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SHORT REVIEW

A Comprehensive Review of the Health Benefits of Calcium and its Bioavailability from Different Leafy Vegetables

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Abstract

Calcium (Ca) is a beneficial mineral for maintaining different physiological activities and is involved in preventing several diseases in human beings. Regular dietary Ca intake is therefore essential. However, the quantity required varies with different age groups. Various leafy vegetables act as a good source of Ca but some natural or physiological factors like phytate and vitamin D may influence Ca absorption from the Gastrointestinal Tract (GIT) thereby affecting the bioavailability of Ca. Methods like boiling and fermentation can be used to increase the bioavailability of Ca. Understanding Ca bioavailability from leafy vegetables can therefore be helpful for a proper recommendation of dietary Ca supplementation.

Keywords: Bioavailability, Calcium, Leafy Vegetables, Phytate, Vitamin D

1. Introduction

Calcium (Ca) is an important micronutrient that maximises peak bone mass throughout the first three decades of our life and minimises eventual bone loss[1]. Ca provides strength to the bones and teeth over the lifetime and also ensures the functioning of muscles and nerves. It helps in blood clotting[2]. Leafy vegetables act as a good source of different secondary metabolites as well as micronutrients. These are affordable, have high-yielding capacity and are regularly consumed as a part of our diet[3]. Several studies have established that leafy vegetables have a rich structure of Ca. However, the consumption of Ca-rich leafy vegetables alone is not enough to increase the Ca level within the body. Bioavailability of Ca refers to the amount of Ca which reaches the blood after the absorption of Ca by the body. It has been observed that various factors influence the absorption and ultimately the bioavailability of Ca from leafy vegetables. This review aims to focus on the diverse health benefits of Ca and the bioavailability of Ca after the intake of leafy vegetables[4].

2. Role of Ca in Humans

Calcium is present in the body in small amounts (2% of the total body weight) and 98% of that is present in the bones. Every 100g of bodily fluid and cells contains 10 to 15mg Ca. The cerebrospinal fluid only has 6mg per 100gm of the blood. To make up for Ca deficiency in the blood, the calcium from the bone can be used and once the deficit is filled, the calcium is then redeposited in the bone. Thus Ca levels in the skeleton, blood and tissues remain balanced. Sometimes for the removal of Ca from the skeleton, parathyroid medication is required. The various forms of calcium in the body are present in an equilibrium. The calcium in the blood exists in two different forms, the non-diffusible form of Ca is attached to the proteins, while the diffusible form is present as undissociated phosphates and carbonates. Ca which is attached to a protein does not diffuse. These ionic forms

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of calcium are engaged in a variety of physiological processes. To sustain overall body health, calcium is necessary. Our body requires it every day to maintain the strength of our bones and teeth over the course of our lifetime as well as to maintain the health of our muscles and nerves. It even facilitates blood clotting. Many people believe they consume enough calcium daily, but this is not true. When blood Ca levels drop, the essential mineral is taken from the bones, which can result in Ca insufficiency. Daily requirements of Ca in the various stages of life are different (Table 1)[1-4].

Ca intake from food is absorbed by the bones. If there is a lack of Ca in the human diet, there may not be enough calcium in the blood to replenish the bones and maintain overall bone health. Ca supplementation is essential for both the prevention of Ca deficiency and its treatment. Some of the body functions which are maintained by Ca are as follows:

- Ca regulates the reactivity of nerves and affects the peripheral neuromuscular system. A muscle can be perfused with Ca-free fluid to cause fibrillary twitching. The automatic ganglia also exhibit hyperarousal.
- The structural integrity of skeletal muscles needs to be preserved. Contractility increases with an increase in ionized Ca and vice versa[1,5,6].
- Ca is crucial to maintain the heart›s tone and contractility. Ca acts as an antidote to the depressant action of potassium.

Table 1. Amount of Ca required in daily life

	Life Stage Group	Daily Intake(mg/d)	Reference
Age	0–6 Mo	210	2
	6-12 Mo	270	
	1-3 Y	500	
	4-8 Y	800	
	9-13 Y	1300	
	14-18 Y	1300	
	19-30 Y	1000	
	31-50 Y	1000	
	51-70 Y	1200	
	>70 Y	1200	
Pregnancy	≤18 Y	1300	2
	19-50 Y	1000	
Lactation	≤18 Y	1300	2
	19-50 Y	1000	

- It helps renin to coagulate milk in the stomach.
- It is necessary for the blood to coagulate. Ca helps to activate various proteins in the platelets which are essential for blood clotting.
- Cellular permeability of neutrophils is reduced by the Ca. Therefore, it helps to lessen exudation in allergic disorders, which can cause rashes and weals. Ca forms a part of the cement that holds these cells together[1,7].
- Calcium is involved in the development of bone and tissue. Normal excretion rates are 25 to 35 % in the urine and the remaining amounts in the faeces[1,8].

An insufficient supply of calcium which exists in the body is known as calcium deficiency. Two types of Ca deficiency are:

2.1 Dietary Calcium Deficiency

Dietary calcium deficiency is a condition in which calcium absorption in the body is inadequate, which can lead to osteoporosis, depletion of calcium stores in bones and thinning and weakening of bones.

2.2 Non-dietary Calcium Deficiency

This refers to low levels of calcium in the blood due to medications such as diuretics, medical procedures or conditions such as hypoparathyroidism or kidney failure. Treatment plans can help to reduce the risk of serious problems due to calcium deficiency, including osteoporosis, high blood pressure and heart rhythm problems.

Different symptoms of Ca deficiency (Figure 1) are as follows.

2.3 Muscle Cramping

Tetany, a neurological disorder characterised by muscle cramping, numbness and tingling in the arms and legs, is one of the first symptoms of a deficiency. If muscles start to cramp up, it indicates a calcium deficit. These pains typically strike at night, particularly in the legs.

2.4 Dry Skin and Brittle Nails

Skin becomes dry and fingernails become brittle in Ca deficiency. These are two prominent manifestations of calcium shortage. Teeth turn yellow. When there is a deficiency of Ca, the teeth and bones can suffer greatly.

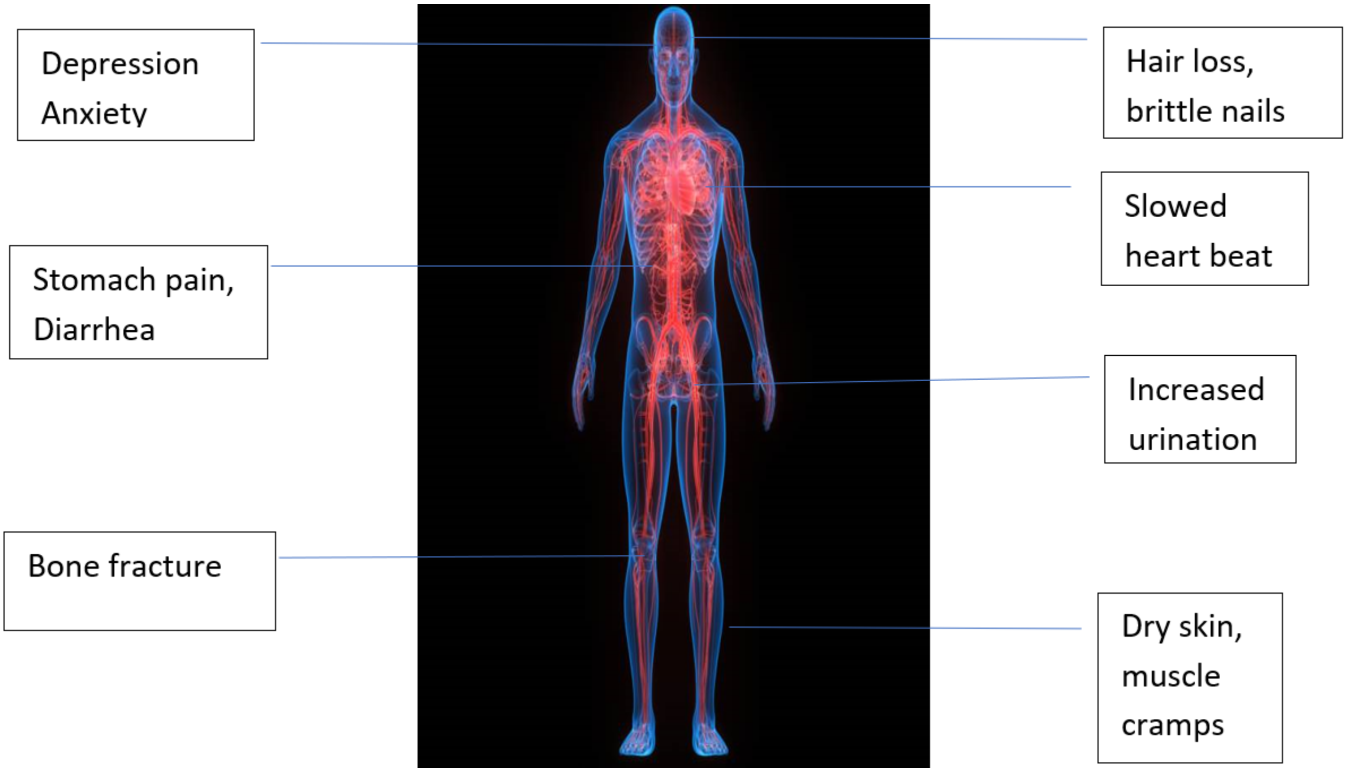

Figure 1. Ca deficiency symptoms.

2.5 Bone Fractures or Breakage

This is a severe symptom of Ca deficiency[1,9].

3. Calcium Absorption and Bioavailability

For absorption, an ionised form of Ca is required. HCl of the stomach helps convert the Ca salts into their ionised form, which can then be absorbed from the duodenum of the small intestine. For bioavailability, Ca is required to be passed through the membrane of the gut, which is a complex process. Ca needs to cross successive layers of epithelial cells, basement membranes, lamina propria and certain vascular endothelium[10]. Transport of Ca can occur by the following processes.

3.1 Paracellular Transport

The intestinal lining is composed of epithelium cells which are connected by a Tight Junction (TJ), which allows the transport of Ca^{2+}. This is transported by passive diffusion that depends on the concentration gradient or electrical gradient across the epithelium cells. It is a nonsaturable process that especially occurs in the jejunum and ileum at adequate or high levels of Ca^{2+} [11,12].

3.2 Transcellular Transport

It consists of three steps for the transport of Ca^{2+}. In the first step, Transient Receptor Potential Vanilloid 5 (TRPV5) and 6 proteins present in the epithelial cells get activated and help in the apical entry of Ca^{2+}. In the next step, cytosolic transport occurs with the help of calbindins. In the final step, extrusion occurs across the basolateral membrane by Plasma Membrane ATPase ($PMCA_{1b}$) and the Na^{2+}/Ca^{2+} Exchanger (NCX1). Calcitriol stimulates all the steps of Ca^{2+} transport. Calcitriol molecules bind to their molecular receptor (VDR) and form a complex known as 1,25(OH)2D3-VDR which interacts with the specific DNA sequences to induce transcription and increase the expression of TRPV5/6, calbindins and extrusion systems (Figure 2)[11,13-17].

By paracellular and transcellular transport, calcium can be reached into the blood from GIT by crossing the intestinal membrane.

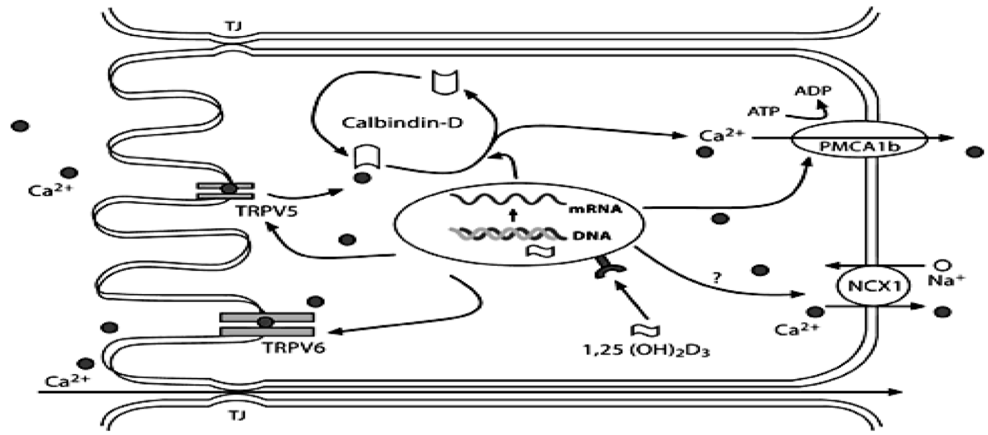

Figure 2. Schematic diagram of Ca absorption mechanism from the small intestine[11].

4. Calcium Content In Edible Plants

The sources of calcium-rich foods are fortified meals, beverages, supplements and edible plants. Calcium sources should be assessed based on their content and bioavailability. There are several edible medicinal plants with various secondary metabolites and micronutrients like Ca, Fe, Zn, etc, responsible for different biological activities. Among these Ca is one of the most significant elements and is widely available in many leafy vegetables (Table 2)[18-33].

5. Factors Affecting Ca Bioavailability

High content of Ca in leafy vegetables, may not be enough to increase the Ca supply in the blood. Several factors involved may influence the absorption of Ca from the GIT and ultimately increase or decrease its bioavailability.

5.1 Factors Increasing Bioavailability of Ca

These factors can be related to the hormones, amino acids, milk and more:

5.1.1 Vitamin D

1,25-dihydroxyvitamin D (1,25$[OH]_2$D), is created when vitamin D is hydroxylated at the 25 position, to convert into 25-hydroxyvitamin D (25[OH]D), followed by its transportion to the kidney where it gets hydroxylated at the 1 position by the 1-hydroxylase. Intestinal calcium absorption is controlled by a large number of calcium transporter proteins that rely on vitamin D. A calcium transporter protein by the name of TRPV6 is present on the luminal surface of the enterocyte. The duodenum expresses TRPV6, regulated by 1,25$(OH)_2$D. Thus in the presence of 1,25$(OH)_2$D, Ca can enter enterocytes more easily leading to higher bioavailability[34].

5.1.2 Parathyroid Hormone

Production of 1 alpha-hydroxylase is stimulated by a Parathyroid Hormone (PTH) in the proximal convoluted tubule. The inactive form of vitamin D, 25-hydroxycholecalciferol, needs to be converted into the active form 1,25-dihydroxycholecalciferol, by the enzyme 1alpha-hydroxylase. Ca absorption in the small intestine is facilitated by Vitamin D, eventually increasing the bioavailability of Ca[35].

5.1.3 Acid Environment

The absorption of Ca in the duodenum depends on the HCl generated in the stomach during digestion. The acidic environment of the stomach is necessary for Ca to convert into the ionised form which is then absorbed through the duodenum[35,36].

5.1.4 Milk Lactose

Milk Lactose promotes the absorption of Ca in newborns. Lactose can be fermented by bacteria in the colon, leading to the production of Short-Chain Fatty Acids (SCFAs) such as acetate, propionate and butyrate. These SCFAs lower the pH in the colon, creating an acidic environment. This acidic environment promotes the solubility of Ca salts, making them more available for absorption in the colon, thus increasing the bioavailability of Ca[36].

Table 2. List of leafy vegetables with their calcium content

Sl. no.	Common name of the leafy vegetable	Scientific Name	Amount of Ca (mg/ 100g)	Reference
	Family: Acanthaceae			
1	*Kalmegh*	*Andrographis paniculata*	318.62	18
2	*Kulekhara*	*Hygrophila auriculata*	27.93	19
	Family: Amaranthaceae			
3	*Palongshak*	*Spinacia oleracea*	136	19
4	*Sada notey*	*Amaranthus viridis*	214	20
5	*Lal notey*	*Amaranthus tricolor*	368	20, 21
6	*Data shak*	*Amaranthus gangeticus*	102	20
7	*Bethushak*	*Chenopodium album*	280	22
8	*Laal shak*	*Amaranth Dubius*	336	20, 22
9	*Kanta notey*	*Amaranthus spinosus*	1035.3	20
	Family: Amaryllidaceae			
10	*Piyazpata*	*Allium cepa*	72	19
	Family: Apiaceae			
11	*Dhonepata*	*Coriandrum sativum*	146	22
12	*Radhunipata*	*Trachyspermum roxburgianum*	67	23
13	*Thankuni*	*Centella asiatica*	171	23
	Family: Araceae			
14	*Kochushak*	*Colocasia esculenta*	272	21
	Family: Asteraceae			
15	Lettuce	*Lactuca sativa*	33	21
	Family: Athyriaceae			
16	*Dheki shak*	*Dryopteris filix-mas.*	32	24
	Family: Basellaceae			
17	*Puishak*	*Basella alba*	109	21, 25
	Family: Brassicaceae			
18	*Kale*	*Brassica oleraceavar. sabellica*	254	26
19	Collard green	*Brassica oleracea*	154	27
20	*Badhakopipata*	*Brassica oleracea*	40	23
21	*Sorseshak*	*Brassica juncea*	115	19
22	*Shalgomshak*	*Brassica rapa*	190	19
23	*Fulkopipata*	*Brassica oleracea*	600	23
24	*Muloshak*	*Raphanus sativus*	140	24
	Family: Chenopodiaceae			
25	*Beet shak*	*Beta vulgaris*	117	28
	Family: Compositeae			
26	*Helenchashak*	*Enhydra fluctuans*	170	19,21, 24
	Family: Convolvulaceae			
27	*Kalmishak*	*Ipomoea aquatica*	416	21, 29
	Family: Cucurbitaceae			
28	*Kumropata*	*Cucurbita maxima*	30.5	26
29	*Laushak*	*Lagenaria siceraria*	80	30
30	*Telakucha*	*Coccinia grandis*	24	24
	Family: Fabaceae			
31	*Methishak*	*Trigonella foenum-graecum*	150	26
	Family: Lamiaceae			
32	*Pudina*	*Mentha spicata*	199	23,31
	Family: Malvaceae			
33	*Paat pata*	*Corchorus oliatorius*	266	23
	Family: Marsileaceae			
34	*Susnishak*	*Marsilea minuta*	53	23
	Family: Meliaceae			
35	*Neem*	*Azadirachta indica*	510	32
	Family: Molluginaceae			
36	*Gima shak*	*Glinus oppositifolius*	1693	19
	Family: Moringaceae			
37	*Sojnepata*	*Moringa oleifera*	1443	21, 22
	Family: Piperacea			
38	*Paan pata*	*Piper betle*	230	22
	Family: Plantaginaceae			
39	*Brahmishak*	*Bacopa monnerii*	202	19
	Family: Rutaceae			
40	*Currypata*	*Murraya koenigii*	659	22, 33

Amaranthaceae, Apiaceae and Brassicaceae are families of green vegetables with high calcium content. The consumption of leafy vegetables can strengthen bones and teeth and also prevent the occurrence of osteoporosis, muscle cramps, brittle nails, etc[16-18,32,33].

5.1.5 Amino Acids

Ca from leafy vegetables is frequently chelated or mixed with some amino acids, which are more soluble. amino acids may also stimulate the activity of Ca transporters in the intestinal epithelial cells, thereby increasing the uptake of Ca from the gut lumen into the bloodstream. increasing the bioavailability. Absorption of Ca is also particularly enhanced by the amino acids, lysine and arginine.

5.1.6 Exercise

Exercise, along with Vitamin D intake helps in increasing Ca bioavailability after consumption of leafy vegetables thereby making bones strong. Physical activity increases blood flow to various tissues, including the bones. This enhances the supply of blood with more nutrients, including Ca, to the bone cells[37].

5.1.7 Vitamin K

It enhances the Ca reabsorption in the bone and thus blood Ca level decreases which facilitates the Ca absorption from the gut to the lumen[38].

5.2 Factors Decreasing the Bioavailability of Calcium

5.2.1 Oxalates and Phytates

Foods rich in oxalic acids, such as spinach and Swiss chard, reduce calcium absorption. Oxalic acid combines with calcium to form a salt crystal, calcium oxalate, which does not get absorbed from the GIT, thereby decreasing the bioavailability of Ca. Similarly, phytic acids found in whole grains and fibre-rich foods also influence Ca absorption by forming insoluble complexes and reducing bioavailability.

5.2.2 Phosphorus

Excess phosphorus in the diet causes Ca to precipitate in the form of calcium phosphate. Ca reacts with phosphate to form an insoluble complex (calcium phosphate) and thus Ca does not remain available for absorption in the digestive tract.

5.2.3 Age

With increasing age, a decrease in TRPV6, which is a gut protein that helps in Ca absorption leads to a decrease in Ca bioavailability.

5.2.4 Stress

Stress can have a negative impact on HCl production in the stomach and the body's normal digestive behaviour and therefore negatively affect the Ca absorption[39].

5.2.5 Caffeine and Medications

Caffeine and medications such as anticoagulants, cortisone and thyroxine reduce the absorption of Ca in the body forming insoluble complexes, inhibiting the activation of Vitamin K, increasing urinary excretion and reducing absorption from GIT respectively.

5.2.6 Lack of Exercise and Vitamin D

Lack of exercise and Vitamin D deficiency leads to reduced calcium absorption[40].

6. Remedies To Increase Ca Bioavailability

Some processes can reduce the factors that interfere with Ca bioavailability. They are as follows:

6.1 Soaking

Soaking in water can reduce the presence of phytate in the form of phytate ions or phytic acid, which can result from the leaching of the phytate ion or acid form into the water under the influence of a concentration gradient[41].

6.2 Boiling/Cooking

Cooking can reduce the soluble oxalate content of leafy vegetables by 30% to 87% by leaching the oxalate in boiling water, although the effects on insoluble oxalates vary in different leafy vegetables. For example, in spinach, the insoluble oxalate is not reduced, but in red and green chard the insoluble oxalate can be reduced up to 75% by cooking. The phytate content of the vegetables can only be reduced at high temperatures[41-45].

6.3 Fermentation

During this process, the bacteria increase the amount of phytase, which reduces phytates and phosphates by breaking down phytic acid or the phosphate-phytate complex. In leafy vegetables, fermented lactic acid produced by *Lactobacillus* sp. produces lactic acid. This lactic acid binds to Ca to form calcium lactate, which increases the absorption and thereby bioavailability[46,47].

7. Leafy Vegetables Having Ca With Good Bioavailability (30% or More)

Consumption of leafy vegetables does not result in total Ca being absorbed into the blood. Figures 3 and 4 show the comparison between the total amount of Ca present in individual leafy vegetables with the percentage of bioavailable Ca in the system[13,48-56].

8. Leafy Vegetables Having Ca With Less Bioavailability (Less than 30%)

Figure 5 shows the comparison between the total amount of Ca present in individual leafy vegetables with the bioavailable Ca ranges of less than 30%[13,48-56].

Figures 3, 4 and 5 represent the amount of Ca present in the leafy vegetables and the bioavailability of Ca, which gives an idea about the Ca bioavailability from different leafy vegetables.

9. Discussion and Conclusion

.The presence of a good amount of Ca found in leafy vegetables is not always enough for good bioavailability. *Amaranthus spinosus, Basella alba, Spinacia oleracea, Amaranthus viridis, Mentha spicata* and *Coriandrum sativum* have a good amount of Ca but the bioavailability is 3.7%, 5%, 5.1%, 8.36%, 21% and 22% respectively.[13,48-56] There are some leafy vegetables with less Ca content but good bioavailability like *Brassica oleracea* which has a Ca bioavailability of 40%. Some of the leafy vegetables have good Ca levels and also good bioavailability like *Brassica juncea, Brassica rapa, Centella asiatica, Alternanthera sessilis, Piper betle, Plectranthus barbatus, Murraya koenigii, Marsilea minuta* and *Moringa oleifera*. Ca bioavailability of some leafy vegetables like *Brassica oleracea and Enhydra fluctuans* is improved because of factors like vitamin K. Plants like *Trigonella foenum-graecum* have less interfering factors like low oxalate that helps to increase their Ca bioavailability. However, the amount of bioavailability increase for these influencing factors is not yet established. Bioavailability is the amount of Ca reaching the systemic circulation after its absorption. Many factors may decrease absorption and thereby reduce bioavailability, like oxalate, phytate, phosphate, etc. Many remedies such as soaking, cooking, boiling and fermentation which may reduce the interfering factors have been explored. However, it is not yet established for many of the leafy vegetables. Further investigation can therefore be carried out to complete the profiling of Ca among the edible leafy vegetables, as well as the profiling of the interfering factors for bioavailability enhancement and to establish the contribution of the (beneficial) remedies to overcome the effect of the interfering factors. Dietary intake of such leafy vegetables having good Ca bioavailability can then replace the requirement for Ca supplementation.

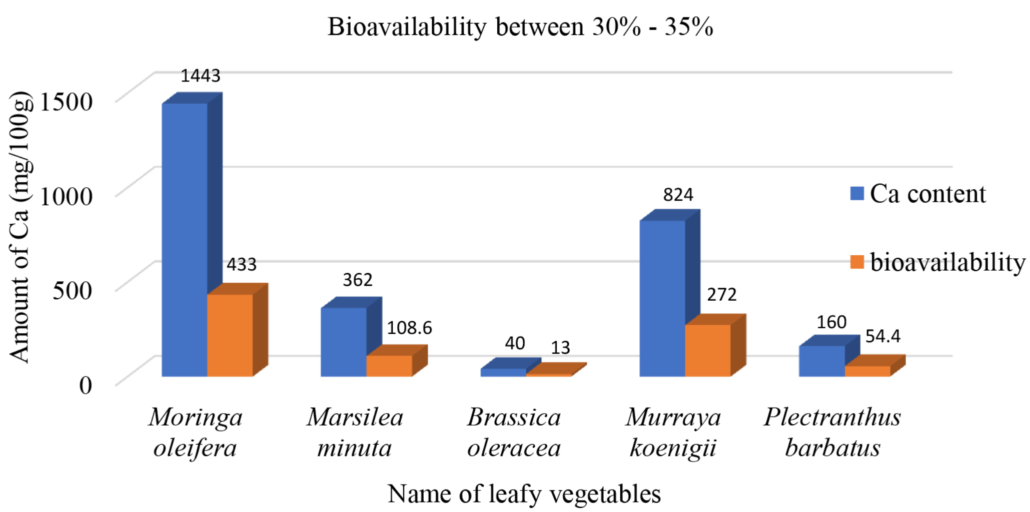

Figure 3. Leafy vegetables with bioavailability between 30% - 35%.

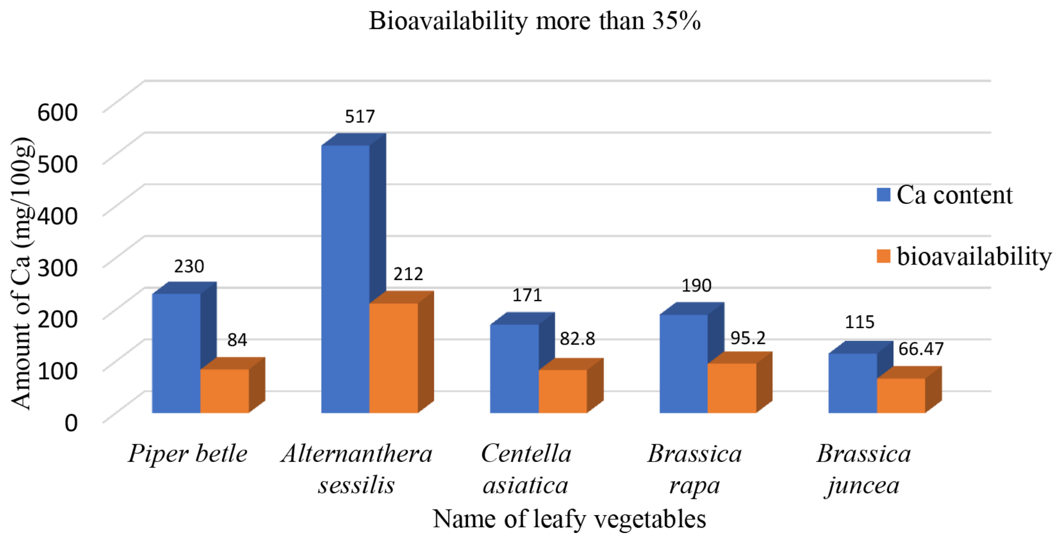

Figure 4. Leafy vegetables with bioavailability of more than 35%.

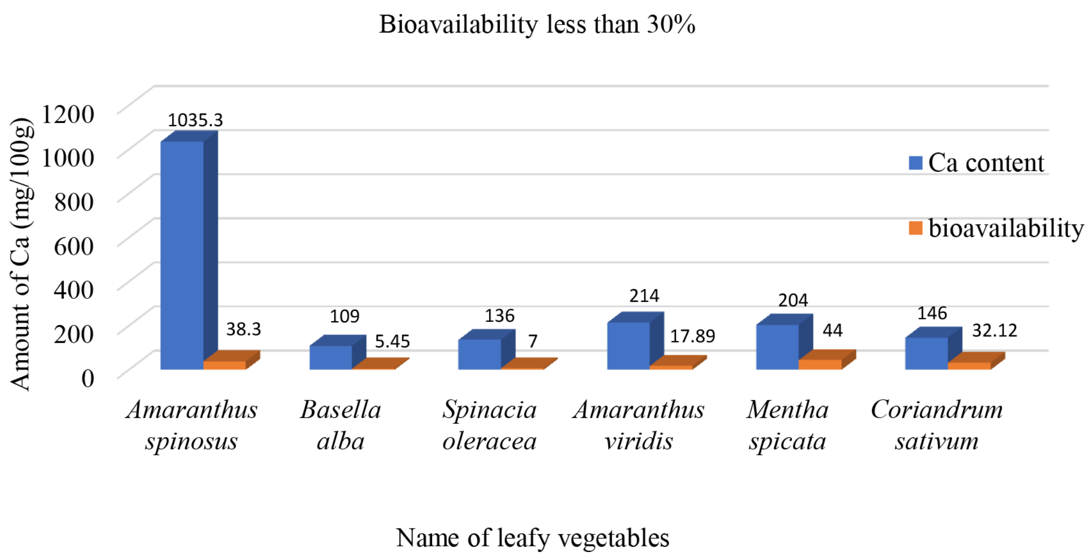

Figure 5. Leafy vegetables with bioavailability less than 30%.

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