%	20%	20%	20%
	Onion	30%	30%	20%	10%
	Fenugreek leaves	-	20%	30%	40%
2.	**Paneer Stuffed Paratha (Cauliflower leaves)**				
	Wheat Flour	80%	70%	60%	50%
	Paneer	20%	20%	20%	20%
	Cauliflower leaves	-	10%	20%	30%

2.1 (d) Organoleptic Evaluation: The organoleptic evaluation of prepared products were done by a panel of 5 judges to assess the acceptability of the products based on the various sensory attributes like colour, appearance, texture, flavour and taste. The evaluation was done on the 9 point hedonic scale based score card (Srilakshmi, 2007) [11].

2.1(e) Statistical analysis: The obtain data was analyzed by using mean, standard deviation and t- test. (Gupta *et al*., 2002).

2.2 Methods

2.2 (a) Nutritional Composition of the Value Added Food Products Developed By Green Leafy Vegetables

Protein content was done by AOAC, using standard procedure Lowery, (1951) [7].

Mineral Analysis- 5 g of sample was ashed and extracted with dilute HCL. Suitable aliquots were used for the estimation of iron and calcium.

Iron was estimated by the colorimetric method (AOAC, 2005) [2] which is based on ferric iron giving a red color was measured with in 20 minute at 560 nm.

Calcium was estimated by titrimetric method (AOAC, 2005) [2]. It was precipated calcium oxalate and was titrated with standard potassium permanganate to definite pink color persisting for at least 1 min.

Total carotene of the sample was estimated using the method prescribed by (Rangnna, 1997) [10].

2.2 (b) Evaluation of Calcium Bioavailability by in *Vitro* Simulated Gastro Intestinal Method: Food items with a high calcium content (analysed by AAS) were further evaluated for calcium bio availability by the *in vitro* simulated gastrointestinal digestion method of Miller *et al*., (1981) [8]. The method included enzymatic digestion of a food sample under simulated gastrointestinal condition, followed by measuring of the released calcium which diffused from the sample to a dialysis bag. The amount of dialysable calcium (analyzed by AAS), expressed as a percentage of total amount of calcium present in the test sample, was used as an indicator of calcium bioavailability. Fig 1 shows the flow diagram of the *in vitro* calcium digestibility.

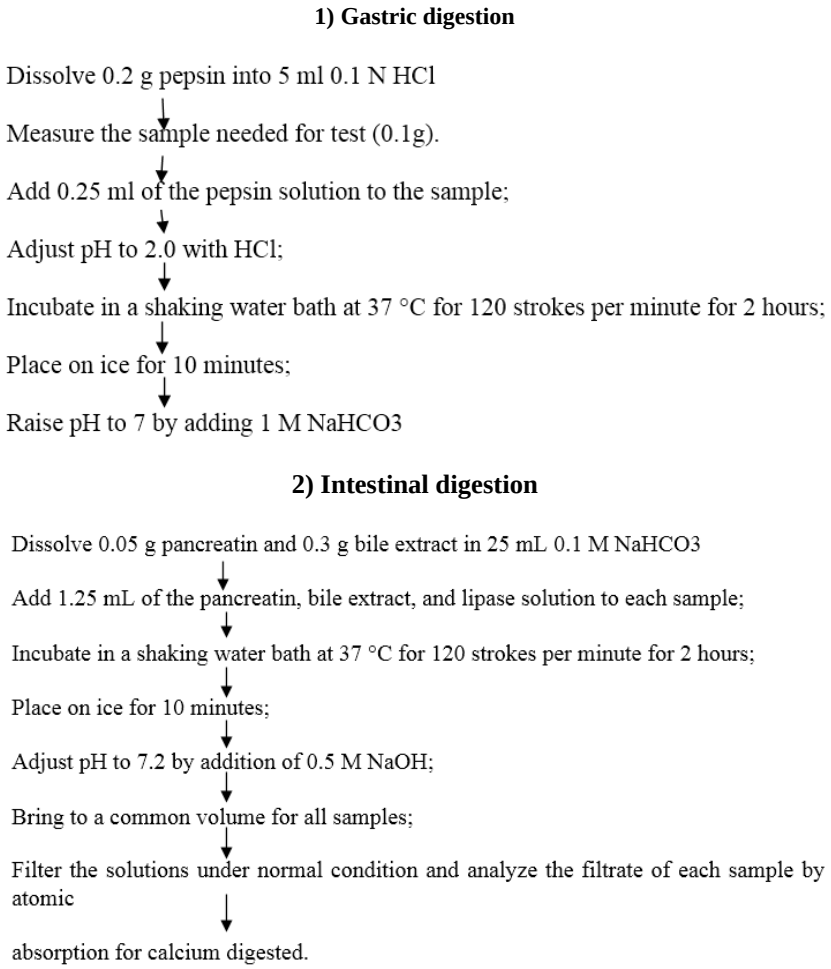

Fig 1: A diagram for an in-vitro digestibility of calcium

3. Results and Discussion

Table 2. Shows using the fresh fenugreek and cauliflower leaves the two value added products for each leaves respectively were prepared and to analyze the proximate protein, calcium, iron and total carotene. Protein content was found approximately similar in both prepared products. Calcium and iron content was highest in *paneer stuffed paratha* T_2 (305.66± 2.8mg/100g) (10.92± 0.29mg/100g) respectively. Total carotene content was found highest in *paneer pakoda* incorporated with fenugreek leaves T_2 (3447.10±123.06µg/100g) followed by *paneer stuffed paratha* incorporated with cauliflower leaves T_2 (39.2±0.32µg/100g) in proximate and mineral composition of the product T_2 was the best product to analyze. This result is supported by the findings of Singh *et al*., (2007) [13]. Result showed that iron content increased as the incorporation level of dehydrated bathua leaves increase in making paratha T_3 i.e. (15% incorporation level) which was also acceptable at sensory score 6.62±0.11 has got highest iron content that is 8.81mg/100g.

Table 2: Average nutrients content in best treatment of prepared products incorporated with green leafy vegetables

Nutrients	*Paneer Pakoda (Fenugreek leaves)*	*Paneer Paratha (Cauliflower leaves)*
Protein (g)	12.04± 0.27	12.65±0.25
Calcium (mg)	300.59± 1	305.66± 2.8
Iron (mg)	2.35± 0.14	10.92± 0.29
Total carotene (µg)	3447.10±123.06	39.2± 0.32

Bioavailability of calcium of the products prepared by incorporating green leafy vegetables (per 100 g)

The *in vitro* method for estimating availability of essential minerals has gained popularity as it is a simple, reliable, rapid and inexpensive method for predicting the availability of minerals. The *in vitro* method can be applied to study both calcium bioavailability and the factors that affect calcium bioavailability in various kinds of foods. With the incorporation of fresh green leafy vegetables (fenugreek leaves and cauliflower leaves) the two different products (*paneer pakoda and paneer paratha* respectively) were prepared and then the *in vitro* bioavailability of calcium were analysed. Table 1 shows the results which revealed that total calcium content in *paneer pakoda* was found highest in best treatment (T_2) 302.68±1mg/100g than control (T_0) 273.47±2.1mg/100g which was incorporated with fenugreek leaves. Total calcium content in *paneer stuffed paratha* incorporated with cauliflower leaves was found highest in best treatment (T_2) 310.66±2.8mg/100 g than control (T_0) 201.1±7.0mg/100 g. our result shows values for calcium concentration in prepared products similar to those found in the study performed by Kumar *et al.,* (2004).

The dialyzability of calcium in *paneer pakoda* incorporated with fenugreek leaves was found highest in best treatment (T_2) 29.5±4.2 and lower in control (T_0) 15.2±2.13 and the dialyzability of calcium in *paneer paratha* was found highest in best treatment (T_2) 15.7±3.56 and least in control (T_0) 9.3±5.5 incorporated with cauliflower leaves. Cauliflower leaves have also good calcium digestibility. Table 2 shows the comparision between the control and best treatments of preparead products. Our result shows values for calcium concentration in prepared products similar to those found in the study performed by Kamchan *et al.,* (2004) [5]. Bioavailability can be described as that portion of a nutrient that can be used. Consequently, the term bioavailability can be used in large concept including digestion, absorption, and incorporation into metabolic process. It can also be used in a narrow sense, meaning that any potentially available part of a nutrient after gastrointestinal digestion should be attributed to its bioavailability. These values were similar those reported by Lucarini *et al.*, (1999) [6] who applied the same *in vitro* method and found the percent dialyzable calcium of *brassica* vegetables ranging from 22.9 percent to 28.9 percent. Similar findings were found in Chinese spinach (Amaranth 5.1 percent calcium absorption) by Weaver *et al*., (1997) [15] using intrinsically labeled ca^{45} *in vivo* method which was lower from the present study on green leafy vegetable's products. Our technique consists of an intra aluminal digestion phase followed by continuous- flow dialysate is completely absorbed in the upper gastrointestinal tract. The gradual pH change from acid to neutral and the continous removal of dialyzed components mimics the passage of chime through the gut and the one way lumen to the mucosal cell pathway for absorption of nutrients in the upper gut. This study also supported with (Bosscher, *et al*., 2001) [3] who studied on *In vitro* availability of calcium, iron, and zinc from first-age infant formulae and human milk. These values were similar those reported by Nalwade *et al*., (2008) [9] who applied the same in vitro method of iron and calcium from 14 leafy vegetables. Calcium nutritional status among some groups in India is suboptimal when judged by calcium intakes and the high prevalence of osteoporosis. Increases in calcium intake will have a significant impact on osteoporosis or other chronic diseases that have been linked to calcium nutriture. *Paneer pakoda* and *paneer stuffed paratha* incorporated with fenugreek leaves and cauliflower leaves reaspectively can help in improving the calcium deficiency in India. In interior areas like villages, peoples can easily accept these good calcium bioavailability prepared products due to feasibility of the product and can combat with the calcium deficiency and related deficiency from calcium.

Table 3: *In vitro* bioavailability of calcium of the products prepared by incorporating green leafy vegetables (per 100 g)

Sr. No.	Products and Treatments	Total calcium (mg) (Mean ±S.E.)	% Dialyzable calcium (Mean ±S.E.)
1.	**Paneer pakoda incorporated with fenugreek leaves**		
	T_0	273.47±2.1	15.2±2.13
	T_2	302.68±1	29.5±4.2
2.	**Paneer paratha incorporated with cauliflower leaves**		
	T_0	201.1± 7.0	9.3±5.5
	T_2	310.66± 2.8	15.5±3.56

± significant error % percentage

Table 4: Comparison between the dialyzable calcium of control and best treatment by using (t) test

Products	T_0	T_2	Difference (T_0-T_2)	T Calculated	T Tabulated	Result
Paneer pakoda	15.2	29.5	-14.3	-429.999	4.303	S
Paneer paratha	9.3	15.5	-6.2	-63.000	4.303	S

S - Significant

4. Conclusion and Recommendations

It is concluded that incorporation of fenugreek leaves and cauliflower leaves in products like *paneer stuffed paratha* and *paneer pakoda* were rich in protein, calcium, iron, and total carotene content. Calcium bioavailability was found more in fenugreek leaves as compared to cauliflower leaves. we can conclude that this *in vitro* method is inexpensive, simple, rapid and reliable and can be used as an index to predict the bioavailability of essential elements of different foods and thus these food products prepared from green leafy vegetables should be included in the diet to overcome various nutritional problems like, iron, calcium and vitamin A deficiency.

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