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In vitro study on the bioavailability of calcium and its absorption inhibitors in raw and cooked pulses commonly consumed in India

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Abstract

Pulses are commonly consumed among Indian people as sources of minerals. The objectives of this research were to assess the content of total and bioavailable calcium, inhibiting factors such as oxalate, phytate, tannin and dietary fiber and effect of cooking on bioavailable calcium in the commonly consumed pulses by Indians. The bioavailability of calcium was studied by an *in vitro* method using equilibrium dialysis after simulating gastric digestion. Horse gram and soya bean with the highest calcium content showed that the lowest calcium solubility and bioavailability percentage. The high inhibiting factors content in horse gram and soya bean favors calcium precipitation and thus decreases its bioavailability. The multiple regression analysis was carried out to explain the significant influence of inhibitors on calcium bioavailability. As per multiple regression analysis, the influence of oxalate, phytate, tannin and dietary fiber, revealed that negative correlation between bioavailable calcium.

Introduction

Calcium is essential for structural integrity of teeth, for the strength of bones, and is also vital in regulating critical functions including nerve impulses, muscle contractions and the activities of enzymes. Interest in dietary calcium has intensified -in recent years as a result of scientific evidence linking it to osteoporosis, fluorosis, hypertension, reduced ability of blood clotting and cancer. The average recommended dietary allowance or adequate intake of calcium is about 900 mg/day (800-1000 mg/day, depending on the country) for adults, rising to 1200 mg/day for adolescents and the elderly (FAO/WHO 2001). Dietary mineral intake is important for human beings in general, particularly for the periods of maximum growth, such as in childhood, adolescence and also during lactation (Bosscher *et al.*, 1998; Unal *et al*., 2005).

Plant foods have almost all of the mineral and organic nutrients established as essential for human nutrition. The calcium intake depends on its concentration in plant foods, but is also greatly influenced by its bioavailability from foodstuffs. The exact level of calcium and their inhibitors will depend upon the individual plant-based foods consumed and their relative proportions in the diet (Kamchan *et al*., 2004; Judprasong *et al.*, 2006; Ghavidel *et al*., 2007). In India, most of the people adopt a vegetarian type of food and heavily depends on cereals and pulses for their diet needs. Around 20 pulses are used as dry grains in appreciable amounts for human nutrition. Among these pulses selected in this study are the most popular, widely consumed, and having been considered as the basic food of the population, both rural and urban areas. However, their role appears to be limited because of several factors including poor calcium bioavailability, thermal processing and / or thermal treatment and high anti-nutritional factors, such as oxalate, phytate, tannin and dietary fiber, which are part of the food and, having a varied nature, exert a toxic or anti-nutritional action; the interactions of all these inhibitors are determinative of the bioavailability of calcium in the pulses (Kamchan *et al*., 2004; Ghavidel *et al*., 2007; Sotelo *et al*., 2010).

Oxalate is widely distributed in plant foods, which are recognized as inhibitors of mineral bioavailability. Phytate is a common constituent of plant-derived foods and acts as chelating agent and forms complexes with several divalent cations of major nutritional significance, such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and Mn^{2+} (Abebe *et al*., 2007). The formation of chelates may reduce the bioavailability of dietary calcium. Tannins are astringent, bitter tasting plant polyphenols, reduce the bioavailability of minerals, mainly calcium through chelation and inhibit the activities of digestive enzymes (Marin

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et al., 2009). Dietary fiber include cellulose, hemicellulose, pectins, gums, and mucilages, which are biosynthesized from pentose, hexose, and uronic acid, and lignin built from phenylpropane units such as cinamyl and couramyl alcohols. Dietary fiber can bind with calcium and may decrease available calcium or lead to negative calcium balance. The potential adverse effect of dietary fiber is probably due to the formation of stable complexes with calcium owing to the anionic character of dietary fiber (Idouraine *et al*., 1996; Elleuch *et al*., 2011).

Bioavailability can be defined as the fraction of the ingested nutrient that is absorbed and utilized for normal physiological functions or storage. It should be determined by *in vivo* methods, but these experiments are labour-intensive, time-consuming and often unethical. Several researchers have developed reliable *in vitro* methods which are proven to give results relating well with *in vivo* tests (Miller *et al*., 1981; Luten *et al*., 1996; Camara *et al*., 2005).

In the present study, pulses samples were collected from the fluorotic villages of Dindigul district of South India. As per Tamil Nadu government records, around 313 villages of Dindigul district are severely affected by different forms of fluorosis. The severity of fluorosis can be reduced by oral administration of calcium. Intake of sufficient amount of calcium through diet reduces the toxic effect of fluoride. Commonly consumed pulses in India were screened through a nutritional survey, for their calcium contents and bioavailability by measuring *in vitro* dialyzability. Bioavailability of calcium in pulses is influenced by various inhibitors like oxalate, phytate, tannins and dietary fiber. Information on the bioavailability of calcium as well as the role of inhibitors from pulses commonly consumed in India is limited. In addition, less is known about the effect of cooking on bioavailable calcium. In this study, bioavailable calcium of the food grains was studied and correlated with the concentrations of inhibitors.

Materials and Methods

Materials

Commonly consumed pulses collected from households of fluorotic villages as well as purchased from different supermarkets of Dindigul District were used. All chemicals used in the experiments were of analytical (AR) grade and were obtained from Sigma Aldrich India Ltd. Millipore - MilliQ distilled water was used for the entire study.

Preparation of the samples

Nine pulses were selected and they are Bengal gram (*Cicer arientinum L.*), Black gram (*Vigna mungo L.*), Green gram *(Vigna radiate)*, Horse gram *(Macrotyloma Uniflorum),* Red gram *(Cajanus cajan (L.) millsp)*, Cowpeas *(Vigna unguiculata)*, Green peas *(Pisum Sativum)*, Green bean *(Dolichos lablab)* and Soya bean *(Glycine max (L.) Merrill).* They were cleaned and washed with Millipore water. Thoroughly drained pulses were separated into two parts. One part was cooked using Millipore - MilliQ distilled water in microwave oven until the water was evaporated and marked. The cooked as well as fresh samples were dried in glass dishes in a hot air oven at 50±5^{0}C. The dried samples were ground to fine powder, stored in airtight containers and were used for the estimation of total, soluble and bioavailable calcium as well as oxalate, phytate, tannin and dietary fiber.

Chemical analysis

Determination of total calcium content

Finely ground pulses samples were ashed in a muffle furnace at 550^{o}C for 10 h and the ash was dissolved in conc. HCl. Calcium content was determined by atomic absorption spectrometer (Perkin – Elmer A Analyst 100). Lanthanum chloride (1%) was added to the mineral solution to avoid interference from phosphate. Calibration of the instrument was performed using commercial standards. All measurements were carried out using standard flame operating conditions, as recommended by the manufacturer.

Evaluation of calcium solubility and bioavailability by in vitro simulated gastrointestinal method

Bioavailability of calcium from selected pulses samples were determined by an *in vitro* method described by Camara *et al.* (2005). The procedure was followed as gastric stage and intestinal stage.

Gastric stage

Ten grams of each pulse were homogenized with 90 g of MilliQ water, and the pH was adjusted to 2.0 with 6 N HCl. To carry out pepsin–HCl digestion, 0.5 g of pepsin solution per 100 g of sample was added. The mixture was then incubated for 2 h at 37^{0}C in a shaking water bath.

Intestinal stage

A dialysis tube (molecular mass cut-off value 12000–14000 Da) containing 25 ml of water and an amount of $NaHCO_3$ equivalent to the titrable acidity was placed in the flasks, together with 20 g aliquots of the pepsin digest. Incubation was continued for

45 min, the pancreatic-bile salt mixture (5 ml) was added, and incubation was continued up to 2 h. After incubation, the segments of dialysis tubes were removed from the flasks, washed and weighed. The titratable acidity was defined as the number of equivalents of NaOH required to titrate the combined pepsin digest pancreatin–bile salts mixture to pH 7.5. The calcium content of the dialysis tubes were analyzed by atomic absorption spectrophotometry (AAS) (Perkin–Elmer Analyst 100).

The soluble calcium percentage (S%) is calculated using the formula %S = [D + Sup /T] x 100 and bioavailability (B%) was calculated as follows: bioavailability (B%) = 100 x D/T, where, D is the calcium content in the dialyzable portion for the bioavailable fraction (mg calcium /100 g pulses), Sup (mg calcium /100 g pulses) the calcium content of the supernatants obtained by centrifugation of the gastrointestinal digestion products, and T is the total calcium content (mg calcium/100 g pulses).

Determination of inhibitory factors

The pulses were analyzed for total oxalates and soluble oxalates by precipitation with calcium oxalate from deproteinized extract and subsequent titration with potassium permanganate (AOAC, 2000). Phytate was extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and the phytate phosphorous content calculated from this value assuming a constant 4Fe:6P molecular ratio in the precipitate. The phytate was estimated by multiplying the amount of phytate phosphorous by a factor of 3.55 based on the empirical formula $C_6 P_6 O_{24} H_{18}$ (Wheeler and Ferrel 1971). Tannin compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of the color is proportional to the amount of tannins. The intensity was measured in a UV-Vis spectrophotometer at 700 nm (Schanderl, 1970). Moisture free pulse samples were analyzed for their TDF, IDF and SDF contents by enzymatic and gravimetric method of the AOAC (2000), using TDF-100 kit obtained from Sigma Aldrich chemical company, India. Along with the test samples, blank and reference samples were also analyzed simultaneously in duplicate for comparison. TDF, IDF and SDF contents of the selected pulses analyzed were calculated and expressed on dry weight basis.

Quality control

To eliminate the risk of contamination, all glasswares used were immersed in 10% (v/v) solution of nitric acid for 24 h, washed with Millipore - MilliQ distilled water before use. For calcium, oxalate, phytate and tannin, recovery of added material was found to be 96.6%, 97.8%, 98.6% and 97.7%, respectively. For dietary fiber, along with samples, blank and reference controls were analyzed in duplicate for comparison. Accuracy and reproducibility of the method for calcium, oxalate, phytate and tannin were checked by adding two levels of known concentration of calcium, oxalic acid, phytic acid and tannic acid (10 and 20 mg L^{-1}) to selected pulses. Pulses were analyzed for three different days for five replicates.

Statistical analysis

All determinations were done in five replicates, the average values, the mean and standard error of mean values were calculated. The data were also analyzed statistically by multiple regression tests, to find out the influence of inherent phytate, oxalate, tannin and dietary fiber on calcium bioavailability using the statistical software SPSS 16 program and Origin 6.

Results and Discussion

The total calcium content in the analyzed raw and cooked pulses ranged from 36.8 mg/100g and 40.5 mg/100g in green peas to 177.9 mg/100g and 180.6 mg/100g in horse gram (Table 1). Pulses in general had high amount of bioavailable calcium. Soluble and bioavailable calcium percentage was calculated in the selected pulses and horse gram registered the lowest 45.2% and 22.2% respectively. Among the selected pulses, green peas registered the highest percentage of soluble and bioavailable calcium that is 58.2% and 40.4% respectively. Bioavailability of calcium from cooked pulses ranged from 22.6% (horse gram) to 41.3% (green bean). The results are comparable with findings of Ghavidel *et al*., 2007.

All selected pulses had comparatively same level of total, soluble and bioavailable calcium in raw as well as in cooked form and little increase was observed in cooked ones. Soluble and bioavailable calcium in the selected pulses in both raw and cooked forms showed a significant ($P<0.01$) correlation between the total calcium content. Among the pulses, the lowest bioavailability of calcium was registered by horse gram, soya bean and black gram although they had the highest concentration of total calcium, and the highest bioavailability of calcium was observed in the case of green peas, green bean and red gram, despite their low concentration of total calcium, indicating that calcium bioavailability is not necessarily dependent on the total concentration of

Table 1. Total, Soluble and bioavailable calcium content in selected pulses

Pulses	Total		Soluble		Soluble %		Bioavailable		Bioavailable %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Bengal gram	65.9 ± 0.5	66.3 ± 0.4	45.3 ± 0.6	46.0 ± 0.6	68.7	69.4	23.0 ± 1.8	24.1 ± 0.6	35.0	36.3
Black gram	109.9 ± 0.8	110.8 ± 0.4	72.0 ± 0.8	74.4 ± 0.7	65.5	67.2	27.6 ± 0.6	28.1 ± 0.5	25.1	25.4
Green gram	79.5 ± 1.1	80.2 ± 0.3	54.9 ± 1.7	56.4 ±1.1	69.1	70.4	24.1 ± 0.5	25.6 ± 0.7	30.3	32.0
Horse gram	177.9 ± 1.7	180.6 ± 0.4	80.5 ± 1.0	82.0 ± 0.5	45.2	45.4	39.5 ± 0.8	40.9 ± 0.4	22.2	22.6
Red gram	73.4 ± 1.3	75.1 ± 0.7	47.5 ± 0.9	49.5 ± 0.8	64.7	65.9	27.0 ± 0.7	27.8 ± 0.8	36.8	37.0
Cowpeas	84.6 ± 1.3	86.3 ± 0.7	42.1 ± 0.9	44.0 ± 1.2	49.8	51.0	28.0 ± 0.9	30.0 ± 0.8	33.1	34.7
Green peas	36.8 ± 1.0	40.5 ± 0.5	22.5 ± 0.5	24.1 ± 0.3	58.2	59.5	15.7 ± 0.5	15.9 ± 0.4	40.4	39.3
Green bean	69.5 ± 1.1	71.0 ± 0.6	36.2 ± 0.8	38.8 ±0.5	52.1	54.6	26.3 ± 0.3	29.3 ± 1.2	37.9	41.3
Soya bean	149.4 ± 0.9	151.1 ± 0.4	81.2 ± 0.9	82.1 ± 0.6	54.3	54.2	37.3 ± 0.6	41.0 ± 0.4	25.0	27.1

Values (mg/100 g) are means ± SD of five independent determinations

Table 2. Total, soluble and insoluble oxalate content in selected pulses

Pulses	Total		Soluble		Soluble %		Insoluble		Insoluble %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Bengal gram	2.6 ± 0.2	1.7 ± 0.2	0.9 ± 0.1	0.4 ± 0.1	34.4	21.2	1.7 ± 0.2	1.3 ± 0.2	65.6	78.8
Black gram	12.6 ± 0.2	7.5 ± 0.3	7.5 ± 0.2	2.8 ± 0.4	59.6	37.2	5.1 ± 0.3	4.7 ± 0.5	41.4	62.8
Green gram	2.5 ± 0.3	1.6 ± 0.1	1.2 ± 0.3	0.6 ± 0.1	46.8	39.7	1.3 ± 0.4	0.9 ± 0.2	53.2	60.3
Horse gram	23.4 ± 0.7	12.4 ± 0.4	10.9 ± 0.4	4.5 ± 0.3	46.6	36.1	12.5 ± 0.6	7.9 ± 0.6	53.4	63.9
Red gram	9.5 ± 0.3	4.9 ± 0.4	4.3 ± 0.1	2.1 ± 0.3	45.5	42.8	5.2 ± 0.4	2.8 ± 0.6	55.5	57.2
Cowpeas	7.5 ± 0.3	2.9 ± 0.3	4.4 ± 0.2	1.9 ± 0.2	59.2	63.7	3.1 ± 0.3	1.0 ± 0.4	40.8	36.3
Green peas	12.5 ± 0.2	5.2 ± 0.3	5.4 ± 0.2	2.2 ± 0.1	43.3	43.0	7.1 ± 0.3	3.0 ± 0.2	56.7	57.0
Green bean	9.3 ± 0.3	4.6 ± 0.5	3.4 ± 0.2	1.6 ± 0.2	36.9	33.8	5.9 ± 0.4	3.0 ± 0.4	63.1	66.2
Soya bean	20.5 ± 0.3	11.1 ± 0.4	5.5 ± 0.3	2.9 ± 0.2	26.9	26.2	15.0 ± 0.3	8.2 ± 0.6	73.1	73.8

Values (mg/100 g) are means ± SD of five independent determinations

calcium in the pulses (Table 1).

Components such as, oxalate, phytate, tannin and dietary fiber negatively affect calcium bioavailability (Kennefick *et al*., 2000; Camara *et al*., 2005). The presence of these inhibitors could explain the relatively low bioavailable percentages obtained from selected pulses. Pulses analyzed contained trace amount of total oxalate content, except horse gram and soya bean. The results of oxalate content of raw and cooked pulses are given in Table 2. The levels of total oxalate in raw pulses varied between 2.5 mg/100g in green gram to 23.4 mg/100g in horse gram and in cooked pulses it varied between 1.6 mg/100g in green gram to 12.4 mg/100g in horse gram. The levels of soluble oxalate in raw pulses ranged from 0.9 to 10.9 mg/100g. Among the raw pulses studied, bengal gram recorded the lowest (0.9 mg/100g) level of soluble oxalate. The soluble oxalate contents in cooked pulses ranged from 0.4 mg/100g in bengal gram to 4.5 mg/100g in horse gram.

Cooking treatment was found to be an effective measure to reduce the oxalate content in the pulses. The contents of total, soluble and insoluble oxalate in pulses registered significant reduction $P< 0.01$ due to cooking by boiling. The percentage of reduction of total oxalate content ranged from 33.6 % in Bengal gram to 60.9% in cowpeas and in the case of soluble oxalate the reduction was registered as 46.6% in green gram to 63.0% in black gram. The higher percentage of oxalate reduction during boiling may be due to its solubility in boiling water (Judprasong *et al*., 2006; Sotelo *et al*., 2010).

In spite of high total calcium content horse gram and soya bean recorded low soluble and bioavailable percentage of calcium (Table 1). The high oxalate content in horse gram (23.4 mg/100g) and soya bean (20.5 mg/100g) could have precipitated calcium precipitation and thus decreased its bioavailability (Table 2). Kennefick *et al.* (2000) observed that oxalate addition to semi synthetic diets decreased

Table 3. Phytate and Tannin content in selected pulses

Pulses	Phytate		Tannin	
	Raw	Cooked	Raw	Cooked
Bengal gram	598.3 ± 2.8	600.3 ± 2.2	215.5 ± 2.2	215.1 ± 2.2
Black gram	891.1 ± 2.5	892.7 ± 1.9	275.8 ± 1.4	274.5 ± 1.9
Green gram	683.8 ± 1.5	689.3 ± 2.0	263.7 ± 2.8	261.2 ± 1.9
Horse gram	589.9 ± 1.6	594.2 ± 2.8	414.3 ± 2.1	413.0 ± 1.6
Red gram	624.5 ± 1.4	626.3 ± 1.8	231.0 ± 2.3	230.0 ± 1.8
Cowpeas	594.9 ± 1.5	600.4 ± 2.6	286.7 ± 2.8	285.8 ± 1.5
Green peas	216.9 ± 2.6	219.4 ± 1.9	387.3 ± 2.9	386.1 ± 2.0
Green bean	752.9 ± 1.3	753.8 ± 2.8	287.3 ± 2.9	285.4 ± 1.6
Soya bean	891.8 ± 1.5	892.3 ± 1.9	325.3 ± 3.2	322.8 ± 1.9

Values (mg/100 g) are means ± SD of five independent determinations

calcium bioavailability in a dose dependent manner. Oxalate can have injurious effects on human nutrition and health particularly by decreasing calcium absorption by forming calcium oxalate. Therefore, reduction in oxalate content while cooking pulses can have positive impact on the health of consumers. Statistically significant differences $P< 0.01$ were found in the total, soluble and insoluble oxalate content among the raw and cooked pulses.

Phytic acid has strong ability to chelate multivalent metal ions, especially calcium, which results in poor bioavailability of such ions as they are precipitated in the form of insoluble complexes as suggested by Zhou *et al*., (1995) Ma *et al*., (2005) Gupta *et al.* (2006) and Ghavidel *et al*., (2007). This study also supports their view. The phytate values are shown in Table 3. The levels of phytate in pulses ranged from 216.9 mg/100g in green peas to 891.8 mg/100g in soya bean in raw pulses and 219.4 mg/100g in green peas to 892.7 mg/100g in black gram in cooked pulses. These results were in agreement with those reported earlier by several researchers (Ma *et al*., 2005; Ghavidel *et al.*, 2007). Green peas with lowest total calcium content showed higher soluble and bioavailable percentage of calcium. Among the pulses examined soya bean and black gram contained the highest amount of phytate but showed the lowest soluble and bioavailable percentage of calcium, due to the inhibitory effect of phytate on calcium bioavailability, which is also pointed out by Frontela *et al.* (2011). Cooking did not significantly change the phytate content of pulses. Phytate is heat-stable, so significant reduction during cooking or any conventional heat-processing method is not expected. During cooking endogenous phytases are inactivated by heat. They are, therefore, unavailable to breakdown phytate which can then only be degraded by high temperature processing, which may not be possible in normal cooking (Yadav *et al*., 2003).

The tannin content of the pulses varied largely and the values are shown in Table 3. The tannin content of the pulses ranged between 215.5 and 414.3 and 215.1 and 413.0 mg/100g in raw and cooked pulses. Cooking process did not significantly affect the tannin content of all selected pulses which was also observed by Sotelo *et al.* (2010). Tannin content of bengal gram and horse gram registered low and high values respectively.

Dietary fiber, an essential non-nutrient component shown in Table 4, is known to interfere in trace mineral absorption. The TDF, which is calculated as the sum of IDF and SDF ranged between 9.9 g/100g (Bengal gram) to 28.4 g/100g (soya bean) and 15.1 g/100g (Bengal gram) to 35.5 g/100g (soya bean) in raw as well as cooked pulses respectively. The SDF content of cooked pulses was higher than raw ones.

Comparison of analyzed raw and cooked pulses showed that proportion of IDF was higher than SDF and the percentage ranged from 81.5% to 92.2 % and 84.5% to 92.9% of the TDF in raw and cooked pulses respectively. The remaining portion comprised of the SDF. Bednar *et al.* (2001) and De Almeida Costa *et al.* (2006) also found very low values of SDF in comparison with IDF. Results confirmed that IDF contents represent the greatest part of the TDF of the pulses.

Difference between IDF, SDF and TDF contents of both raw and cooked pulses was significant ($P<0.001$). Similar results were reported by other researchers also (Ghavidel *et al.*, 2007; Hemalatha *et al*., 2007). Ramulu *et al.* (1997) have reported that cooking cereals and pulses resulted in a significant ($p<0.01$) increase in their TDF and IDF contents. High values observed in the case of cooked pulses may be due to the formation of resistant starch (Ramulu *et al.*, 1997).

Multiple regression analysis was carried out to explain the significant influence of inhibitors on calcium bioavailability Table 5. In the analyzed pulses, horse gram had highest total calcium content and green peas had highest bioavailable percentage of calcium. Significant correlations ($p<0.01$) between total calcium and soluble calcium ($r = 0.94$ and 0.93) as well as dialyzable calcium fractions ($r = 0.96$ and 0.94) of raw and cooked pulses respectively were found. Soluble and bioavailable calcium found to increase with the total calcium content of the selected pulses. As per data from multiple regression analysis, the influence of oxalate, phytate, tannin and dietary fiber, revealed negative correlation between bioavailable calcium percentage and the above mentioned inhibitors (Table 5).

Several reports show a negative correlation of

Table 4. Total, soluble and insoluble dietary fibers in selected pulses

Pulses	IDF		SDF		TDF		SDF %	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Bengal gram	8.4 ± 0.8	13.1 ± 1.3	1.4 ± 0.5	2.1 ± 0.6	9.9 ± 1.5	15.1 ± 0.9	14.4	13.6
Black gram	17.6 ± 0.6	23.8 ± 1.7	1.5 ± 0.7	2.6 ± 0.4	19.1 ± 1.5	26.3 ± 1.5	8.0	9.7
Green gram	23.5 ± 1.3	29.3 ± 1.8	2.4 ± 0.6	2.9 ± 0.6	25.9 ± 1.8	32.2 ± 1.1	9.4	9.1
Horse gram	15.4 ± 0.9	21.0 ± 1.5	2.6 ± 0.8	2.9 ± 0.7	18.1 ± 1.6	23.9 ± 1.7	14.5	12.1
Red gram	11.9 ± 1.5	16.8 ± 1.9	2.7 ± 0.9	3.1 ± 0.9	14.6 ± 1.8	19.9 ± 1.1	18.5	15.7
Cowpeas	19.0 ± 1.2	21.1 ± 1.2	2.5 ± 0.5	2.8 ± 0.7	21.4 ± 1.5	23.9 ± 1.5	11.5	11.9
Green peas	16.2 ± 0.8	18.9 ± 1.6	1.6 ± 0.6	2.5 ± 0.6	17.7 ± 0.9	21.4 ± 1.7	8.9	11.7
Green bean	16.0 ± 0.7	22.8 ± 1.0	2.5 ± 0.5	3.2 ± 1.2	18.5 ± 0.8	25.9 ± 1.3	13.7	12.2
Soya bean	25.0 ± 1.2	31.6 ± 1.6	3.5 ± 0.7	3.9 ± 1.1	28.4 ± 1.5	35.5 ± 1.8	12.2	11.1

Values (g/100 g) are means ± SD of five independent determinations

Table 5. Regression equations and correlation coefficients for influence of various inhibitors in relation to calcium bioavailability of selected pulses

Y	X	Regression equation	Correlation coefficient
Raw pulses			
Bioavailable calcium	Total oxalate	Y = 36.95-0.524X	-0.554
	Soluble oxalate	Y = 37.39-1.274X	-0.583
	Insoluble oxalate	Y = 35.28-0.668X	-0.462
	Phytate	Y = 44.64-0.020X	-0.622
	Tannin	Y = 39.09-0.027X	-0.263
	TDF	Y = 41.60-0.510X	-0.519
	SDF	Y = 38.91-3.344X	-0.333
	IDF	Y = 40.80-0.531X	-0.512
Cooked pulses			
Bioavailable calcium	Total oxalate	Y = 38.63-1.153X	-0.639
	Soluble oxalate	Y = 39.70-3.597X	-0.639
	Insoluble oxalate	Y = 37.53-1.530X	-0.607
	Phytate	Y = 43.63-0.017X	-0.511
	Tannin	Y = 42.22-0.034X	-0.324
	TDF	Y = 45.34-0.507X	-0.530
	SDF	Y = 37.14-1.759X	-0.134
	IDF	Y = 44.57-0.537X	-0.541

phytate, oxalate and dietary fiber contents of different pulses with the percentage of calcium bioavailability and also showed that the unabsorbable complexes of calcium with uronic acid in hemicellulose fraction of dietary fiber and with phytic acid and oxalic acid reduce the bioavailability of calcium (Kamchan *et al.*, 2004; Judprasong *et al.*, 2006; Ghavidel *et al.*, 2007). High negative correlation coefficient values of bioavailable calcium between oxalate, phytate, tannin and dietary fiber in all samples presented in Table 5 also support the findings in this regard.

Conclusions

To conclude, this study was carried out in the fluorotic area of the Dindigul district Tamil Nadu. The inhibition of fluoride absorption by calcium would take place through the formation of a chemical complex, this effect of calcium is generally attributed to the formation of insoluble CaF_2 within the intestinal lumen by consuming diet rich in calcium. From this study, we found that high content of inhibiting factors such as oxalate, phytate, tannin and dietary fiber decreases bioavailable calcium in the commonly consumed pulses. Among the pulses analyzed, the lowest bioavailability of calcium was observed in horse gram, soya bean and black gram in spite of their high total calcium content, and the highest bioavailability of calcium in the case of green peas, green bean and red gram, with comparatively lower concentration of total calcium indicated that calcium bioavailability is not necessarily depend on the total concentration of calcium in the pulses. Horse gram and soya bean with high calcium content showed lower calcium solubility and bioavailability percentage, which is attributed to high content of inhibiting factors. The multiple regression analysis was carried out to explain the significant influence of inhibitors and it revealed a negative correlation between bioavailable calcium and inhibiting factors.

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References

Abebe, Y., Bogale, A., Hambidge, K.M., Stoecker, B.J., Bailey, K. and Gibson, R.S. 2007. Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability. Journal of Food Composition and Analysis 20(3-4): 161–168.

AOAC. 2000. Official methods of analysis Washington, DC. Association of Official Analytical Chemists, (17th Ed.).

Bednar, G.E., Patil, A.R., Murray, S.M., Grieshop, C.M., Merchen, N.R. and Fahey, G.C. 2001. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability *in vitro* in a canine model. Journal of Nutrition 131(2): 276-286.

Bosscher, D., Dyck, K.V., Robberecht, H., Caillie-Bertrand, M.V. and Deelstra, H. 1998. Bioavailability of calcium and zinc from cow's milk-based versus soya-based infant food. International Journal of Food Sciences and Nutrition 49(4): 211-283.

Camara, F., Amaro, M.A., Barbera, R. and Clemente, G. 2005. Bioaccessibility of minerals in school meals: Comparison between dialysis and solubility methods. Food Chemistry 92(3): 481–489.

de Almeida Costa, G.E., Queiroz-Monici, K.S, Machado Reis, S.M.P. and Oliveira, A. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chemistry 94(3): 327-330.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. and Attia, H. 2011. Dietary fibre and fibre-rich by-products of food processing: characterization, technological functionality and commercial applications: a review. Food Chemistry 124(2): 411 – 421.

FAO/WHO. 2001. Human Vitamin and Mineral Requirements, 2nd ed. Geneva, Switzerland.

Frontela, C., Ros, G. and Martínez, C. 2011. Phytic acid content "in vitro" iron, calcium and zinc bioavailability in bakery products: the effect of processing. Journal of Cereal Science 54(1): 173-179.

Ghavidel, R.A. and Prakash, J. 2007. The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT 40(7): 1292–1299.

Gupta, S., Lakshmi, A.J. and Prakash, J. 2006. *In vitro* bioavailability of calcium and iron from selected green leafy vegetables. Journal of the Science of Food and Agriculture 86(13): 2147–2152.

Hemalatha, S., Platel, K. and Srinivasan, K. 2007. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. Food Chemistry 102(4): 1328–1336.

Idouraine, A., Khan, M.J. and Weber, C.W. 1996. In Vitro Binding Capacity of Wheat Bran, Rice Bran, and Oat Fiber for Ca, Mg, Cu, and Zn Alone and in Different Combinations. Journal of Agricultural and Food Chemistry 44(8): 2067-2072.

Judprasong, K., Charoenkiatkul, S., Sungpuag, P., Vasanachitt, K. and Nakjamanong, Y. 2006. Total and soluble oxalate contents in Thai vegetables, cereal grains and legume seeds and their changes after cooking. Journal of Food Composition and Analysis 19(4): 340–347.

Kamchan, A., Puwastien, P., Sirichakwal, P.P. and Kongkachuichai, R. 2004. *In vitro* calcium bioavailability of vegetables, legumes and seeds. Journal of Food Composition and Analysis 17(3-4): 311–320.

Kennefick, S. and Cashman, K.D. 2000. Investigation of an *in vitro* model for predicting the effect of food components on calcium availability from meals. International Journal of Food Sciences and Nutrition 51(1): 45– 54.

Luten, J., Crews, H., Flynn, A., Dael, P.V., Kastenmeyer, P., Hurrel, R., Deelstra, H., Shen, L.H., Fairweather-Tait, S.J., Hickson, K., Farre, R., Schlemmer, U. and Frohlich, W. 1996. Inter laboratory trial on the determination of *in vitro* dialysability from food. Journal of the Science of Food and Agriculture 72(4): 415-424.

Ma, G., Jin, Y., Piao, J., Kok, F., Guusje, B. and Jacobsen, E. 2005. Phytate, Calcium, Iron, and Zinc Contents and Their Molar Ratios in Foods Commonly Consumed in China. Journal of Agricultural and Food Chemistry 53(26): 10285-10290.

Marin, A.M.F., Siqueira, E.M.A. and Aarrud, S.F. 2009. Minerals, phytic acid and tannin contents of 18 fruits from the Brazilian savanna. International Journal of Food Sciences and Nutrition 60(7): 177-187.

Miller, D.D., Schricker, B.R., Rasmussen, B.S. and Van Campen, D. 1981. An *in vitro* method for estimation of iron availability from meals. American Journal of Clinical Nutrition 34(10): 2248-2256.

Ramulu, P. and Rao, R.U. 1997. Effect of proccesing on dietary fiber content of cereals and pulses. Plant Foods for Human Nutrition 50(3): 249-257.

Schanderl, S.H. 1970. In: Method in Food Analysis, Academic Press, New York. 709.

Sotelo, A., Gonzalez-osnaya, L., Sanchez-chinchillas, A. and Trejo, A. 2010. Role of oxate, phytate, tannins and cooking on iron bioavailability from foods commonly consumed in Mexico. International Journal of Food Sciences and Nutrition 61(1): 29–39.

Unal, G., El, S.N.S. and Kilic, S. 2005. *In vitro* determination of calcium bioavailability of milk, dairy products and infant formulas. International Journal of Food Sciences and Nutrition 56(1): 13-22.

Wheeler, E.L. and Ferrel, R.E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chemistry 48: 312 – 320.

Yadav, S.K. and Sehgal, S. 2003. Effect of domestic processing and cooking on selected antinutrient contents of some green leafy vegetables. Plant Foods for Human Nutrition 58(3): 1–11.

Zhou, J.R. and Erdman, J.W. 1995. Phytic acid in health and disease. Critical Reviews in Food Science and Nutrition 35(6): 495–508.