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In vitro calcium availability from *brassica* vegetables (*Brassica oleracea* L.) and as consumed in composite dishes

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Abstract

In vitro calcium availability from four varieties of *Brassica oleracea* L. (broccoli, cauliflower, green cabbage and kale) was evaluated. The effect of including some *brassica* vegetables in composite dishes (macaroni and broccoli, macaroni and cauliflower) was also studied. *Brassica* vegetables were rich in calcium (20.6–35.3 mg/100 g) and in organic acids. Dietary fibre content in *brassica* vegetables (2.4 g/100 g) was lower than in composite dishes, the latter having a higher content of the soluble fraction. Uronic acids represented 50% of the soluble fibre fraction of *brassica* vegetables and only 24% of that of composite dishes. Approximately 25% of the total calcium of *brassica* vegetables was dialysable; ionic dialysable calcium was about 7% of the total calcium. The addition of macaroni to vegetables significantly lowered only calcium dialysability ($p < 0.001$). Unlike vegetables, composite dishes had a percentage of soluble calcium higher ($p < 0.001$) than that of dialysable calcium. The presence in dialysates of higher amounts of bound calcium compared to free ionic calcium implies that most of the absorbable calcium was bound to low molecular weight compounds. Organic acids might constitute the main association of calcium in *brassica* vegetables. *Brassicaceae* can be regarded as a good source of available calcium; their consumption in the diet may contribute to an adequate calcium nutriture.

Keywords: Calcium; Availability; Brassica vegetables

1. Introduction

Increased calcium availability can be achieved by including, in the meal, foods having a high calcium availability, as well by reducing the presence in the meal of those factors inhibiting calcium absorption. It is widely assumed that calcium absorption from milk and dairy products is markedly higher than from other foods or inorganic calcium salts (Smith, Kolars, Savaiano, & Levitt, 1985; Sheikh, Santa Ana, Nicar, Schiller, & Fordtran, 1987; Recker, Bommi, Barger-Lux, & Heaney, 1988). Vegetables are another important source of dietary calcium, especially in Italy where the consumption of vegetables is quite high (about 290 g/person/die) (Turrini, Saba, & Lintas, 1991). However, calcium absorption from vegetables is generally considered low because they contain substances (phytate, oxalate, dietary fibre components) which bind calcium in unabsorbable compounds. Studies have clearly shown that foods with high concentrations of oxalic acid and phytic acid may strongly reduce calcium availability (Weaver, Martin, Ebner, & Krueger, 1987; Heaney, Weaver, & Fitsimmons, 1991; Lombardi-Boccia, Lucarini, Di Lullo, Ferrari, Del Puppo, & Carnovale, 1998). Dietary fibre is another food constituent interacting with minerals with consequence on their absorption. Among dietary fibre components, pectins have been shown to bind calcium. The ability of pectin to complex ions depends on the negatively charged uronic acid residues (James, Branch, & Southgate, 1978). Uronic acids are therefore the major component of dietary fibre involved in the binding of calcium.

Most literature data on calcium availability derives from in vivo studies; little work has been done to evaluate, in vitro, the availability of calcium, especially from vegetable foods (Rejkdal & Lee, 1991; Lombardi-Boccia et al., 1998). The present investigation aimed to evaluate the in vitro potential calcium availability from four varieties of *Brassica oleracea* L. (broccoli, cauliflower, green cabbage and kale). *Brassica* vegetables were chosen for two reasons: (i) *brassica* vegetables are of great

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importance in Italy, being consumed not only as vegetables but also in main courses, together with macaroni, according to traditional recipes, (ii) in order to estabilish a comparison between results deriving from in vitro experiments and those deriving from in vivo studies, *brassica* vegetables being a subject of some human studies (Heaney & Weaver, 1990; Heaney, Heaney, Weaver, Hinders, Martin, & Packard, 1993). In addition, particular attention is focused now on *Brassica oleracea* L. because much evidence suggests that *brassica* vegetables possess anticarcinogenic properties. Two bioactive sulfur-containing phytochemicals in *Brassica oleracea* L., glucosinolates and S-methyl cysteine sulfoxide, appear to have potential value as cancer chemopreventive agents (Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997).

In this study measures of dialysable (D), soluble (S), ionic dialysable (ID) and ionic soluble (IS) calcium were used as indicators of the potential calcium availability. Because the availability of minerals from a single food or when included in a meal can vary greatly, we also studied the effect of including *brassica* vegetables in composite dishes prepared following traditional Italian recipes (macaroni and broccoli, macaroni and cauliflower).

2. Materials and methods

Brassica vegetables (broccoli, cauliflower, cabbage, kale) and macaroni were purchased locally.

All reagents were of analytical grade and deionized water was used throughout. Glassware was acid-washed in concentrated HCl and rinsed with deionized water.

2.1. Cooking procedures

About 800 g of vegetables were washed with deionized water and then pressure-cooked for 8 min in deionized water (150 ml). Cooked vegetables were freeze-dried before subsequent analysis. Composite dishes were prepared by boiling 80 g of macaroni (durum wheat semolina) in 1 L of deionized water with 2 g NaCl; macaroni was then strained and mixed with cooked broccoli or cauliflower (110 g, raw weight). The composite dishes were then freeze-dried.

2.2. Calcium analysis

This was performed by atomic absorption spectrometry on a Varian SpectrAA 400 following liquid ashing of the samples (4 ml HNO_3 + 1 ml H_2O_2) in a microwave digestion system. A nitrous oxide–acetylene flame was used in order to avoid the formation of refractory compounds, and 0.2% caesium chloride was added to samples to suppress ionization. Certified Standard NBS 1567a, wheat flour, (National Bureau of Standards, Gaterborough, MD 20899), was analysed as a check on the accuracy of the analysis. Experimental values were not statistically different from certified values (19.2 ± 0.05 mg/100 g; 19.1 ± 0.4 mg/100 g, respectively).

2.3. Dietary fibre, uronic acids

Total, soluble and insoluble dietary fibre and uronic acid contents were determined in duplicate using the method of Englyst and Cummings (1988). Cabbage Reference Samples from the Dietary Fibre Collaborative trial, Part IV (MAFF Trial 48) was used as check on the accuracy of the analysis.

2.4. Organic acids

Analyses were performed by HPLC. Samples were extracted in 0.05N H_3PO_4 and then centrifuged at 2000×***g***. The supernatant was filtered through a 0.45 μm filter (Millipore Corporation, Bedford, MA) and 20 μl injected into an HPLC system. A Waters instrument equipped with a 510 pump model was used. Absorbance (220 nm) was measured by a model 481 Absorbance detector, connected to a Millennium 2010. Separation was performed with a 5 μm Hypersil ODS C18 column (250×4.5 mm, Sigma Aldrich, USA). The HPLC solvent system was potassium dihydrogen orthophosphate buffer (0.015 M, pH 2.8). Flow rate was 0.8 ml min^{-1}. Identification and quantification was done by comparison of sample peaks with those of external standards.

2.5. Dialysable calcium (D)

This was assessed by using the in vitro method of Miller, Schricker, and Cederblad (1981). Aliquots of samples were blended with 0.1 N HCl, the pH was adjusted to 2.0 ± 0.05, and 5 ml of pepsin solution (10 g pepsin in 100 ml 0.1 N HCl) were added. The final volume of the homogenates was brought to 100 g by adding deionized water, and the samples were incubated at 37°C for 2 h in a shaking water bath. Aliquots (20 g) of the pepsin digests were transferred into 100 ml beakers. Segments of dialysis sac (MW cut-off 6-8000 Spectrapor I, Spectrum Medical Industries, Los Angeles, CA), containing 0.5 N $NaHCO_3$ (in a volume tested in a previous trial to give a pH 7.5) and sufficient deionized water to obtain the volume of 20 ml, were placed in each beaker and incubated for 30 min. When the pH reached 5.0, 5 ml of a pancreatin-bile solution (0.8 g pancreatin, 5 g bile in 200 ml 0.1 N $NaHCO_3$) were added and the incubation continued for a further 2 h. The dialysates were weighed and the amount of calcium in the dialysis bag was expressed as percentage of the total calcium.

2.6. Soluble calcium (S)

This was determined in the retentates deriving from the in vitro gastro-intestinal digestion. At the end of the simulated gastro-intestinal digestion the retentates were centrifuged at 3500×*g* for 20 min. Supernatants were weighed, and analysed for calcium content by Atomic Absorption Spectrometry. Soluble calcium was expressed as percentage of total calcium.

2.7. Ionic calcium

Ionic-dialysable calcium (ID) and ionic-soluble calcium (IS) were determined by a calcium selective electrode (Model 93-20, Orion, Boston, MA). Ionic strength of standards and samples was adjusted to 0.08 M with KCl before measurements. Ionic strength adjustor (4 M KCl) and 0.1 M $CaCl_2$ standard solution were purchased from Orion (Boston, MA). ID and IS calcium were expressed as percentages of total calcium.

Statistical analysis was by one-way analysis of variance and Duncan's multiple range test was used to separate significantly different means (Duncan, 1955).

3. Results and discussion

All selected vegetables were rich in calcium (Table 1), with broccoli and kale showing the highest values. Composite dishes (macaroni and broccoli, macaroni and cauliflower) had slightly higher amounts of total calcium compared to broccoli and cauliflower. Cauliflower had the highest amount of both citric and malic acids (Table 1). The amounts of the organic acids detected in composite dishes were strictly dependent on the vegetable ingredients. Table 2 shows the dietary fibre and uronic acids content of the samples. *Brassica* vegetables were similar in dietary fibre content (about 2.4 g/100 g) and composition. All of them contained high amounts of uronic acids in the soluble fibre fraction, representing about 50% of this fraction. This finding is consistent with data from Southgate (1987) who reported that uronic acids constitute about 45% of the non-cellulosic fraction in vegetables and fruits. In composite dishes, the total dietary fibre content was slightly higher than that reported for *brassica* vegetables; these values reflected a higher content of the soluble fibre fraction (Table 2). Nevertheless, the uronic acids content of this fibre fraction was approximately half the amount determined in *brassica* vegetables, representing only 24.4% of the soluble fibre fraction. This was dependent on the contribution of macaroni to the dietary fibre composition of composite dishes; cereals indeed contain very low amounts of uronic acids; these were estimated to be about 10% of the non cellulosic fibre fraction (Southgate, 1978).

Table 1
Calcium, citric acid and malic acid contents in cooked *brassica* vegetables and composite dishes (mg/100 g, f.w.)

Food source	Total Ca	Citric acid	Malic acid
Vegetables			
Broccoli	35.3 ± 2.1	15.0 ± 2.8	65.5 ± 10.1
Cauliflower	20.6 ± 1.8	64.0 ± 4.2	74.0 ± 9.8
Cabbage, green	22.8 ± 1.9	49.0 ± 8.0	68.0 ± 10.8
Kale	34.2 ± 2.2	21.1 ± 1.9	41.0 ± 2.7
Composite dishes			
Macaroni and broccoli	37.5 ± 2.1	7.8 ± 3.2	34.2 ± 7.4
Macaroni and cauliflower	32.5 ± 1.8	41.9 ± 6.4	38.2 ± 9.0

Each value represents the Mean ± SD of three determinations.

Percentages of dialysable (D), ionic-dialysable (ID), soluble (S) and ionic-soluble (IS) calcium for both cooked vegetables and composite dishes are given in Table 3. In *brassica* vegetables approximately 25% of the total calcium was dialysable (D), with kale showing the highest value ($p < 0.05$). Ionic dialysable calcium (ID) ranged from 5.7 to 8.1% of the total calcium. The finding that the percentage of dialysable calcium (D) was higher than the percentage of ionic dialysable calcium (ID), indicated that in vitro digestion released, more than ionic calcium, high amounts of calcium bound to low molecular weight compounds. The percentage of soluble calcium (S) in digested vegetables was slightly higher than that of dialysable (D) calcium, only for kale was a significant difference found ($p < 0.05$). The amounts of ionic soluble calcium (IS) were similar to those reported for ionic dialysable calcium (ID). The similar concentration found for both total (D and S) and ionic (ID and IS) calcium on both sides of the dialysis membrane indicated that, during the in vitro digestion, the dialysis equilibrium was reached.

The addition of cereal products (macaroni) to vegetables significantly lowered ($p < 0.001$) only calcium dialysability (D; Table 3). Unlike vegetables, composite dishes had a percentage of soluble calcium (S) significantly higher ($p < 0.001$) than that of dialysable calcium (D). On the other hand, ionic dialysable calcium (ID) and ionic soluble calcium (IS) were found to be in dialysis equilibrium (Table 3). The finding that, in composite dishes, soluble calcium (S) was higher than dialysable calcium (D) indicated that not all soluble complexes containing calcium were dialysable, and that some calcium was likely bound to soluble complexes of molecular weight higher than 8000 Dalton. These higher molecular weight soluble calcium complexes found in composite dishes might still be absorbable. The same finding was reported in a previous study dealing with calcium dialysability from beans (Lombardi-Boccia et al., 1998). This could be due to differences in the chemical form of calcium between seeds (cereal, legume) and green vegetables.

Table 2
Total, soluble and insoluble fibre and uronic acids content in cooked *brassica* vegetables and composite dishes (g/100 g, f.w.)

Food source	Dietary fibre	Soluble fibre		Insoluble fibre	
	Total	Total	Uronic acids	Total	Uronic acids
Vegetables					
Broccoli	2.3 ± 0.4	1.0 ± 0.1	0.58 ± 0.2	1.3 ± 0.3	0.09 ± 0.3
Cauliflower	2.4 ± 0.1	1.1 ± 0.4	0.60 ± 0.1	1.3 ± 0.5	0.06 ± 0.1
Cabbage, green	2.5 ± 0.8	1.0 ± 0.5	0.59 ± 0.1	1.5 ± 0.2	0.09 ± 0.1
Kale	2.3 ± 0.1	0.9 ± 0.2	0.45 ± 0.2	1.4 ± 0.4	0.05 ± 0.1
Composite dishes					
Macaroni and broccoli	2.6 ± 0.1	1.4 ± 0.1	0.33 ± 0.04	1.2 ± 0.1	0.08 ± 0.07
Macaroni and cauliflower	2.6 ± 0.1	1.3 ± 0.1	0.33 ± 0.01	1.3 ± 0.1	0.04 ± 0.02

Determined on duplicate samples (Mean ± SD).

Table 3
In vitro dialysable (D), ionic-dialysable (ID), soluble (S) and ionic-soluble (IS) calcium in cooked *brassica* vegetables and composite dishes (fresh basis)

Food source	D Ca (%)	ID Ca (%)	S Ca (%)	IS Ca (%)
Vegetables				
Broccoli	22.9 ± 1.3a	8.1 ± 0.9a	27.5 ± 4.4a	6.9 ± 1.3a
Cauliflower	23.4 ± 1.6a	8.0 ± 2.2a	27.1 ± 5.1a	8.8 ± 2.5a
Cabbage, green	24.8 ± 0.8a	5.7 ± 1.2a	28.5 ± 1.3a	4.7 ± 0.5a
Kale	28.9 ± 1.4b	6.9 ± 1.1a	39.7 ± 0.7a	10.9 ± 3.2a
Composite dishes				
Macaroni and broccoli	18.7 ± 0.3c	10.1 ± 1.1a	32.0 ± 8.4a	12.5 ± 5.7a
Macaroni and cauliflower	19.0 ± 0.7c	12.9 ± 1.5a	29.2 ± 6.0a	15.0 ± 1.9a

Each value represents the Mean ± SD of three determinations.
Values, in the same column, with different letters are significantly different (a vs b, $p < 0.05$; a and b vs c, $p < 0.001$).

Brassica vegetables are essentially phytate- and oxalate-free vegetables; therefore dietary fibre components and organic acids are the constituents that could mainly influence calcium availability. James et al. (1978) showed that the binding by the non-cellulosic fraction of dietary fibre will reduce the absorption of calcium from the small-intestine. In in vitro experiments, in which pectin degradation does not take place, we expected a negative effect on calcium availability. This did not occur. In *brassica* vegetables, therefore, the uronic acids content, though high, seems do not impair calcium availability. Benway and Weaver (1993) demonstrated that pectinase treatment of kale did not liberate appreciable amounts of calcium. Probably a high percentage of uronic acids in *brassica* vegetables are methyl-esterified; therefore the high level of calcium dialysability found in this study might be because, in these vegetables, little calcium was bound to uronic acids. On the other hand, the presence in dialysates of higher amounts of bound calcium compared to free ionic calcium (Table 3), suggests that most of the potentially absorbable calcium was bound to low molecular weight complexes. Thus components, such as organic acids, may act as promoters of calcium availability (Table 1). Organic acids in *brassica* vegetables could likely bind calcium and, consequently, might be responsible for the high calcium dialysability determined in these vegetables. Studies carried out on calcium fortification of foods with citrate and citrate–malate salts (Ca-citrate and CCM) indicate that these supplementations allow increased calcium absorption (Smith, Heaney, Flora, & Hinders, 1987; Mehansho, Kanerva, Hudepohl, & Smith, 1989; Lacour, Tardivel, & Drueke, 1997).

Composite dishes differed from vegetables essentially in the percentage of dialysable calcium (D). This difference in calcium dialysability was likely dependent on the presence in composite dishes of wheat constituents affecting the bioavailability of calcium. Among these, phytate might be the most involved factor. Macaroni generally contains about 2.6 mg g^{-1} of phytate (Harland & Oberleas, 1987; Plaami & Kumpulainen, 1995); its negative effect on calcium bioavailability is well known (Lonnerdal, Sandberg, Sandstrom, & Kunz, 1989; Heaney et al., 1991; Lombardi-Boccia et al., 1998).

The values of the potential calcium availability found in this study are lower than those reported for kale and broccoli in in vivo studies (Heaney et al., 1990, 1993). On the other hand, it must be taken into account that a portion of calcium absorption take place from the colon. In vivo studies (Favus et al., 1985; Nyman & Asp, 1988) have demonstrated that some of the calcium bound to indigestible components is released into the large intestine and might still be absorbable. A previous study (Lombardi-Boccia et al., 1998), dealing with calcium dialysability from beans, showed that in vitro fermentation of retentates released from 24 to 72.3% of the total calcium. The results of this study clearly cannot account for the calcium released by microbial fermentation; this potentially absorbable calcium might be responsible for the observed differences from in vivo results.

Since the main determinants of calcium absorbability are the amounts of free ionic calcium and of soluble complexes containing calcium in the intestine during digestion, the in vitro method is useful for a better knowledge of the chemical forms of calcium in foods and the interactions influencing calcium availability. The in vitro method would therefore provide important suggestions for improving calcium absorption from meals and diets.

In conclusion, our findings suggest that *brassica* vegetables can be regarded as a good source of available calcium. Also, the consumption of these vegetables together with foods of low calcium availability (e.g. macaroni) contributes to maintain the potential calcium availability at a reasonable level.

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