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Determination of Calcium in Pulses by Complexometric Titration and Overview of Nutritional Parameters

Dr. Shashwati V. Pradhan[1], Chandresh R. Bari[1], Dr. Amol P. Kharche[2], Dr. Sanjiwani S. Sonar[2]*

[1]Department of Chemistry, R.D. and S.H. National College and S.W.A. Science College, Bandra (W), University of Mumbai, India.

[2]Pimpri Chinchwad College of Engineering (An Autonomous Institute affiliated to SPPU, Pune), Sector -26, Pradhikaran, Nigdi, Pune - 411 044.

Email: *sanjiwani.sonar@pccoepune.org*

Abstract - Pulses, like cereals, are commonly consumed as a source of protein. They are high in carbohydrates, protein, and dietary fiber, as well as micronutrients. Its importance and use for a range of health conditions have been referenced in ancient writings and the ayurvedic medical system. This paper details a research of calcium measurement in several pulse samples, as well as a discussion of various nutritional aspects. The acid digestion method was used to create sample solutions for the determination of calcium. After that, an aliquot of the sample solution was titrated using the complexometric method by using Disodium salt of Ethylenediamine tetra acetic acid (EDTA). Patton-Reeder's reagent (3-hydroxy-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)diazenyl] naphthalene-2-carboxylic acid) was utilized as an indicator.

Keywords: Titration, Complexometric, EDTA, Calcium, Patton and Reeder's indicator, Pulses.

Abbreviations:

Kcal/100g	Kilo Calories per 100 grams
ml	Milliliters
EDTA	Ethylene diamine tetra acetic acid
PR	Patton and Reeder's reagent
$molL^{-1}$	Moles per liter
kg/ha	Kilogram per hectare
L.	Linnaeus
GIT	Gastrointestinal tract
DASH	Dietary Approaches to Stop Hypertension
CVD	Cardiovascular Disease

Introduction:

Pulses, a type of dietary legume that is high in protein, vitamins, and minerals, continue to play a significant role in human nutrition, especially in underdeveloped nations. The nutritional content of pulses has long been emphasized, in addition to production, flexibility, and yield stability. One of the greatest remedies for protein-calorie deficiency is to augment cereals with protein-rich legumes. India produces over 12 million tons of pulses per year, making it the world's largest producer. The primary pulse crops farmed and consumed in India include pigeon pea, chickpea, mung bean, and urad bean.**[15]** Pulses go through two stages of processing before being used as human food: (1) primary processing, or dehulling, which turns the whole seed into dhal, and (2) secondary processing, which involves soaking, autoclaving, germination, roasting, and fermentation (Teresa Nestares et al., 1999). The amount of secondary processing varies by food type and consumption area.

Since the turn of the century, calcium has been reported in studies of food nutrient composition. Morris and Oliveira (1933) were among the first to identify the presence of this mineral in over 60 different meals.**[12]** Calcium is a mineral that is most commonly linked with healthy bones and teeth, but it also aids in blood clotting, muscular contraction, and the regulation of proper heart rhythms and neuron activities. About 99% of the calcium in the body is stored in bones, with the remaining 1% in blood, muscle, and other tissues. Calcium is obtained in two ways by the body. One way is to consume calcium-rich foods or supplements, and the other is to extract calcium from the body. The body will eliminate calcium from bones if you don't eat enough calcium-rich foods. The calcium that is "borrowed" from the bones should, in theory, be restored at a subsequent time.

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Pulses have several nutritional benefits and can be an excellent source of energy. Most pulses have an energy level of between 300 and 540 Kcal/100g, according to research.[15]The nutritional importance of pulses arenumerous, they will be a valuable source of energy. The energy content of most pulses are found to be between 300 and 540 Kcal/100g.[15] Energy is required for metabolic processes. The energy of pulses comes from the nutrient supply of protein, fat and carbohydrate.

With growing clinical evidence showing that plant-based foods have many potential health benefits, their consumption increases at a rate of 5 – 10% each year. In addition, many world health organizations have recommended increasing consumption plant foods to improve health and prevent chronic diseases.

Materials and method:

The classical method of determining calcium and other suitable cations is titration with a standardized solution of ethylenediaminetetraacetic acid (EDTA). EDTA has the structure shown below. Each acid hydrogen on EDTA can be removed, producing H_3EDTA^{-1},H_2EDTA^{-2},$HEDTA^{-3}$, and $EDTA^{-4}$ ions. The disodium dihydrate salt of EDTA ($Na_2H_2EDTA.2H_2O$) is commonly used to prepare standard EDTA solutions.

This salt is readily available from many commercial sources, and often in such a high purity that solutions need not be standardized for routine work. Primary standard calcium carbonate can be used to standardize EDTA solutions. Of the various EDTA species, only the $EDTA^{-4}$ ion (the completely deprotonated anion of EDTA) forms a 1:1 complex with metal ions. To increase the fraction of $EDTA^{-4}$, the pH needs to be increased to basic condition (around 10). The endpoint of an EDTA titration is determined with a metallochromic indicator. These indicators are complexing agents that change color when combined with metal ions. A variety of indicators can be used for EDTA titrations. A blue dye called Patton and Reeder's indicator (PR) is used as the indicator. This blue dye also forms a complex with the calcium ions changing color from blue to pink/red in the process, but the dye–metal ion complex is less stable than the EDTA–metal ion complex. As a result, when the calcium ion–PR complex is titrated with EDTA the Ca^{2+} ions react to form a stronger complex with the EDTA.[2]

For the titration, the indicator is added to the sample solution containing the calcium ions and forms the pink/red calcium ion-indicator complex (Ca-PR). This solution is then titrated with EDTA. The endpoint occurs when the solution turns blue; indicating that the Ca-PR complex has been completely replaced by the calcium ion-EDTA complex and the PR indicator reverts to its blue color.[2]

The reaction is:

$$\text{Ca-PR} + \text{EDTA} \rightarrow \text{PR} + [\text{Ca} - \text{EDTA}]^{2-}$$

Ca-PR is pink/red and PR is blue.[2]

Calconcarboxylic acid (IUPAC name 3-hydroxy-4-[(2-hydroxy-4-sulfonaphthalen-1-yl)diazenyl] naphthalene-2-carboxylic acid; commonly called Patton and Reeder's Indicator) is an azo dye that is used as an indicator for complexometric titrations of calcium with ethylenediamine tetra acetic acid (EDTA) even in the presence of magnesium. Structurally, it is similar to eriochrome blue black R, which is obtained from calconcarboxylic acid by decarboxylation and reaction with sodium hydroxide. Calconcarboxylic acid is soluble in water and a variety of othersolvents,including sodiumhydroxide, ethanol and meth anol. It has a violet color in dissolved form in ethanol. The melting point of calconcarboxylic acid is at approximately 300 °C, where it undergoes thermal decomposition.[36][37]

Though the determination of calcium and magnesium by complexometric titration with standard solutions of disodium dihydrogen tetraacetate, utilizing Eriochrome Black T as indicator is widely accepted and quite adequately understood, it, like other complexometric titration methods, suffers from the limitations of having an indistinct endpoint and/or having to separate the metals before titration can occur. Calconcarboxylic acid was thus adopted as a superior alternative due to its ability to give a good and visual endpoint and its rapid performance even with the presence of magnesium. As described by James Patton and Wendell Reeder in 1956, calconcarboxylic acid can be synthesized by coupling diazotized 1-amino-2-naphthol-4-sulfonic acid with 2-hydroxy-3-napthoic acid. Calconcarboxylic acid is used for the determination of calcium ion concentration by complexometric titration. Free calconcarboxylic acid is blue color, but changes to pink/red when it forms a complex with calcium ions. EDTA forms a more stable complex with calcium than calconcarboxylic acid does, so addition of EDTA to the Ca–calconcarboxylic acid complex causes formation of Ca-EDTA instead, leading to reversion to the blue color of free calconcarboxylic acid. This method of complexometric titration is dependent on the pH of the solution being sufficiently high to ensure that magnesium ions precipitate as magnesium hydroxide before the PR indicator is added to prevent interference with the results, as if magnesium were present, the EDTA would also form complexes with it. Concentrated sodium hydroxide or potassium hydroxide is usually added to the solution to this end.

Experimental: The accuracy of this method may also be affected by the presence of metal ions such as copper, iron, cobalt, zinc or manganese in sufficiently high concentrations.

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- Apparatus: 10 and 20 mL pipettes, 250 mL conical flasks; 100, 250- and 500-mL volumetric flasks, pH indicator paper, 10 mL and 100 mL measuring cylinders, burette and stand, etc.
- Solutions needed: EDTA solution, Patton-Reeder's indicator, sodium hydroxide solution (8 $molL^{-1}$), dilute hydrochloric acid (1-2 $molL^{-1}$), dilute sodium hydroxide solution (1-2 $molL^{-1}$).

Sample preparation:

Calcium samples in solution, such as tap water and milk, do not require any additional treatment.

To eliminate solid debris like sand and algae, seawater may need to be filtered. Solid samples, such as limestone, eggshells and food samples, must be first dissolved in acid.

1g of powdered sample was taken in a conical flask, and about 20 ml dilute hydrochloric acid was added to dissolve the sample completely. This solution was kept for 24 hrs. for dissolution.

The unreacted acid was neutralized with dilute sodium hydroxide solution until the pH of the solution became almost 7 (according to pH indicator paper).

This solution was the transferred to a 100 ml volumetric flask and dissolved up to the mark.

Titration:

An aliquot of sample solution was taken in a conical flask.

Now, 40 ml of water and 4 ml of 8 $molL^{-1}$ sodium hydroxide solution was added and allowed to stand for about 5 minutes with occasional swirling. A small amount of magnesium hydroxide may precipitate during this time.

A small quantity of Patton and Reeder's indicator is added the solution swirled to dissolve the indicator.

This was then titrated against EDTA solution. The end point is a color change from pink/red to blue. This process was repeated further to obtain constant results.

Nutritional aspects of Pulses:

Pulses are eaten all around the globe. Consumption is higher in those parts of the world, where animal proteins are scarce and expensive. Pulses offer a major fraction of the protein necessary for adults and children in certain regions of the world. Pulses provide around 20% of the protein now accessible to humans in developing nations. [14]

The carbohydrate supply:

The carbohydrate content of pulses is high (Table 1) (Reddy et al., 1985; Oke et al., 1995). The high carbohydrate content contributes a great deal to energy supply of pulses. A large percentage of pulses occur as starch, about 1.8-18% occurs as oligosaccharides while 4.3-25% occur as dietary fiber (Table 2). Although the oligosaccharides, which are made up of raffinose, stachyose, verbascose, cause flatulence in human, they are presently believed to have some beneficial effects. They can shorten transit time and promote the growth of bifido bacteria in human. In fact, researchers in japan have actually suggested that oligosaccharides from soybeans could be used as substitute for table sugar. Pulses are also hypothesized to improve longevity and reduce colon cancer risk (Hayakawa et al., 1990; Koo and Rao, 1991). The high dietary fiber content of pulses, are postulated to have some important physiological effects, such as reducing the transit time in the mammalian gut (Sathe et al., 1984). This would help to relieve gastrointestinal conditions such as constipation and diverticular disease. It is also capable of lowering the blood cholesterol level due to its ability to bind with cholesterol in the human gut (Burkitt and Trowell, 1985). This feature is being suspected as being capable of reducing colonic cancer in man (Davis and Stewart, 1987; Hangen and Bennick, 2002). Pulses also have low glycemic indices, which makes them valuable foods for diabetics. The cotyledon of legumes like locust bean and gaur (gaur gum) reduces postprandial glucose and insulin concentrations in man (Fairchild et al., 1996; Gatenby, 1991; Feldman et al., 1995).

Protein Supply:

Pulses have high protein content (Table 3), the value is about twice that in cereal and several times that in root tuber (FAO, 1968), so they can help to improve the protein intake of meals in which cereals and root tubers in combination with pulses are eaten (Kushwah et al., 2002). Pulse when eaten with cereals, can also help to increase the protein quality of the meal. In human, protein helps in the repair of body tissue, synthesis of enzymes and hormones and also in the supply of energy. In children, the consumption of pulses should be encouraged, particularly where animal protein is scarce and expensive, as this would help to furnish the child with the necessary amino acids required for growth. (Ofuya , Z M; Akhidue, V, 2005)

Fat supply:

The fat content of pulse varies in different species. Most species contain about 1% fat, while groundnut and soybeans have very high fat content, about 30% for soybean and 49% for peanut. The fat content besides contributing to the energy needs, provides the needed essential fatty acids required in human nutrition. A pulse like soyabean, contains linolenic acid, which is an omega-3 fatty acid. This fatty acid is currently being studied for its ability to reduce the risk of heart disease and cancer.(Ofuya , Z M; Akhidue, V, 2005)

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Table 1: starch and total carbohydrate content of pulses

Reddy et al. (1985); Frank-Peterside, Dosumu, and Njoku (2002), Ofuya (2002); Oke, Tewe, and Fetuga (1995).

Common name	Scientific name	Total carbohydrates %	Starch %	Amylose content of starch %
Lentil	Lens culinaris	59.7	34.7-52.8	20.7-45.5
Black gram	Vigna mungo	56.5-63.7	32.2-47.9	43.9
Bengal gram	Cicer arietinum	60.1-61.2	37.0-50	31.8-45.8
Mung gram	Vigna radiata	53.3-61.2	37.0-53.6	13.8-35.0
Red gram	Cajanus cajan	57.3-58.7	40.4-48.2	39.6

Table 2: Dietary Fibre Content of Pulses (per 100g of whole mature seeds)

Legume	Dietary fibre
Chickpea	25.6
Groundnut	6.1
Kidney bean	25.4
Mung bean	15.2
Pea	16.7
Soya bean	11.9
Cluster bean	4.3
Lentils	11.7
Pigeon pea	15.0

Kamath and Belvady (1980) by Paul and Southgate (1978)

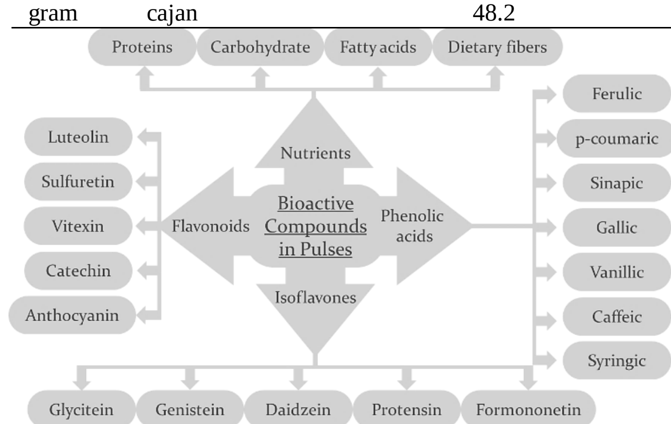
Table 3: Protein content of pulses

Common name	Scientific name	Protein content g/100g DM	
		Mean	Range
Broad bean	Vicia faba	24.0	22.0-38.2
Chickpea	Cicer Arietinum	22.2	19.1-31.2
Common bean	Phaseolus vulgaris	23.9	15.2-36.0
Common pea	Pistum sativum	23.1	14.2-36.1
Cowpea	Vigna unguiculata	24.0	20-34.2
Pigeon pea	Cajanus cajan	21.0	17.9-31.0
Groundnut	Arachis hypogaea	26.2	17.1-31.0
Soya bean	Glycine max	40.3	28.7-50.1
African yam bean	Streptpstylis stenocarpa	18.4	18-22

Ofuya (2002); Frank-Peterside, Dosumu and Njoku (2002); Oke, Tewe and Fetuga (2002); Amartiefo et al. (2002)

Micronutrient supply:

Vitamins: The vitamins present in appreciable quantities in pulses are thiamin, riboflavin, pyridoxine and folic acid; vitamin E and K are also found in pulses. The B-vitamins act as co-enzymes in biological processes. Vitamin E is known to play a role as an antioxidant inhibiting the oxidation of vitamin A in the GIT and of polyunsaturates in the body tissues. It is also believed to maintain the stability of cell membranes. Vitamin K functions primarily in the liver where it is necessary for the formation of blood clotting factors.(Ofuya , Z M; Akhidue, V, 2005)



Pulses and production:

○ Chickpeas (*Cicer arietinum* L.) are an ancient crop cultivated and consumed throughout the world, also known as Bengal gram. After dry beans and field peas, chickpeas are the most cultivated crop globally. India was the world's largest producer of chickpeas (6.38 million metric tons) during the years 2006–2009, which accounted for 66% of the world chickpea production.[14] Chickpeas are enriched with carbohydrates (starch, dietary fibre, glucose, sucrose, and oligosaccharides) and proteins (all essential amino acids excluding Sulphur-containing amino acids). They have a high fat and fibre content, while protein makes up about 22% of the chemical composition. [14] The protein quality of chickpea hydrolysates and isolates has been explored to improve the nutritional and functional properties of chickpea proteins. Compared to other pulses, chickpeas have a relatively high content of fat, composed of polyunsaturated fatty acids with less than 1% saturated fatty acids.[14] Chickpeas contain sterols such as campesterol, β-sitosterol, and stigmasterol in their oil and possess beneficial unsaturated fatty acids, namely linoleic and oleic acids. Minerals such as magnesium, calcium, phosphorous, and potassium, and essential vitamins including niacin, riboflavin, thiamine, β-carotene (a precursor for vitamin A), and folate are also present in chickpeas. They also contain proteinase inhibitors like trypsin and chymotrypsin. (Wood and Grusak, 2007)

○ Pigeon pea (*Cajanus Cajan* L.) is the oldest crop in India and is commonly referred to as red gram or pigeon pea. [14] The Cajanus Cajan cultivation area covers 11.8% of the total pulse growing area in India.[14] Cajanus Cajan is grown both as a food crop and cover/forage crop, having high levels of proteins and important amino acids like methionine, lysine, and tryptophan. It has a high content of proteins (22.3%) and carbohydrates (57.6%). [14] In addition, it contains calcium, magnesium, iron, and fats. Considerable progress has been achieved regarding understanding red gram's biological activity and medicinal applications. Flavonoids and stilbenes are found to be higher than in the leaves of Cajanus Cajan compared to pods. In addition, tannins, saponins, resins, reducing sugars, and terpenoids are also present in Cajanus Cajan. The presence of 2'-2'-methyl Cajanone, 2'-hydroxy genistein, isoflavones, cajanin, cahanones, etc., in C. Cajan results in antioxidant properties. Genistein and genistin are found in C. Cajan roots. They also contain hexadecanoic acid, α-amyrin, β-sitosterol, pinostrobin, longistylin A, and longistylin C, which show antibacterial activity. [14] C. Cajan contains a significant amount of cajaninstilbene acid, pinostrobin, vitexin, and orientin, which are responsible for antiplasmodic activity. (Dilipkumar et al, 2011)

○ The green gram (*Vigna radiata*(L.) R. Wilczek) commonly known as the mung bean, originated in India and Central Asia. Green gram has high nutritive protein content and is considered an easy natural food to digest. The green plants, harvested pods, and seed husks are used as animal feed and fodder. Green gram protein content may vary from 20.97–31.32%; however, these beans are best suited for complementing cereal proteins because of their amino acid profile with high lysine/low methionine, which is the inverse of cereals. [14] Green gram is a good source of carbohydrates (56%) and dietary fibre (4.1%).[14] In addition, it also possesses high concentrations of calcium, phosphorus, and iron. It provides about 15–45% of the recommended daily allowance (RDA) of calcium and iron, respectively. Green gram is also rich in linoleic acid, tocopherols, and antioxidants. (Anwar et al., 2007)

○ Black grams (*Vigna mungo* L.) are small and black-colored beans featuring prominently in Asian cuisine, which basically originated from India. [14] Botanically, they belong to the Fabaceae or Leguminosae family. Black gram is the major pulse crop cultivated all over India and has a protein (24%) and carbohydrate-rich (59.6%) composition. [14] Moreover, it also contains high amounts of calcium, phosphorus, and iron. Black gram is used as feed for milch cattle. Black gram beans are a very important source of protein and hold 341 calories of energy and provide 25.21 g of protein per 100g after consumption. [14] Though these beans have a low caloric value compared to other pulses, they have a relatively high protein composition. Black gram beans have unique features as they possess soluble mucilaginous polysaccharides and high dietary fibre content. Dietary fibre plays an important role in the reduction of blood cholesterol by decreasing re-absorption of cholesterol-binding bile acids and helps to protect the colon mucosa by decreasing its exposure time to toxic substances by acting as a bulk laxative that binds to cancer-causing chemicals in the colon. Black gram is mainly rich in various isoflavones which include glycitein, genistein, daidzein, and formononetin. Isoflavones have been found to reduce post-menopausal cancers and osteoporosis. Also, black gram is an excellent source of vitamin B-complex vitamins such as vitamin B6 (22% of RDA), thiamine (23%), pantothenic acid (18%), riboflavin (20%), and niacin (9%). Most of these vitamins work as co-factors for the enzymes in carbohydrate, protein, and fat metabolism. [14] Black gram is a rich source of iron, comprising 7.57 mg of the recommended daily intake. Iron improves memory power and cognition and helps prevent anemia. Other minerals include calcium (14%), copper (10%), magnesium (67%), zinc (30%), phosphorus (54%), and potassium (21%) per 100 g. (Mudryj et al., 2014)

○ Lentils (*Lens culinaris* Medikus) originated in Turkey through South Iran and serve as a nutritious food source for humans. Lentil-based foods are suggested for patients due to their rapid digestion and increased biological value. Different parts of the lentil plant (dry leaves, pods, and stems) are utilized as cattle feed. Lentils are classified as either bold-seeded or small seeded based on the size and weight of the seeds. India has the largest area of lentil cultivation and is second in lentil production worldwide. New Zealand stands first in global productivity (2667 kg/ha) and is followed by China (2239 kg/ha). Lentils are a rich source of protein (24–26%), carbohydrate (57–60%), and fibre (3.2%).[14] Moreover, lentils also possess a higher amount of vitamins A and C than other pulses as well as elevated levels of micronutrients such as calcium, phosphorus, and iron.(Tiwari & Shivhare, 2016)

Use of Pulses in Special Diets

As a result of their nutrient content and other properties, pulses can play a role in several special diets:

- Gluten-free diet: If a person with celiac disease consumes gluten (a protein found in wheat and some other cereal grains), an immune reaction is triggered in the small intestine, which can cause damage and poor absorption of nutrients. Pulses contain no gluten; therefore, people with celiac disease can use chickpeas, lentils or peas as an ingredient in recipes.
- Diabetic diet: For people with diabetes, consuming lentils, peas and beans may help with blood glucose management. Compared with some other carbohydrate sources, pulses have a lower glycemic index. Some studies have shown that consuming pulses may result in more stable blood glucose levels after meals. [15]
- Vegetarian diet: Pulses are good sources of protein, vitamins and minerals (especially iron and zinc), which makes them an excellent food choice for vegetarians. They contain eight essential amino acids. Consuming lentils with rice provide the full complement of amino acids needed for growth.
- Weight management diet: Although more studies are needed, consuming pulses may help with weight management.

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For people trying to lose weight, pulses are high in fiber and protein, low in fat and moderate in calories. One cup of cooked lentils or dry peas contains about half of the daily fiber recommendation for adults. Foods higher in fiber content usually help people feel "full" or satiated at mealtime.

Results and discussion

The present researches was designed to estimate the amount of Calcium present in the different pulse samples easily available in the market and are important part of diet in Indian cuisine. The investigated calcium content in the samples is given in Table 4.

The experimental data shows the titre volumes and amount of calcium calculated and the reference range. The various nutritional composition of pulses shows that the pulses contain carbohydrate and proteins as a major component along with dietary fibers. Pulses are a low-fat source of protein with high levels of protein and fibre. Pulses also contain important vitamins and minerals like iron, potassium and folate. Many dietary guidelines recommend pulses as a part of healthy diet. Different researches show that person who eats pulses everyday have higher intake of fibre, protein, calcium, potassium, folate, zinc, iron, and magnesium. Many diets around the globe rely on pulses as a source of protein. The amount of protein in beans, lentils, chickpeas and peas is 2-3 times the levels found in cereal grains like wheat, rice, quinoa, oats, barley, and corn. Compared to animal and many other plant-based sources of protein, pulses are a more affordable and sustainable protein source. Pulses also contain resistant starch, a type of carbohydrate that behaves like fibre in the body; and has been shown have similar health benefits such as reduced circulating cholesterol and blood sugar levels as well as improved gut health.

Table 4: Observational data of Calcium with reference

Sample	B.R. (cm^3)	Amount (mg)	%	Reference
Cicer arietinum	0.15	6.012	0.60%	93-197mg/100g
Lens culinaris	0.2	8.01	0.80%	57.5-76.2mg/100g
Cajanus cajan	0.9	36.07	3.6%	81.4-140mg/100g
Vigna radiata	0.03	1.20	0.12%	105-140mg/100g
Vigna mungo	0.2	8.01	0.80%	375.01-485.38mg/100g

A number of nutrient and non-nutrient components of pulses have been connected to reduction of cancer risk. Additionally, epidemiological evidence as well as animal studies supports the protective link between pulse consumption and diseases such as cardiovascular disease (CVD) and diabetes. Pulses are also a key component in a number of healthy diets, such as the Mediterranean diet, which is associated with reduced risks of developing heart disease, hypertension, type 2 diabetes, cancer, Parkinson's disease, and Alzheimer's disease. The DASH diets, for those with high blood pressure, as well as the Gluten-Free Diet, for people who suffer from Celiac disease, also include pulses as significant components of their diets. When combined with a complementary protein source, pulses are also an optimal food source for vegetarians who wish to find an alternative to meat.

Conclusion

Calcium present in the different pulse samples is determined. Pulses mainly contain carbohydrate and proteins as a major component along with dietary fibers.

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