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BIOCHEMICAL CHARACTERISATION AND ENRICHMENT OF NUTRIENTS IN PULSES USING TERMITE MOUND SOIL

A PROJECT REPORT

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BONAFIDE CERTIFICATE

Certified that this project report "**BIOCHEMICAL CHARACTERISATION AND ENRICHMENT OF NUTRIENTS IN PULSES USING TERMITE MOUND SOIL**" is the bonafide work of **K.GOKUL (0710204013), G.KARTHIKA (0710204016), M.PRADEEPA (0710204031) and N.PRATHAP (0710204032),** who carried out the project work under my supervision.

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ABSTRACT

Pulses are a vital ingredient of the human diet in India. Pulses are important because they provide the essential proteins and elements. Red gram and hyacinth bean are the two most commonly used food pulses, in south India. The termite mound soil, owing to the presence of the some enzymes (due to the termites present), and the mineral content in the soil, brings a change in the proximate values and in the mineral contents. The pulses were mixed with the mound soil (processing) and the processed samples were tested for proximate values and minerals. It was found that there was an increase in the proteins in samples processed with the red termite soil, and the carbohydrate content was increased in all the processed pulse samples. Minor increase was observed in the minerals calcium, iron and potassium.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPLANATION
1.	mg	Milligram
2.	ml	Milliliter
3.	U/ml	Units per milliliter
4.	h	Hour
5.	L	Litre
6.	°C	Degree Celsius
7.	min	Minutes
8.	rpm	Revolutions per minute
9.	mM	Milllimolar
10.	M	Molarity
11.	OD	Optical density

INTRODUCTION

Pulses have been grown since millennia and have been a vital ingredient of the human diet in India.Even "balanced food" – as defined over 1000 years ago – consisted of pulses, besides cereals,vegetables and fruits, and milk products (Ayachit, 2002). Today, nutritionists tell us that pulses are important because they provide the essential proteins. Even today, pulses and milk provide the full complement of proteins to people who avoid eating meat. (Y. L. Nene, 2006).

Termites are important components of biologically mediated feedback to land-use change in the tropics. Carbon, nitrogen, and potassium levels in the termite mounds were significantly elevated, by 33, 28, and 38%, respectively (44 mg,2.5 mg, and 33 mg), while no significant difference in phosphorus, magnesium, iron, zinc, or copper concentrations was observed. Calcium was depleted by 27% in the termite mounds at 0.026 mg . Aluminum concentrations and acidity were significantly higher in the termite mound material (0.23 mg , pH 4.3) than surrounding soils (0.15 mg, pH 4.4). (Fernandes *et al.*, 2007).

Termite mound soils had higher concentrations of all elements tested than soils from woodlands, and termite mounds differed from woodland plots in terms of plant species composition. Trees growing on termite mounds had higher concentrations of all nutrients except sodium and crude protein. (Biotropica, 2004).

Red gram (*Cajanus cajan*) is an important pulse crop in India. It is also known as Pigeonpea, Arhar and Tur. Red gram is mainly cultivated and consumed in developing countries of the world. This crop is widely grown in India. India is the largest producer and consumer of Red gram in the world. Red gram is a protein rich staple food. It contains about 22 percent protein, which is almost three times that of cereals. The biological value improves greatly, when wheat or rice is combined with Red gram because of the complementary relationship of the essential amino acids. It is particularly rich in lysine, riboflavin, thiamine, niacin and iron.(Singh *et al.*,2002).

Garden bean (*Lablab purpureus*) originated in India and grows in the wild in Bengal and Assam. It is also known as Lablab bean, vaal, and sem. The mean crude protein content of lablab herbage was 17%. Lablab has the potential to supply rumen degradable nitrogen in excess of requirements. In a feeding trial, found that the improved plane of nutrition resulted

in a digestible crude protein intake 3-5 times that of the maintenance requirement.(Jakhmola and Pathak ,1981)

In South India, there is a tradition of processing the pulses with the termite soil to separate the seed coat easily from the pulse. These pulses were found to have an enhanced taste, than the normal pulse. This paved the way for our study of treating the pulses with the termite soil mound, to check for the variation in the proximate and mineral values.

OBJECTIVES

The present study was carried out with the objective to

- To collect pulses varieties from organically formed market and process the pulse samples with different termite mound soil
- To understand the nutrient and mineral contents transferred from termite mound soil to pulses.
- To evaluate biochemical analyses and to compare proximate values in raw and termite mound soil treated samples.

LITERATURE REVIEW

2. LITERATURE REVIEW

Pulses are the major sources of dietary protein in the vegetarian diet in India. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture (Kannaiyan, 1999).

2.1 Types of pulses

Table 2.1 Various pulses grown and consumed in India: (Nene, 2006).

Family name	Scientific name	Common name	Local name
Fabaceae	*Cicer arietinum*	Desi chana	Chickpea
Fabaceae	*Cicer arietinum*	Kabuli chana	Chickpea
Fabaceae	*Lens culinaris*	Masur	Lentil
Fabaceae	*Lens culinaris*	West Asian lentil	Masur
Fabaceae	*Cajanus cajan*	Tur	Pigeonpea, Arhar
Fabaceae	*Vigna mungo*	Urd	Black gram
Fabaceae	*Vigna radiata*	Mung	Green gram
Fabaceae	*Lablab purpureus*	Sem	Lablab bean, Vaal
Fabaceae	*Vigna aconitifolia*	Moth	Moth bean
Fabaceae	*Dolichos uniflorus*	Kulthi	Horse gram
Fabaceae	*Pisum sativum*	Matar	Pea
Fabaceae	*Lathyrus sativus*	Khesari	Grass pea
Fabaceae	*Vigna unguiculata*	Lobhia	Cowpea, Chowli
Fabaceae	*Vicia faba*	Baqla	Faba bean

It has been reported that cultivated plants with high chemical inputs such as fertilizers, plant growth regulator, herbicides etc has lost their natural taste, appearance and nutritive values (Sekaroglu et al., 2006).

For this reason we took two types of pulses pigeon pea and hyacinth bean and processed with termite soils, and analyzed for their proximate and mineral values, before and after processing to test the change in the values, if found.

Table 2.2 Description of *Cajanus cajan*

Order	Fabales
Family	Fabaceae
Genus	*Cajanus*
Species	*Cajan*

Fig .2.1 *Cajanus cajan*

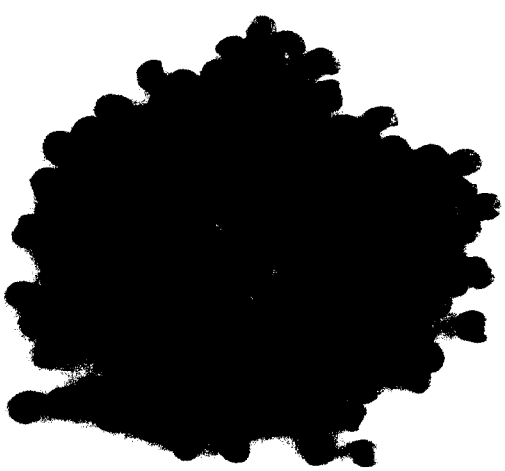
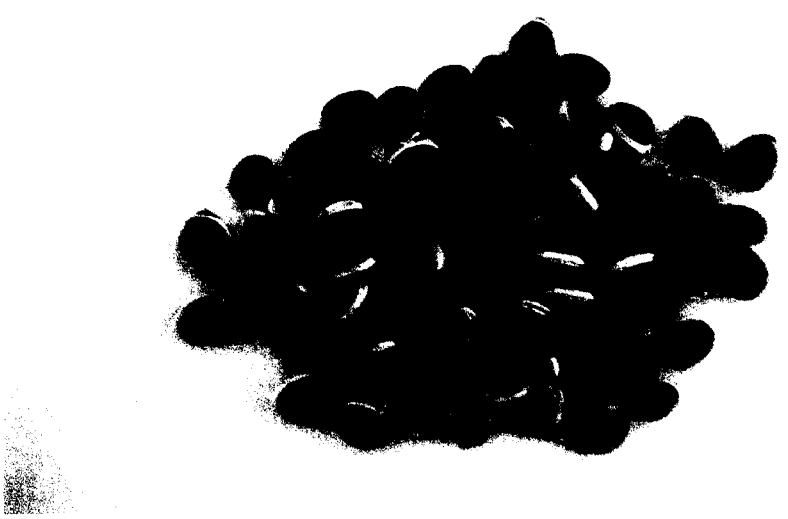


Table 2.3 Description of *Lablab purpureus*

Order	Fabales
Family	Fabaceae
Genus	*Lablab*
Species	*purpureus*

Fig 2.2 *Lablab purpureus*



2.2 Termite mound soil-Properties

Termites are one of the most abundant animals on earth. They inhabit 2/3 of the lands surface, mostly in the tropical regions. They live on cellulose, which is why they eat wood. However, termites do not produce cellulases, the enzymes that break down cellulose. These enzymes are produced by protozoa in the termite gut that take in the ingested cellulose chips,digest them and produce acetate and other products that the termites can use for energy and carbon (Prasad *et al.*, 1987).

Termites have been identified as common biological agents that produce significant physical and chemical modifications to tropical and subtropical soils (Semhi *et al.*, 2008). Termites go through a sequence of actions, from fetching, carrying, to cementing mineral particles into mounds by using their salivary secretion (Lopez-Hernandez, 2001). Also, it has been shown that termite activity increases the content of organic matter in the soils that they use for the construction of their nests and also modifies the clay mineral composition of these soil materials (Jouquet *et al.*, 2002; Amsaleg *et al.*, 2004).Studies emphasized the role of termite on soil texture and chemical properties (Wood *et al.*, 1983), soil nutrient cycling and soil metabolism (Menaut *et al.*, 1985; Abbadie & Lepage, 1989).

2.3 Pulses description

Pulses are the edible seeds of plants, such as peas, beans and lentils which are members of the legume plant family. Pulses are great for energy because they are high in fiber, protein, vitamins, minerals and complex carbohydrates. They are also healthy because they are low in fat and have no cholesterol. Food that is high in fiber, complex carbohydrates and essential nutrients are great for long-term energy. Pulses have been grown by farmers since millennia, and these have contributed in providing nutritionally balanced food to the people of India. Pigeonpea, black gram, green gram, lablab bean, moth bean, chickpea and lentil and horse gram have definitely originated and domesticated in the Indian subcontinent (Y L Nene, 2006).

2.4 Properties of *Cajanus cajan*

Pigeon pea (*Cajanus cajan*) is known under different names in different languages of the world. In addition to pigeon pea, other English names are cango pea, angola pea, goongo, Puertorico pea, and red gram. (Morton, 1976). Pigeon pea is among the most heat and drought resistant legume crop, capable of surviving even on the poor soils of the driest savana (Aron, 1972). Although, little of the crop enters world trade, pigeon pea is the fifth most important crop in the world. It is mainly a subsistance crop in the tropics and subtropics of India, Africa, South-east Asia and the Caribbean, but also an important cash crop in West Indies (Whiten *et al.*, 1985). Pigeon pea is a rich source of protein, carbohydrates and certain minerals. It is made of three anatomical structures; the seed coat, the cotyledons and the embryonic tissue (Singh *et al.*, 1968).

The cotyledons contain about 90% of the protein, 95% of the fat, 86% of the carbohydrates, 83% of the minerals and most of the phosphorus of the whole seed. Although, germ is rich in protein (48.1%), fat (13.5%), and minerals, its contribution is neglible on total seed weight basis.The cotyledons are the major sources of nutrients (Salunkhe *et al.*, 1986) .

Akbar et al. (1973) reported 10.2 per cent moisture for Desi variety of pigeon pea. Kadwe *et al.*, (1974) reported moisture content in the range of 8.5% to 9.4% for some varieties of *Cajanus cajan.* Shrivastava and Bajpai (1980), however, reported that the moisture content of some varieties varied from 12.22% to as low as 6.37%. Armando (1935) had earlier reported the moisture content of 10.20% to 15, 63% for philipinian varieties of *Cajanus cajan.* Khalil *et al.*, (1986) recorded a value of 25.6 %- in protein in dhal. (Salunke *et al.*, 1986) reported that whole pigeon seed contained 10.1% moisture. 19.2% protein, 57.3 carbohydrate, 1.5% fat, 8.1% crude fiber and 3.8% ash compared to 15.2% moisture, 22.3% protein, 57.2% carbohydrate, 1.7% fat and 3.6 ash in dehusked splits of pigeon pea. Khalil *et al.*, (1986) determined the average values for the proximate composition of two lots of dry mature seeds of pigeon pea from Pakistan. According to him, pigeon pea contained 10% moisture, 21.3% crude protein, 1.2% fat, 4.5% ash, 8.2% crude fibre.

In a comparative study of the chemical composition and nutritive value of some common Indian pulses, Pant and Kapur.(1963) reported that *cajanus cajan* contained 11.20% moisture, 22.31% protein , 1.452 Fat and 3.21% ash. The early maturing varieties of pulses have been reported to contain more protein in seeds than the late maturing cultivar (Tipathi *et al.*, 1975)

Pigeon pea seeds contain 57.3% to 58.7% total carbohydrates of which major proportion is contributed by starch (Salunkhe *et al.* 1986). Shrivastava and Bajpai, (1980) determined carbohydrates of several varieties of pigeon pea by the method of Nelson (1949) and found that it ranged from 41.34% to 47.96%. The legumes were relatively high in calcium, iron, magnesium, phosphorus, and potassium, and low in sodium. Singh *et al* (1977) reported that red gram contained 0.34% phosphorus, 1.47% potassium, 0.176% calcium and 0.172% magnesium. Among the trace minerals Singh *et al.*, (1977) reported that *Cajanus cajan* contained 22.66 ppm zinc, 9.63 ppm copper, 20.39 ppm iron and 21.53 ppm manganese. Nwokolo, (1937) reported that pigeon pea meal had very high content of potassium (12500 mg/kg), calcium and magnesium and low content of iron, zinc, copper and manganese.

Pulse legumes are a major source of dietary proteins and calories in food and food products throughout the world. Daniel *et al.*, (1970) observed that the incorporation of 8.50% red gram dhal in poor rice diet and 16.7% red gram dhal in poor ragi diet, along with vitamins and minerals, markedly improved the overall nutritive value of the diet as judged by the growth of young rats.

2.5 *Lablab purpureus*

Lablab (*Lablab purpureus*, formerly *Dolichos lablab*), also called hyacinth bean, Egyptian bean, and (in Japan) Fuji mame, is a popular legume vegetable in South Asia, China, Japan, West Africa, and the Caribbean . With a protein range between 21% and 28%, *LabLab* is high quality forage.

The mature seeds of five cultivars of dolichos bean (*Dolichos lablab*) were analysed for some nutritional and antinutritional factors. The percentage of crude protein varied from 22·4 to 31·3, crude fibre, 7·62 to 9·63 and total carbohydrate, 54·2 to 63·3. The amounts (mg/100 g) of calcium, phosphorus and iron ranged from 36·0 to 53·5, 388 to 483, and 282 to 380 respectively

2.6 Health benefits of pulses

10 Reasons to use pulses:

- Excellent source of fiber.
- Good source of protein.
- Low-fat.
- Low-sodium.
- Good source of iron.
- Excellent source of folate.
- Good source of potassium.
- Low glycemic index.
- Gluten-free.
- Cholesterol-free.

These foods usually have low glycemic index (GI) value. The lower the GI value, the longer it takes for your body to digest the food for energy. This is a good thing, especially if you are diabetic because glucose is absorbed at a steadier rate, reducing blood sugar spikes. Pulses are also great for weight management because they digest slower which keeps the hunger away. The nice things about pulses are that they are versatile, inexpensive, and easy to

store and have longer shelf-life. The lower GI of legumes has been attributed to the viscosity of food, high un-absorbable carbohydrate content or delayed gastric emptying (Hemraj Chandalia *et al.*, 1992).

The glycemic index or GI is a measure of the effects of carbohydrated on blood sugar levels. Carbohydrates that break down quickly during digestion and release glucose rapidly into the bloodstream have a high GI; carbohydrates that break down more slowly, releasing glucose more gradually into the bloodstream, have a low GI.

A lower glycemic index suggests slower rates of digestion and absorption of the foods' carbohydrates and may also indicate greater extraction from the liver and periphery of the products of carbohydrate digestion. A lower glycemic response usually equates to a lower insulin demand but not always, and may improve long-term blood glucose control and blood lipids. The insulin is also useful for providing a direct measure of the insulin response to a food (David *et al.*, 1981).

Pulses supply many bioactive substances found in minor amounts in food, but which may have significant metabolic and/or physiological effects. These compounds have long been classified as antinutritional factors, but many studies have reconsidered their impact on health. Some could play a role in the prevention of the major diseases of affluent societies. As these compounds can be beneficial or adverse, depending on conditions, an assessment of their various physiological effects is necessary to determine whether they should be preserved or eliminated in each main nutritional situation (Martine M.J. Champ).

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

2.6.1 Pulse as a 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. Pulses and the gluten-free diet.

Pulses are a healthy option for people with celiac disease. Compared to many of the other gluten-free grain alternatives, pulses are an excellent source of dietary fibre, protein, iron, and other minerals and vitamins. For example, pulses have two major advantages in the gluten-free market. One is that pulses are high in iron, as iron deficiency anemia is a common nutritional concern for celiacs, along with vitamin B deficiencies. Secondly, pulses help with another problem that arises as a result of a diet low in whole wheat fibre, wheat bran and other dietary fibres: constipation. Pulses are a good source of fibre.

In the gluten-free diet, all forms of wheat, rye and barley must be strictly avoided. This can be a major challenge, as gluten is found in so many different foods, such as: breads, baked products, cereals, pastas, soups, sauces, seasonings, salad dressings, snack foods, prepared meats (hot dogs, deli meats, and hamburger patties), flavored coffees and teas, candy and some medications. (Shelley Case, 2003).

2.6.2 Pulse as a 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.

2.6.3 Pulse, a major 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 provides the full complement of amino acids needed for growth.

2.6.4 Pulse in weight management diet

Although more studies are needed, consuming pulses may help with weight management. 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. low fat, low glycemic diet led to a significant reduction in body mass and fat mass whereas lean body mass-reduction was negligible. This diet seems to be a promising method for the treatment of obesity (Bahadori B, *et al.*, 1998)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 GLASSWARE AND CHEMICALS

Good quality glassware and chemicals were used for all tests. All the glassware was of brand Borosil or Corning. They were washed with good detergent, rinsed in tap water and soaked in chromic acid clearing solution.

3.2 CLEARING SOLUTION (Mahadevan & Sridhar, 1996)

Potassium dichromate - 60g

Conc H_2SO_4 - 60ml

Distilled water - 1 L

Potassium dichromate was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning glassware. Then they were rinsed thrice in tap water, finally rinsed in distilled water and dried in hot air oven. Dried glassware was sterilised in an autoclave for 15 minutes at 15lb/sq inch pressure. These sterilisation and cleaning methods were used for further experiments.

3.3 CHEMICALS

Analytical grade chemicals supplied by Loba, S.D.Fine Chemicals, E.Merck, Qualigens and Sigma Chemicals(U.S.A.) were used in the study.

3.4 PULSE COLLECTION AND IDENTIFICATION

The pulses are collected from Tamilnadu Agricultural University (TNAU), Coimbatore. The pulses collected are Co(Gb)14[hyacinth bean] and Co(Rg)7[red gram]. The pulses were collected after thorough investigation to check for any pathological disorders and from contamination of other seeds and shade dried.

3.5 SOIL COLLECTION

Two soil samples were collected. Red termite soil from the termite mound present in the cultivation fields of Kunnathur, near Annur, Coimbatore. Black termite soil was taken from the termite mounds in the cultivation lands of Pogalur, near Annur, Coimbatore district.

3.6 PREPARATION OF SAMPLES

A total of 100 grams of seeds (pulses- hyacinth bean and red gram) were air dried and then ground into fine powder individually for further experiments.

3.7 SAMPLE PROCESSING

The pulses were washed and cleaned with distilled water. The washed pulses were mixed for processing with termite mound soils (red termite soil and black termite soil), with a little of water to keep the mixture intact. The mixture was kept at room temperature for 2 days. After 2 days the mixture was kept under the sun for a day. The pulses and the soil were separated and they were used for further analysis.

3.8 DETERMINATION OF NUTRITIVE VALUES

Pulses are major food sources and are rich source of protein in the vegetarian diet of the Indians. So it was decided to determine the nutritive values of the pulses. The amount of carbohydrate, protein, fibre, fat, ash, moisture content and few minerals were determined.

3.8.1 DETERMINATION OF CRUDE PROTEIN

Reagents

Reagent A:

2% sodium carbonate in 0.1N sodium hydroxide

Reagent B:

0.5 % copper sulphate in 1% potassium sodium tartarate

Alkaline copper reagent:

Mix 50 ml of A and 1 ml of B prior to use

Folin –Ciocalteau reagent:

Folin –Ciocalteau solution was diluted with equal amount of water .

Protein solution (stock standard):

Weigh accurately 50 mg of bovine serum albumin and dissolve in distilled water and make upto 50 ml in standard flask.

Working standard:

Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask.1 ml of the solution contains 200mg protein.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay .weigh 500 mg of the sample and grind well with a mortar and pestle in 5-10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of protein

Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes. Pipette out 0.1 and 0.2 ml of sample extract in 2 test tubes. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank. Add 5 ml of reagent C to each tube including the blank. Mix well and allow standing for 10 minutes. Then add 0.5 ml of reagent D . mix well and incubate in room temperature in the dark for 30 minutes. Blue colour is developed. Take the readings at 660nm. Draw the standard graph and calculate the amount of protein in the sample.

3.8.2 Carbohydrate estimation

Reagents

Phenol 5%:

Redistilled phenol (50gm) dissolved in water and diluted to 1 litre

Sulphuric acid 96%

Standard glucose:

Stock 100 mg in 100 ml of distilled water

Working standard:

10ml of stock diluted to 100ml with distilled water.

Procedure

Weigh 100mg of sample into boiling tube. Hydrolyse by keeping it in a boiling water bath for 3 hrs with 5ml of 2.5 N HCl and cool to room temperature. Neutralise it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100 ml and centrifuge. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes. Pipette out 0.2 and 0.2 ml of sample solution into separate test tubes. Make up the volume in each test tube to 1 ml with water. Set the blank with 1 ml water.

Add 1 ml of phenol solution to each tube. Add 5 ml of 96% sulphuric acid to each tube and shake well. After 10 min shake the contents in the tube and place in a water bath at 25^0 to 30^0C for 20 minutes. Read the colour at 490 nm. Calculate the amount of total carbohydrate present in sample solution using standard graph.

3.8.3 ESTIMATION OF MOISTURE

Procedure

The moisture content of the various materials used in the study were estimated following the method described by Johnson and Ulrich.

About 5 g of freshly collected samples were placed in bottles and dried in a hot air oven for 10-12 hrs. The weights of the samples were noted after 12 hrs. The difference in weight was expressed as percentage of moisture on oven dry basis.

Calculation

Weight of samples before drying in hot air oven = A

Weight of samples after drying in hot air oven = B

Loss in weight of samples = A-B

Moisture content = (A-B) x 100/A

3.8.4 ESTIMATION OF ASH CONTENT

Procedure

5 g of the sample was taken in a crucible. Weight of the empty crucible was noted. Then it was kept in a muffle furnace at a temperature of 600^0C for 4 hours. The inorganic matter present was determined gravimetrically. The inorganic matter was washed with 1:1 HCl and transfer to a standard flask for future analysis.

Calculation

Weight of crucible with sample before heating = A

Weight of crucible with sample after heating in muffle furnace =B

Weight of ash = A-B

Ash content in g/ 100 g of sample = (Wt of ash / wt of sample) * 100

3.8.5 DETERMINATION OF CRUDE FAT

Reagents

Petroleum ether of boiling range 40^0 C to 60^0 C.

Hexane, food grade confirming to IS:3470-1966.

Procedure

The prepared sample (5g) was dried in a hot air oven at 105^0 C for at least 2 h and was extracted with petroleum ether or hexane in a soxhlet extracter. The extraction was done at a condensation rate 5 to 6 drops per sec for 4 h and 2 to 3 drops per sec for 16 h. The extract was dried on a steam bath for 30 minutes, cooled in a dessicator and weighed. Alternative drying and weighing were done at 30 min intervals until the difference between two successive weighing was less than 1 mg and the lowest mass were noted.

Calculation

Percentage of crude fat =100 * (M1 –M2)/M

(On moisture free basis)

Where,

M1= Mass in g of the extraction flask with dried extract

M2= Mass in g of the extraction flask

M = Mass in g of dried sample taken for the test

3.8.6 Determination of crude fibre (IS, 1990)

Reagents

Sulphuric acid – 0.255N (1.25% v/v), accurately prepared

Sodium hydroxide solution 0.313 N (1.25% m/v), accurately prepared

Procedure

The dried material (2g) was extracted for fat content with petroleum ether or hexane , using soxhlet extracter (alternatively , the residue from the crude fat determination can be used). The fat free dry residue was transferred to 1 litre conical flask. Boiling dilute sulphuric acid (200 ml) was added to the flask with the fat free material and immediately the flask was connected with a reflux condenser and heated for 30 min. The contents were filtered through fine linen held in a funnel. The residue was washed with boiling water. The residue on the linen was washed into 200 ml of boiling sodium hydroxide solution.

Immediately the flask was connected with the reflux condenser and boiled for 30 min. The solution was filtered, washed with boiling water and transferred to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. The residue was washed first with hot water and then with 15ml of 95% (by volume) ethyl alcohol. The Gooch crucible and the contents were dried at 105^0C in the hot air oven to constant mass. It was cooled and weighed. The contents of the Gooch crucible were incinerated at 600^0C in a muffle furnace until all the carbonaceous matter was burnt. The crucible containing the ash was cooled in a dessicator and weighed.

Calculation

Percentage of crude fibre = 100 *(M1-M2) / M

(On moisture free basis)

Where,

M1 = Mass in g of Gooch crucible and contents before ashing.

M2 = Mass in g of Gooch crucible containing asbestos and ash.

M = Mass in g of the dried sample taken for the test.

3 .9 Estimation of trace elements

3.9.1 Phosphorus estimation

Reagents

Vanadate-Molybdate composite reagents

Dissolve 20 mg ammonium molybdate in 400 ml warm water (50^0C) and cool. Dissolve 1g of ammonium vanadate in boiling distilled water, cool and add 140ml conc. Nitric acid gradually with stirring. Then add the molybdate solution gradually to the acid vanadate solution with stirring and dilute to 1 litre with water.

Standard phosphate solution

Prepare a stock solution containing 3.834 g of potassium dihydrogen phosphate per litre. Dilute 25 ml to 250 ml.

Preparation of standard graph

To a series of 100 ml volumetric flasks add 0, 2.5, 5, 10, 20, 30, 40, 50 ml of standard phosphate solution and dilute each to 50-60 ml with water. Add a few drops of ammonia solution (0.88) and make just acid with nitric acid (1:2). Add 25 ml of vanadate-molybdate reagent, dilute to the mark and mix. Allow to stand for 10 min and measure the optical density in a 2.5 or 10 mm cell at 470 nm.

Procedure

Transfer a suitable volume of the sample to a 100 ml volumetric flask. If the determination is carried on the ash, boil the ash with 10 ml of 5N hydrochloric acid and wash the solution into the 100 ml flask with water, filtering if necessary. Neutralize with dropwise addition of 0.88 ammonia, and proceed as for the standard graph i.e. make just acid with dilute nitric acid, add 25 ml of vanadate-molybdate reagent, dilute to the mark and measure the optical density after allowing to stand for 10 minutes.

3.9.2 Calcium and magnesium estimation

Reagents

Buffer solution

Dissolve 16.9 g of NH_4Cl in 143 ml of NH_4OH. Add 1.25g magnesium salt of EDTA to obtain sharp change in colour of indicator and dilute to 250 ml. If magnesium salt of EDTA is unavailable, dissolve 1.179g disodium salt of EDTA (AR grade) and 780 mg

$MgSO_4.7H_2O$ or 644 mg $MgCl_2.6H_2O$ in 50 ml distilled water. Add to above solution of NH_4Cl in NH_4OH and dilute to 250 ml.

Inhibitor:

Dissolve 4.5 g hydroxylamine hydrochloride in 100 ml of 95% ethyl alcohol or isopropyl alcohol.

Eriochrome black T indicator:

Mix 0.5 g dye with 100g NaCl to prepare dry powder.

Murexide indicator:

Prepare a ground mixture of 200 mg of murexide with 100 g of solid NaCl.

Sodium hydroxide:

Dissolve 80 g NaOH and dilute to 1000 ml.

Standard EDTA solution 0.01M:

Dissolve 3.723 g EDTA sodium salt and dilute to 1000 ml. Standardize against standard Ca solution, 1 ml=1 mg calcium carbonate.

Standard calcium solution:

Weigh accurately 1g AR grade calcium carbonate and transfer to 250 ml conical flask. Place a funnel and add HCl till $CaCO_3$ dissolves completely. Add 200 ml distilled water and boil for 20-30 min to expel CO_2. Cool and add few drops of methyl red indicator. Add NH_4OH 3N drop wise till intermediate orange colour develops. Dilute to 1000 ml to obtain 1 ml= 1 mg $CaCO_3$

Procedure

Total hardness

Take 25 or 50 ml well mixed sample in porcelain dish or conical flask. Add 1-2 ml of buffer solution followed by 1 ml inhibitor. Add a pinch of Erichrome black T and titrate with standard EDTA (0.01M) till wine red colour changes to blue. Note down the volume of EDTA required (A). Run a blank. Note the volume of EDTA (B).

Calculate the volume of EDTA required by the sample,
C= (A-B) from volume, of EDTA required in steps 3 & 4.

Calcium hardness

Take 25 or 50 ml of sample in a porcelain dish. Add 1 ml of NaOH to raise pH to 12.0 and add a pinch of murexide indicator. Titrate immediately with EDTA till pink colour changes to purple. Note the volume of EDTA required (A'). Run a reagent blank. Note the ml of EDTA required and keep it aside to compare end points of sample titrations. Standardize the EDTA (0.1M) solution following the procedure of calcium hardness from 1-4 using standard calcium solution.

Calculation

Calcium hardness as $CaCO_3$,

(mg/l) $$= \frac{A' * D * 1000}{\text{ml sample}}$$

Where,

A' = volume of EDTA used by sample

D = mg $CaCO_3$ equivalent to 1 ml EDTA titrate.

Magnesium hardness as $CaCO_3$ = Total hardness as $CaCO_3$ - Calcium hardness as $CaCO_3$

3.9.3 ATOMIC ABSORPTION SPECTROSCOPY

ESTIMATION OF IRON, POTASSIUM AND ZINC

Reagents

1. Deionized water (resistivity of 1 megohm or better), referred to as H_2O.
2. Reagent grade concentrated nitric acid (HNO_3), 70%.
3. Reagent grade concentrated hydrochloric acid (HCl), 37%.
4. Diluted HCl (approximately 9.25% HCl prepared from reagent grade HCl by diluting Concentrated HCl (37%) 25 mL to 100 mL with H_2O.

Procedure:

1. With each set of samples, prepare a blank in the same manner.

2. Weigh the amount of sample required (about 5 g of dry matter) into a crucible. Note: crucible weight, and crucible plus as is sample weight.

3. Dry the sample in an oven for about 16 hours (overnight) at 105°. Note: crucible plus dry sample weight.

4. Place the samples in the furnace set to 200°. Keep the doors slightly ajar to allow the smoke to escape. Allow the samples to reach 200°.

5. If there is little or no smoking, raise the temperature (50o increment).

6. Repeat step 5 until the temperature reaches 350°, and then close the furnace door, set the temperature to 450°, and ash about 16 hours (overnight).

7. Remove the samples from the furnace and allow them to cool. Wet the ash with water and add sufficient nitric acid to cover the ash(typically 2-4 ml). Cover with a watch glass and reflux on a hot plate for about 1 hour, then remove the watch glass and if necessary reduce the heat to gently evaporate the acid. Failure to remove the acid may cause ignition of the organic residue. Return the samples to the furnace and ash at 375^0c for 1 hour.

8. Repeat step 7, if necessary until white ash is obtained.

9. Add 2-5 mL diluted HCl and dissolve the ash by gently boiling the solution, cool and make up to the appropriate volume with H_2O.

10. Determine elements by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

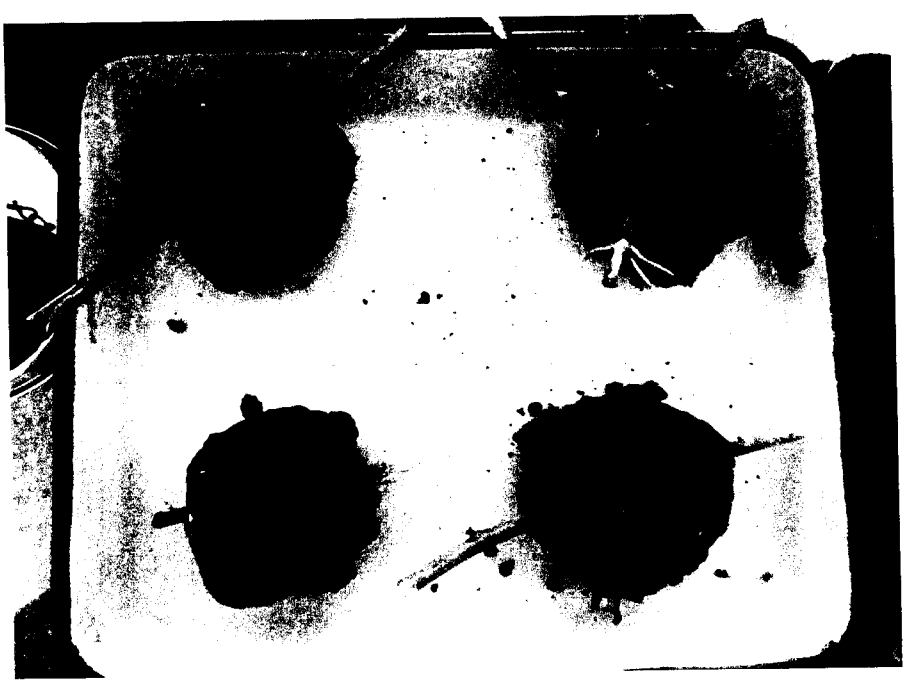
4. RESULTS AND DISCUSSION

The pulses were collected from Tamilnadu Agricultural University (TNAU), Coimbatore. The pulses collected are Co(Gb)14[hyacinth bean] and Co(Rg)7[red gram]. The pulse samples were collected after thorough investigation to check for any pathological disorders and from contamination of other seeds and shade dried.

Two soil samples were collected. Red termite soil from the termite mound present in the cultivation fields of Kunnathur, near Annur, Coimbatore. Black termite soil was taken from the termite mounds in the cultivation lands of Pogalur, near Annur, Coimbatore district.

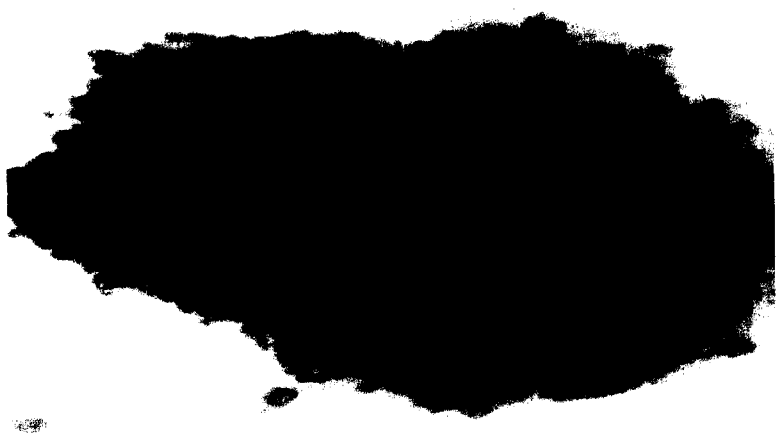
A total of 100 grams of seeds (hyacinth bean and red gram) were air dried and then ground into fine powder individually for further experiments. The pulse samples were washed and cleaned with distilled water. The washed pulses were mixed for processing with termite mound soils (red termite soil and black termite soil), with a little of water to keep the mixture intact. The mixture was kept at room temperature for 2 days. After 2 days the mixture was kept under the sun for a day. The pulses and the soil were separated.

Fig.4.1 Sample during processing



The pulse samples were powdered and used for the proximate analysis. For mineral analysis, the pulse samples were ashed and acid hydrolyses using Hydrochloric acid and then mixed with Sulphuric acid, centrifuged and filtered for measurement in Atomic absorption spectrophotometer, for estimation of elements present, quantitatively.

Fig. 4.2 Ashed pulse sample



As expected the proximate values of *Cajanus cajan*, after processing with red and black soil showed a considerable changes in the values as observed in Fig. 4.5.

Table 4.1 shows the change in the proximate value of *Lablab purpureus* after processing with the red and the black soil, showing an increase in the carbohydrate values and decrease in the protein value.

***Cajanus cajan*, when treated** with red soil, shows an increase in protein content and moisture content, whereas the other values such as carbohydrates and crude fibre content decreases. The minerals like phosphorous and magnesium shows a slight increase. *Cajanus cajan* treated with the black soil increases its carbohydrate content whereas the protein content decreases. The mineral values, calcium and zinc show a considerable change.

Lablab purpureus with red soil shows a considerable increase in carbohydrate and moisture content, whereas the protein content has decreased. The magnesium content of *L.purpureus* was slightly increased. When processed with black soil, the carbohydrate content was increased whereas protein and fat content remains the same. Calcium content of *L.purpureus* was increased when treated with black soil.

Table 4.1 Comparison of the proximate values of raw sample and treated samples of *Lablab purpureus*

Proximate values	Raw pulse sample	Pulse sample processed with red soil	Pulse sample processed with black soil
Protein	21.13	13.19	13.13
Fat	1.53	0.82	0.83
Ash content	4.03	2.88	2.27
Moisture content	7.77	10.85	10.2
Crude fibre	2.61	1.92	1.92
Carbohydrate	62.93	70.34	71.65

Fig. 4.3 Comparison of the proximate values of raw sample and treated samples of *Lablab purpureus*

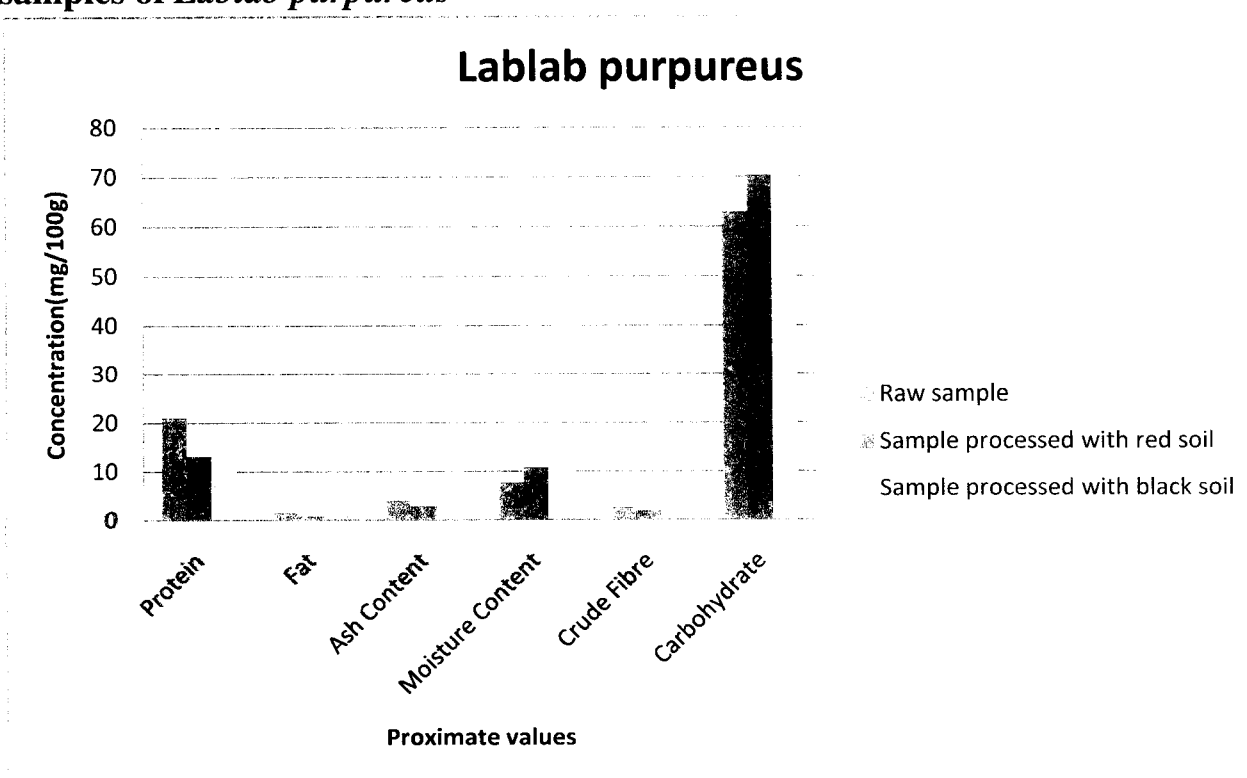


Table 4.2 Comparison of the proximate values of raw sample and treated samples of *Cajanus cajan*

Proximate values	Raw pulse sample	Pulse sample processed with red soil	Pulse sample processed with black soil
Protein	13.75	15.63	9.75
Fat	0.76	0.93	0.74
Ash content	3.75	3.06	2.53
Moisture content	7.65	9.35	10.96
Crude fibre	2.37	1.52	1.7
Carbohydrate	71.72	69.51	74.32

Fig. 4.4 Comparison of the proximate values of raw sample and treated samples of *Cajanus cajan*

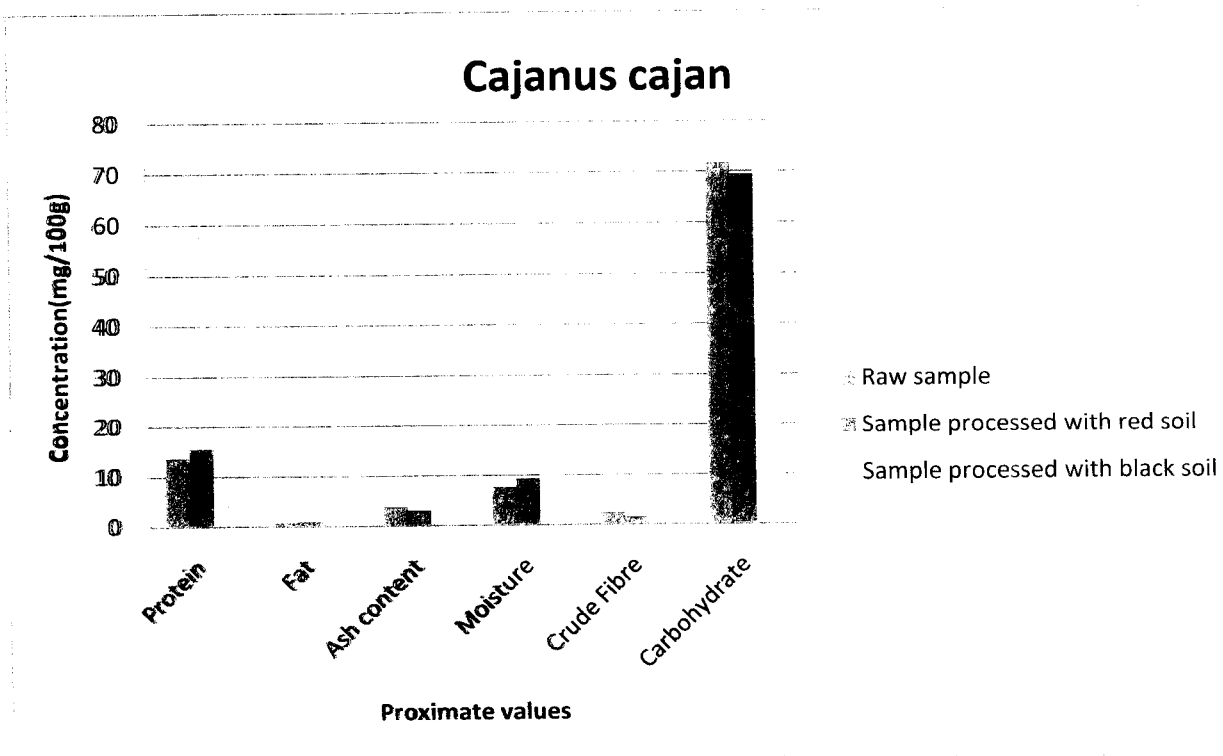


Table 4.3 Comparison of protein concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	13.75	21.13
Processed with red soil	15.63	13.19
Processed with black soil	9.75	13.13

Fig. 4.5 Comparison of protein concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

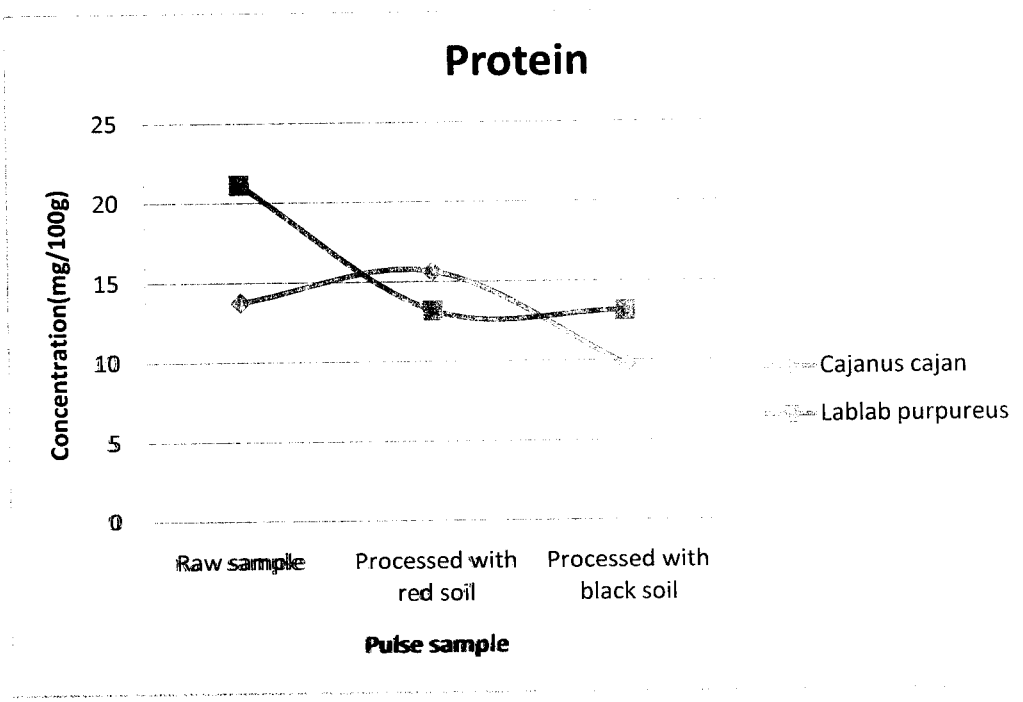


Table 4.4 Comparison of fat concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	0.76	1.53
Processed with red soil	0.93	0.82
Processed with black soil	0.74	0.83

Fig. 4.6 Comparison of fat concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

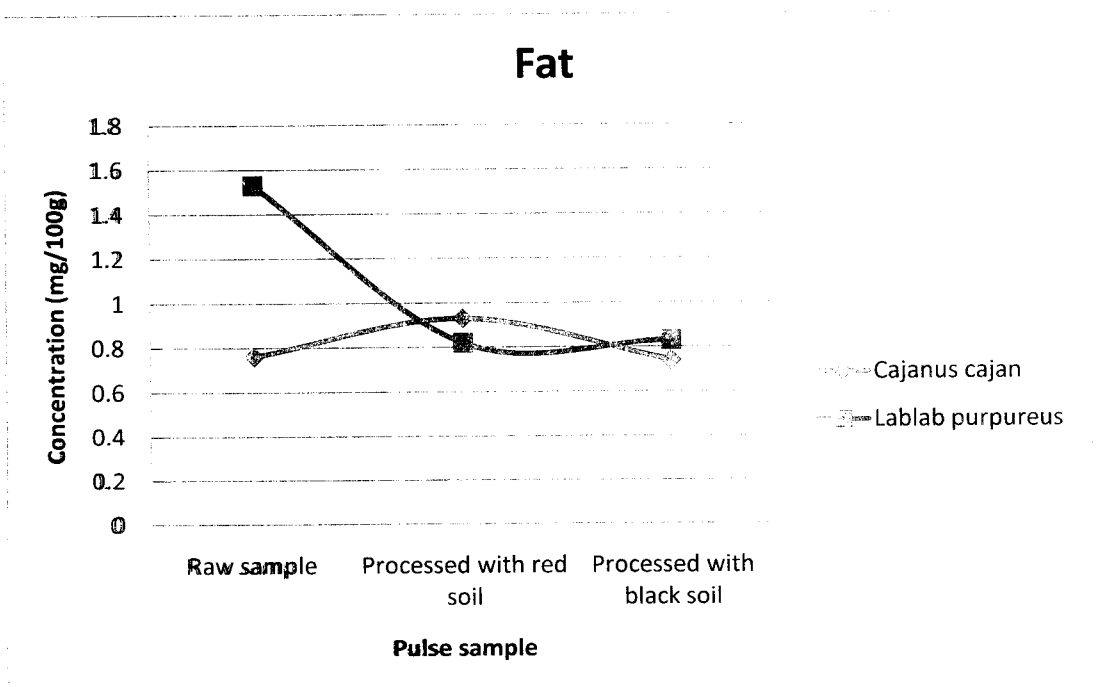


Table 4.5 Comparison of Ash content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	3.75	4.03
Processed with red soil	3.06	2.88
Processed with black soil	2.53	2.27

Fig. 4.7 Comparison of Ash content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

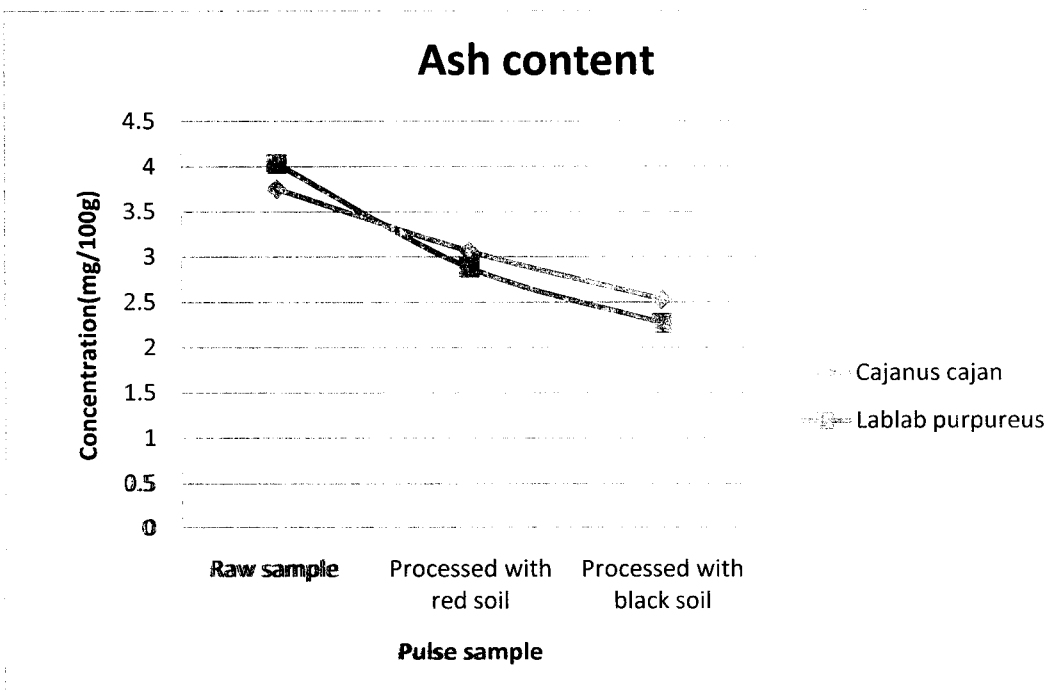


Table 4.6 Comparison of Moisture content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	7.65	7.77
Processed with red soil	9.35	10.85
Processed with black soil	10.96	10.2

Fig. 4.8 Comparison of Moisture content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

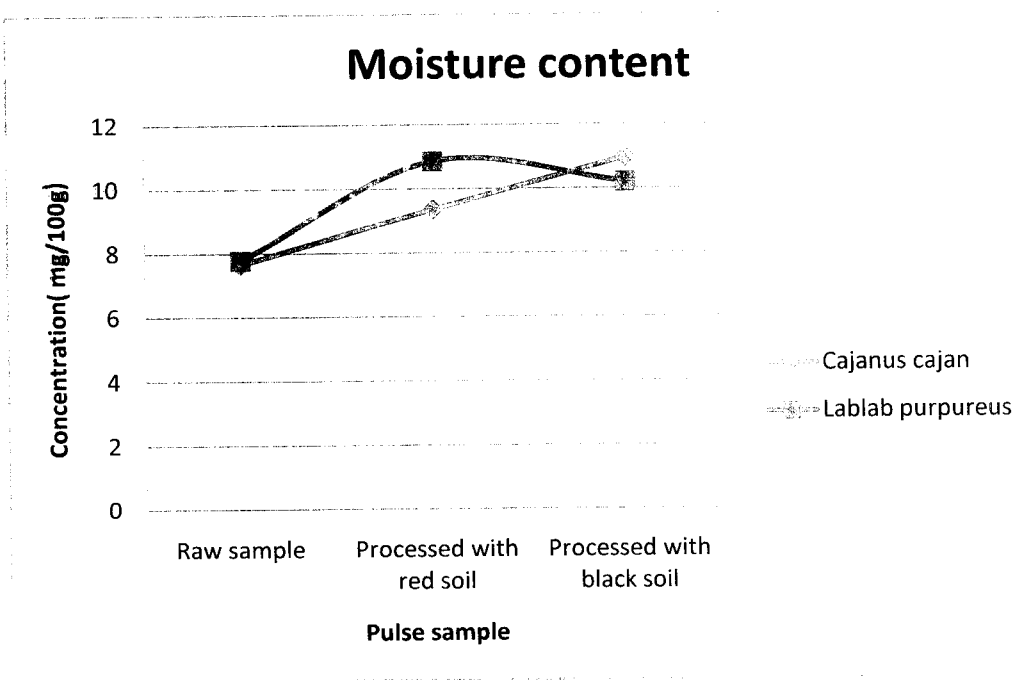


Table 4.7 Comparison of Crude fibre concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	2.37	2.61
Processed with red soil	1.52	1.92
Processed with black soil	1.7	1.92

Fig. 4.9 Comparison of Crude fibre concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

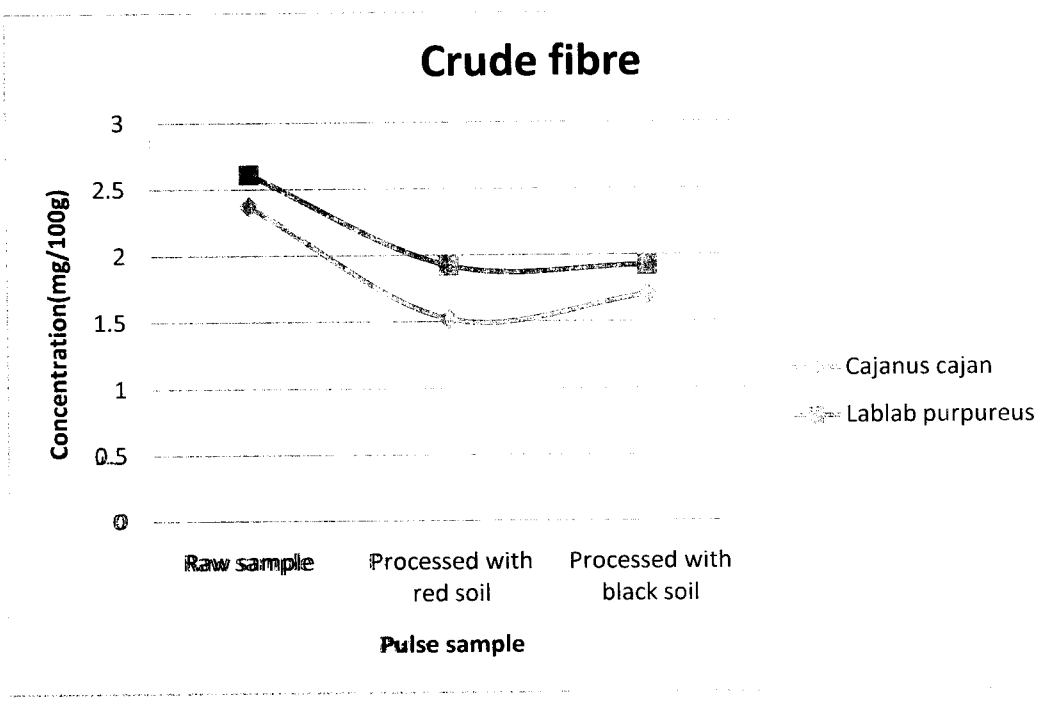


Table 4.8 Comparison of Carbohydrate concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	71.72	62.93
Processed with red soil	69.51	70.34
Processed with black soil	74.32	71.65

Fig. 4.10 Comparison of Carbohydrate concentration in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

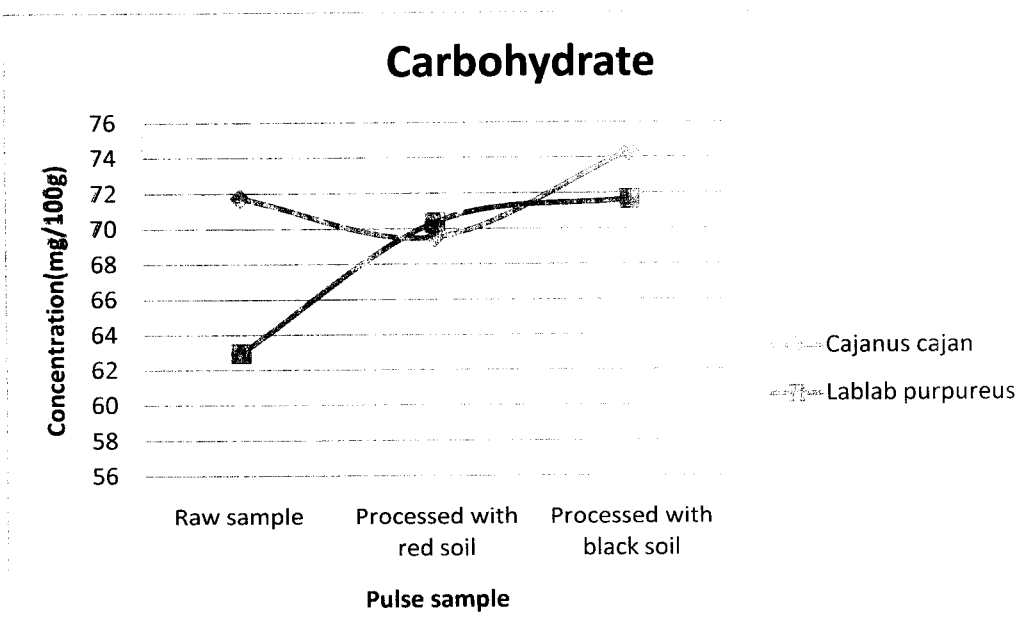


Table 4.9 Comparison of the elemental values of raw sample and treated samples of *Lablab purpureus*

Elements	Raw pulse sample	Pulse sample processed with red soil	Pulse sample processed with black soil
Iron(mg)	3.9	3.9	4.2
Phosphorous(x 10^{-2}g)	34.5	35.2	34.2
Calcium(mg)	98	97.5	99.5
Potassium(x 10^{-2}g)	57.143	57.66	56.89
Magnesium(mg)	42.1	44.5	41.8
Zinc(mg)	0.8	0.78	0.8

Fig. 4.11 Comparison of the elemental values of raw sample and treated samples of *Lablab purpureus*

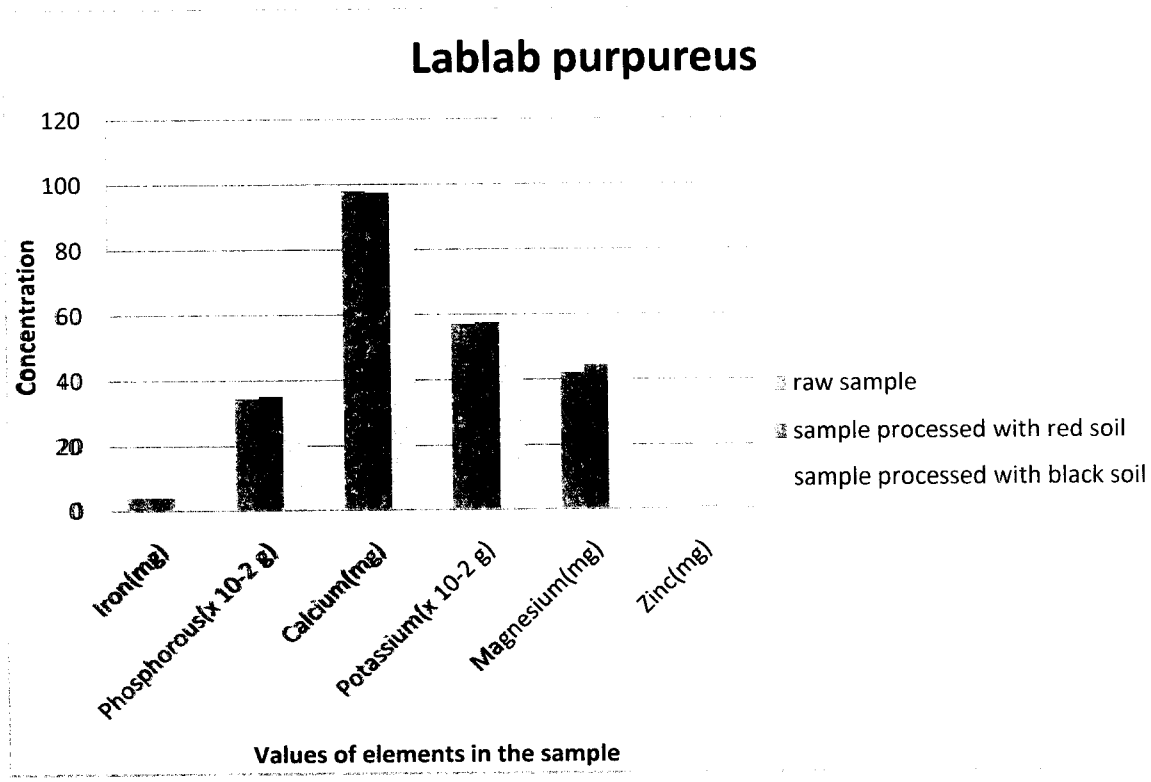


Table 4.10 Comparison of the elemental values of raw sample and treated samples of *Cajanus cajan*

Elements	Raw pulse sample	Pulse sample processed with red soil	Pulse sample processed with black soil
Iron(mg)	2.9	2.76	3.3
Phosphorous(x 10^{-2}g)	25.26	25.42	25.14
Calcium(mg)	72.4	70.8	73.5
Potassium(x 10^{-2}g)	57.68	57.75	58.6
Magnesium(mg)	69.4	70.2	72.8
Zinc(mg)	1.6	1.5	1.9

Fig 4.12 Comparison of the elemental values of raw sample and treated samples of *Cajanus cajan*

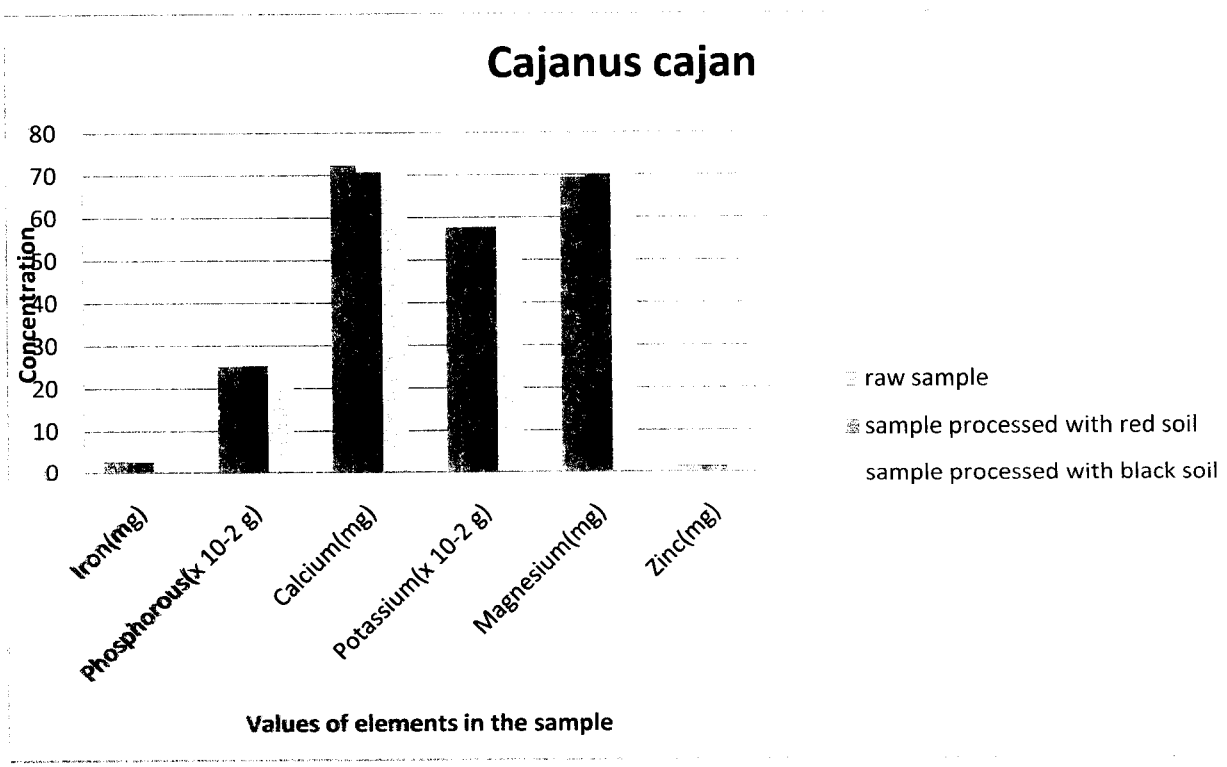


Table 4.11 Analysis of Iron content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	2.9	3.9
Processed with red soil	2.76	3.9
Processed with black soil	3.3	4.2

Fig 4.13 Analysis of Iron content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

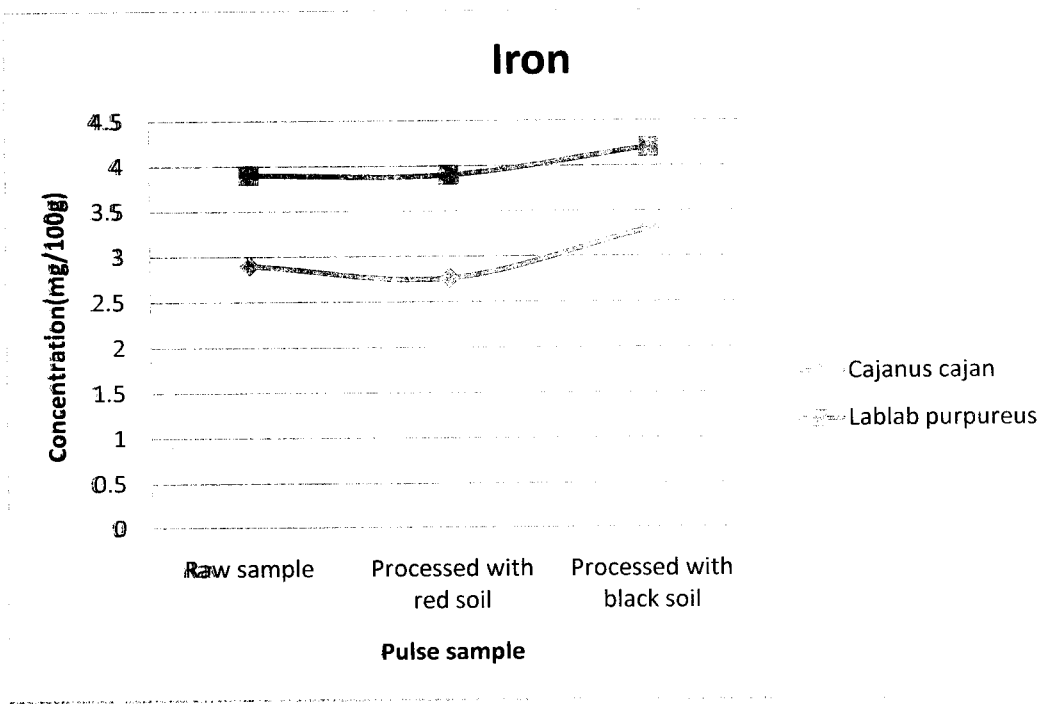


Table 4.12 Analysis of Phosphorous content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	252.6	345
Processed with red soil	254.2	352
Processed with black soil	251.4	342

Fig 4.14 Analysis of Phosphorous content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

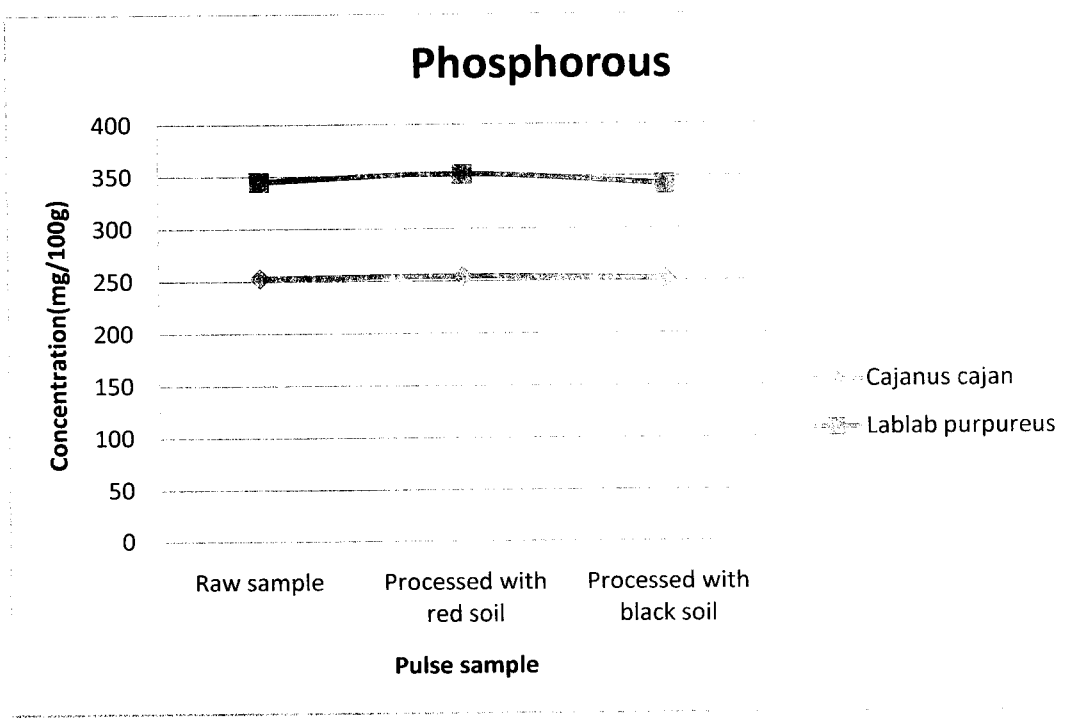


Table 4.13 Analysis of Calcium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	72.4	98
Processed with red soil	70.8	97.5
Processed with black soil	73.5	99.5

Fig 4.15 Analysis of Calcium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

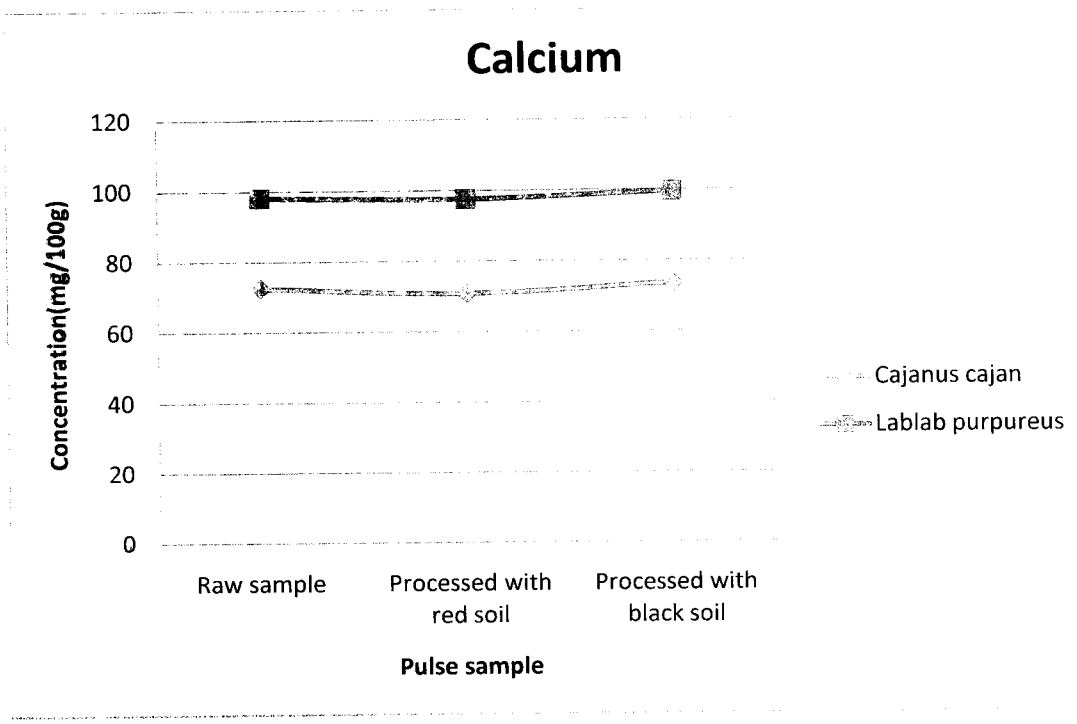


Table 4.14 Analysis of Potassium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	72.4	98
Processed with red soil	70.8	97.5
Processed with black soil	73.5	99.5

Fig 4.16 Analysis of Potassium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

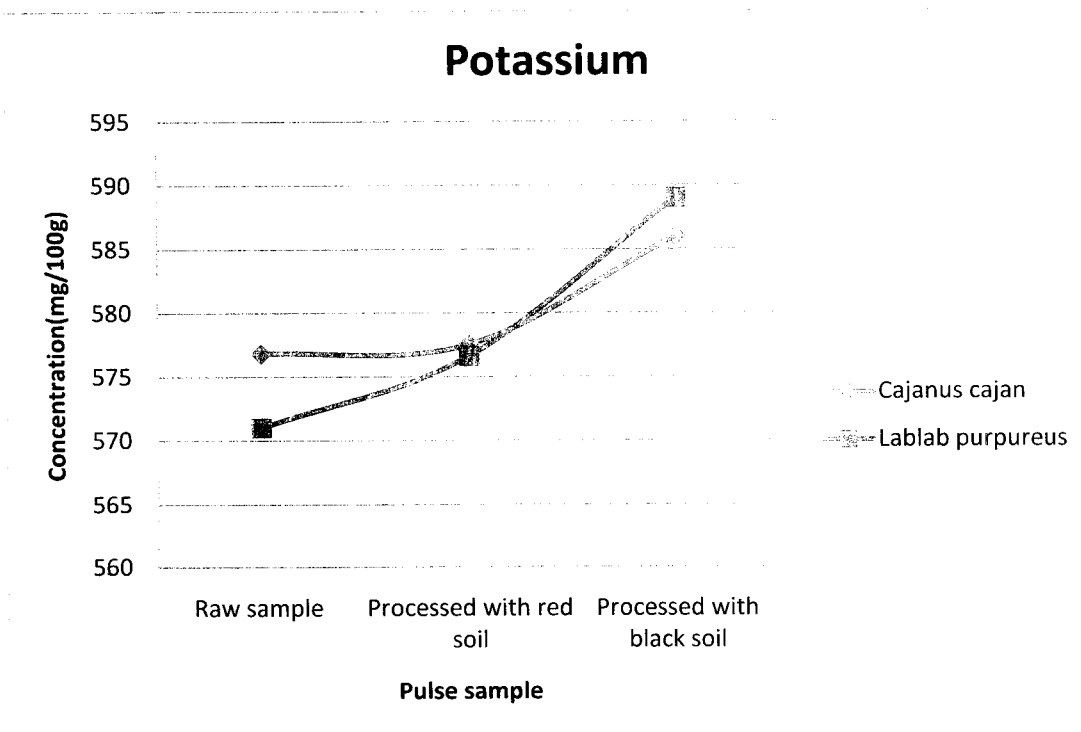


Table 4.15 Analysis of Magnesium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	69.4	42.1
Processed with red soil	70.2	44.5
Processed with black soil	72.8	41.8

Fig 4.17 Analysis of Magnesium content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

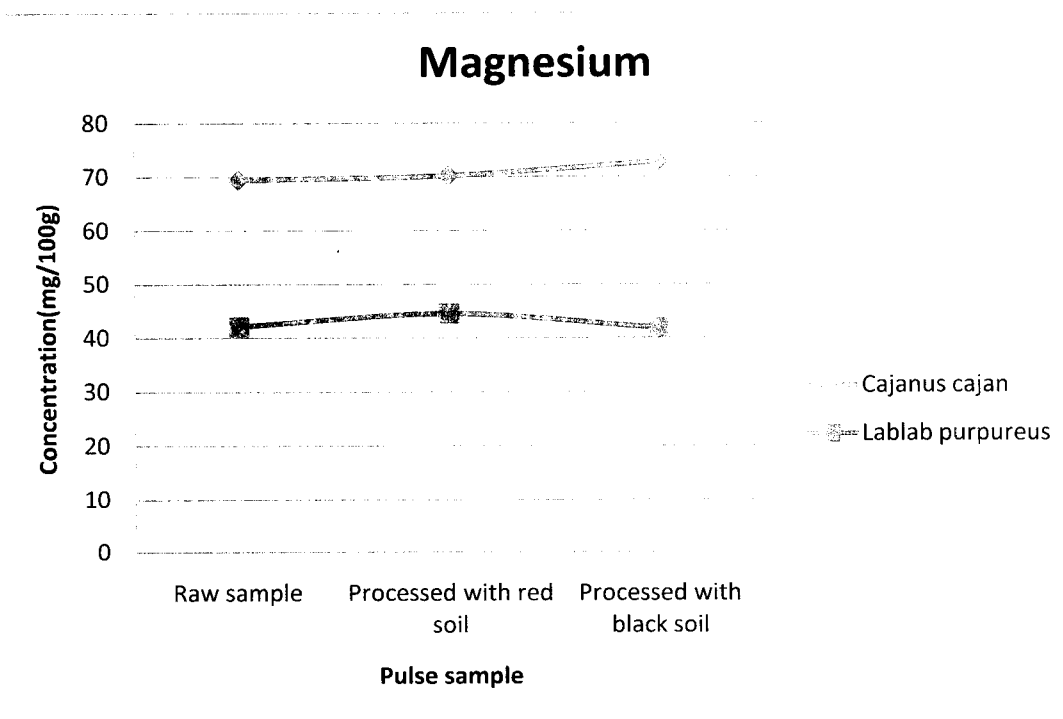
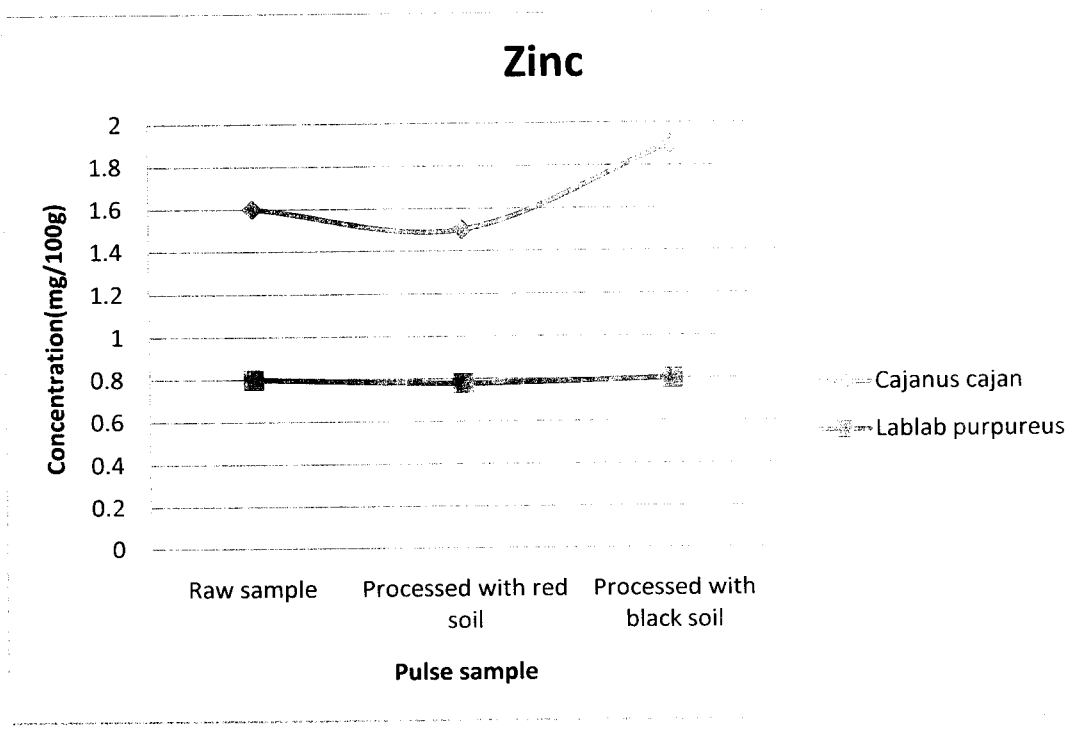


Table 4.16 Analysis of Zinc content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*

	Cajanus cajan	*Lablab purpureus*
Raw sample	1.6	0.8
Processed with red soil	1.5	0.78
Processed with black soil	1.9	0.8

Fig 4.18 Analysis of Zinc content in raw and processed samples of *Cajanus cajan* and *Lablab purpureus*



CONCLUSION

CONCLUSION

Cajanus cajan and *Lablab purpureus* were collected and processed with termite mound soil to enhance the nutritional values. The study was conducted in two different soil samples, black and red. The proximate values were analysed and minerals were estimated by atomic absorption spectroscopy. The results from the present studies showed that the essential minerals like iron, potassium and calcium has increased in pulses processed with the black soil than that in the raw sample and red soil processed sample. The proximate values were also found to be increased in the processed pulse samples. Hence this method holds a key in the area of functional foods in the future, by developing a standard method of processing the pulses with organic substances, to enrich the nutrient or minerals (or both). This method also has the advantage that organic substances are used for processing. A new technology could be derived, to increase any particular nutrient or minerals, as needed.

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