INVESTIGATIONS ON NUTRITIONAL ASPECTS OF CASSAVA (*Manihot esculenta* Crantz) LEAF AND ITS ENRICHMENT

By

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(2014-09-106)

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DECLARATION

I hereby declare that the thesis entitled **"Investigations on nutritional aspects of cassava (*Manihot esculenta* Crantz) leaf and its enrichment"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS

%	Percentage
A260	Absorbance at 260 nm wavelength
A280	Absorbance at 280 nm wavelength
cm	centimetre
et al.	et alia
kg	Kilogram
L	Litre
m	Meter
M	Molarity
mg	milligram
min	Minute
ml	Millilitre
mM	Millimolar
KOH	Potassium Hydroxide
NaCl	Sodium Chloride
NaOH	Sodiumhydroxide
H_2SO_4	Sulphuric acid
nm	Nanometer
^oC	Degree Celsius
OD	Optical density

rpm	revolutions per minute
s	second
UV	Ultraviolet
α	alpha
β	beta
w/v	weight/volume
µg	Microgram
µl	Microlitre
N	Normality
BSA	Bovine serum albumin
ppm	Parts per million
LPC	Leaf Protein Concentrate
HCN	Hydrocyanic acid
TAN	Tropical Ataxic Neuropathy
CLP	Cassava Leaf Protein
LPC	Leaf Protein Concentrate
CTTAD	Coefficient of Total Tract Apparent Digestibility
PNPG	p – nitrophenyl β – D – glucoside
TCA	Trichloroacetic acid

INTRODUCTION

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae is a stable crop for sustainable agricultural practice and food security in developing countries. It is a perennial shrub that varies widely in height and degree of branching. Cassava is an out breeding species possessing 2n = 36 chromosomes, and thus it is considered to be an amphidiploid or sequential allopolyploids. It is inherently tolerant to stressful environment and is cultivated mainly in resource limited countries of tropical and sub-tropical regions by farmers for its starchy roots, which are used as human food either in a fresh form by removing its cyanogen content or in many processed forms and products, mostly starch, flour, and for animal feed. For rural industrial development the improved utilization of cassava products acts as a catalyst for increasing the incomes of producers, processors and traders. It can also contribute to the food security status of its producing and consuming households (Plucknett *et al.*, 1998).

The cassava plant has sympodial branching and it propagates through stem cuttings. Though the height of the plant ranges between 1-5m it grows normally upto 2.4 metres tall and usually does not exceed 3m. Stem is the vegetative part of the plant formed by the alternation of nodes and internodes and the leaves are arranged in a spiral fashion around the stem. In each node axillary buds are present. The stem has a characteristic nobby appearance due to a single structure in which the petioles are borne on raised structures. The cassava plant exhibits apical dominance. The leaf size is a typical characteristic of each plant as it primarily depends on the environmental conditions. The leaf colour also depends on plant age. Cassava is a monoecious plant and the induction of flowering greatly influences the environmental conditions. Storage part of cassava is the root and its principal characteristic is their capacity for starch storage.

Cassava has a reasonable yield in fields where the cultivation of other food crops is difficult. It is tolerant to poor soils, diseases and drought (Chauynarong *et al.*, 2009). Under prolonged drought conditions for more than 6 months the crop reduces its leaf canopy and transpiration rate but the leaves remain photosynthetically active. The rapid stomatal closure under stress conditions is the main physiological mechanism protecting the leaf against dehydration. The plant takes away the available soil water slowly during long dry periods. The mean temperature at which cassava grows best is 25-29°C and a soil temperature of about 30°C. The plant stops growing at a temperature below 10°C. It grows well in soil having a lower pH value. The cassava root growth is poor in clay soil or poorly drained soils and will results in frequent root rot. Stony soils hinder the penetration power of the cassava roots and results in poor growth. The best growth is observed in sandy loam soils with good drainage capability. For germination and establishment cassava requires moist soil with low water holding capacity. It is also observed that they are very well adapted to regions with low rainfall. Cassava is planted by placing the stem cuttings of size 10-30 cm in soil having enough moisture content. The cuttings are obtained from the mature parts of the stem which gives a better yield compared to younger parts.

Cyanogenic glucosides are the nitrogenous secondary metabolites produced by the plant. They are non-uniformly distributed in different tissues of cassava plant. The highest concentration of cyanogenic glucosides is found in the peel's cortex, then the leaves and roots. The cyanide content is found to be higher in younger tissues than the mature tissues. Free radicals are the reactive species that causes oxidation of various bio molecules present in the organism and the substances that combat free radicals are termed as Antioxidants. The presence of synthetic antioxidants that are used in food preservation can cause undesirable

effects in animals and human. The antioxidant substances such as β-carotene, Vitamin C and polyphenol content are high in cassava leaves which increases with leaf maturity when the chlorophyll level is low. In leaves the presence of the photosynthetic pigment chlorophyll is also directly related to the nutritional state of the plant. The leaves at the age of 14 months present the highest antioxidant activity. The limited availability of energy with the highest number of populations resulted in a recent surge of interest in cassava as a human food and also for animal feed stuffs and industrial uses. Due to its high availability it is an important ingredient in human and animal feeding. Under low input conditions various research works are being carried out inorder to develop disease resistant and high yielding varieties of cassava. The forms by which cassava consumed varies widely by country and regions. Cassava roots are commonly used for the production of feed stuffs such as root chips and pellets. Various researches are also conducted in cassava leaves for their protein content and nutritional value. In 60% of countries the leaves are consumed as a vegetable. In many African country's cassava leaves are served as part of a sauce and can be eaten with starchy dishes like Chikwange, cassava fufu and boiled cassava roots. A typical cassava leaf dish is made in Sierra Leone, a country in west africa by including ingredients such as ground nuts, onions, fish and capsicums into the diet that provides a considerable protein requirement up to 150 g per day for an adult, or 75 g for a child (Lancaster & Brooks, 1983). Even though cassava is readily available and widely grown in many countries it is not generally consumed. So researches should be carried out in order to establish simple cassava leaf processing methods for its safe and effective consumption without losing the nutrient level. The present study is done with screening of cassava genotypes for suitability of leaf meal and characterization of leaf protein, nutrient content and its enrichment.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 CASSAVA LEAVES

The leaves are the organs in which the photosynthetic reaction occurs. They are simple and consists of leaf blade and petiole. The palmate, petiolate leaves are arranged spirally and are variable in size, colour, number and shape of lobes, although generally the fully developed leaves are 5-7 lobed and the lobes are usually within the range 4-20 cm in length by 1-6 cm in width (Purseglove, 1968). Depending on factors such as rainfall, planting density and cultivar, the obtainable yield of cassava leaves varies considerably. The leaf yield is affected by the age of the cassava plant. The high crude fibre content of leaves is dependent on the age of cutting the leaves during harvesting. As the duration for cutting the leaves increased, the production will be higher but inversely proportional to the quality of feed. Protein production of cassava leaves has also showed significant variations at different cutting frequencies. The dry matter, organic matter, crude protein, total digestible nutrients of cassava leaves increased as the cutting time increased to four times. To get the highest production for proteins and total digestible nutrients the leaves can be cut two to until four times.

The increased cyanide content of cassava leaves has precluded their use as a vegetable in Nigeria even though they are rich in carotenes, proteins, vitamins B1, B2 and C (Oke, 1968). The nutritional value is limited due to the presence of antinutritional factors such as high fibre, tannin and cyanide content. They are good sources of minerals and are rich in calcium, magnesium, iron, manganese and zinc.

2.2 CASSAVA LEAVES AS ANIMAL FEED

Due to the increased availability cassava is becoming an important ingredient in diets. Efficient use of cassava products resulted in reduced feed costs. However, the consumption of cassava leaves as feed is reduced due to several anti

nutritional factors like presence of hydrocyanic acid, high fibre content etc. With proper processing the inclusion levels of cassava leaves in diets can be increased and can be used as an alternative energy source. Due to good protein and micronutrient content the cassava leaves are consumed as a vegetable in countries such as Malaysia, Philippines and Indonesia. An estimated level of production of cassava leaves for human consumption is 0.5-0.7 million tons/year (Wargiono *et al.*, 2002). According to the taste preferences, economic status, availability of other food crops it is consumed in different forms in different parts of the world. For the usage as feed the leaves should be processed into a dehydrated leaf meal.

2.3 ANTINUTRITIONAL FACTORS AND ITS DETOXIFICATION

The chemical substances present in the diet which interfere with feed utilization, reduced production, health of the animal due to by themselves or their metabolic products arising in the system. Hence, they are also called as toxic factors. Cyanogenic glycosides are natural chemical plant toxic compounds that are present in several plants such as almonds, stone fruit, pome fruit, cassava, bamboo shoots, linseed/flaxseed, lima beans, coco yam, chickpeas, cashews, and kirsch etc. These chemical compounds are nitrogen-containing secondary metabolites that are contained in foods and have an ability to produce highly toxic hydrogen cyanide when it is chewed or digested. Cyanogenic glycosides are not toxic on its own. It becomes toxic when these compounds are hydrolysed during grinding, pounding or any other kind of disintegration processes. The toxicity of these compounds are dependent or are estimated in terms of the quantity of free cyanide generated following hydrolysis. β glucosidase is the enzyme involved in the process of hydrolysis which results in the production of sugars and a cyanohydrin that spontaneously decomposes to HCN and a ketone or aldehyde. Taxiphyllin in bamboo shoots and linamarin in cassava are the different kinds of cyanogenic glycosides found in plants. In animals including humans the cyanide toxicity can occur if the dosage level is between 0.5 and 3.5 mg HCN per kilogram body weight

(CFS (Centre for Food Safety), 2007). Symptoms of cyanide toxicity in humans have been reported to include vomiting, stomach-ache, diarrhoea, convulsion, and in severe cases death.

2.3.1 Cyanogenic glycosides in cassava and cassava leaves

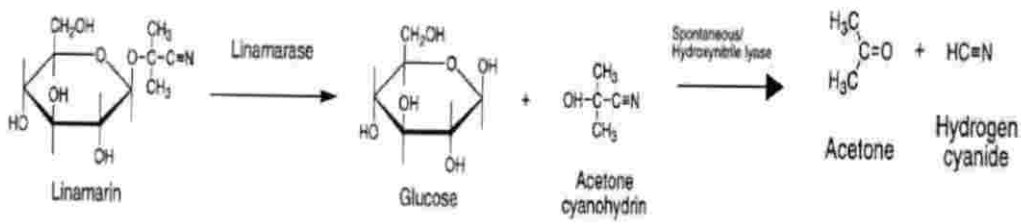
Cassava is potentially toxic to human consumers due to the presence of cyanogenic glycosides. All cassava tissues, except seeds, contain abundant amounts of cyanogenic glycosides, linamarin and lesser amounts of lotaustralin. The highest quantities of cyanogenic glycosides are present in leaves and peel of cassava roots (Balagopalan *et al.*, 1988; Brimer and Dalgaard, 1984; Bradbury and Egan, 1992). Varieties of cassava are often classified as either sweet or bitter, signifying the absence or presence of cyanogenic glucoside toxic levels.

Cassava leaves cyanogenic potential is 5 to 20 times higher than that of roots. During processing the leaves has the ability to rapidly loose the cyanogens content and the toxicity occurring due to the consumption of cassava leaves is greatly reduced. Linamarase is the enzyme which is responsible for the removal of cyanogens in cassava and its derivatives. Cassava leaves has the higher concentration of linamarase enzyme. Dehydrated cassava leaf meal may have potential in animal feeding. Almost 90% of the HCN present in cassava leaves has been eliminated by simple drying (Ravindran, 1985). The cyanide content of the leaves reached a level of 5mg per 100g by boiling for a period of one hour (Gondwe, 1974). The hydrocyanic acid content (mg/100g) of the tender and mature varieties of M4, H1687, H-165, H-226 are 123.8 and 43.6, 127.8 and 54.2, 193.1 and 72.9, 223.8 and 73.6 respectively (Padmaja, 1989).

2.3.2 Linamarin

Linamarin belongs to the family of cyanogenic glucosides found in over 2500 species of plants. It represents more than 80% of the cyanogenic glucosides of

cassava. The roots and the leaves has the higher concentration of linamarin and it is present in all parts of the cassava plant. It is a ß-glucoside of acetone cyanohydrin and ethyl-methyl-ketone-cyanohydrin. Under high pressure, high temperature, and by the use of mineral acid results in the breakage of the β linkage of linamarin, while its enzymatic breakdown occurs by an endogenous enzyme linamarase under optimal conditions at 25° C temperature and pH ranging from 5.5 to 6.0. Ingested and absorbed linamarin is readily excreted in the urine and the glucoside itself does not appear to be acutely toxic. It appears to be toxic when it produces a highly toxic compound hydrogen cyanide which can be hazardous to the consumer.



2.3.3 Protease inhibitors

Protease inhibitors is an antinutritional factor present in many forage crops. In animals the proteolytic activity of the enzymes of the gastrointestinal tract is inhibited by protease inhibitors. The antinutrient activity of protease inhibitors results in growth inhibition and pancreatic hypertrophy. It can be easily denatured by heat processing due to its particular protein structure. According to Wobeto *et al.*, (2007) the trypsin inhibitor (ITU/mg) content of cassava leaves at 12, 15 and 17 months are 2.8, 1.9 and 2.8 respectively.

2.3.4 Crude fibre and tannins

The fat-free organic substances present in feeding stuffs which are insoluble in acid and alkaline media that limits the nutritional value of food are termed as

crude fibre. It is the residue of plant materials remaining after solvent extraction followed by digestion with dilute acid and alkali and is composed of indigestible cellulose, pentosans, lignin, and other components present in food. A minimal amount of these components in food stuffs are necessary for proper peristaltic action in the intestinal tract and hence the digestion.

Stage of maturity is the major factor that determines the fibre content of cassava leaves. Over 26% of crude fibre content has observed in mature leaves when compared to very young leaves that has a content of 8.3% (Ravindran and Ravindran 1988). The inclusion of petioles during sampling procedures will also influence the fibre levels in cassava leaf meal. The diet with high-fibre content has low volumetric weight, such as roughage with low effective energy content as the compensatory intake increasing, resulting in an increase in the animal digestive tract volume and the weight of the digestive organs to be more adaptable (Wang *et al.*, 2014). The fibre content and its level present in cassava foliage affected the relative lengths of the digestive tract in many bird species such as Geese. The high fibre content present in the leaves can be mechanically separated to give a fibre free leaf protein concentrate (LPC). These protein concentrate will serve as a high-quality feed for ruminants. As the crude fibre content increases the crude protein content decreases.

Tannins are the polyphenolic substances with moderately high molecular weight contains enough phenolic hydroxyls and other suitable groups that effectively forms strong complexes with protein and other macromolecules. By forming indigestible complexes with dietary proteins or by inactivating the proteolytic enzymes these compounds lowers the protein digestibility and amino acid availability (Kumar and Singh, 1984). The tannin content will be increased with increase with maturity. Free tannin contents of cassava leaves are also considerably lowered during drying and the tannin content (g tannic acid/100g) of

varieties M4, H-1687, H-165, H-226 are 4 and 3.5, 3.7 and 3.4, 3.1 and 3.8, 3.0 and 3.1 respectively (Padmaja, 1989).

2.3.5 Lignin

Lignin is a class of complex organic polymers that form key structural materials in the support tissues of vascular plants and it is important in the formation of cell walls because it lends rigidity and do not rot easily. Lignin fills the cell wall spaces between components of cellulose, hemicellulose, and pectin, particularly in vascular and supporting tissues such as xylem tracheids, vessel elements, and sclereid cells. It is covalently linked to hemicellulose and thus cross-links various plant polysaccharides, giving the cell wall mechanical strength. Due to its negative impact on nutritional availability, lignin is considered an anti-quality component in forages. It differs from other classes of anti-quality components in forages that it is a structural component other than secondary metabolites. Lignin concentration of forages is negatively correlated with digestibility (Jung and Deetz, 1993).

2.3.6 Toxicity

The cassava leaves that are insufficiently processed may cause diarrhoea, headache, dizziness, fatigue, nausea, vomiting, stomach pains and weakness (Nhassico *et al.*, 2008). Goitre and Cretinism are caused by iodine deficiency due to high cyanide exposure (Nhassico *et al.*, 2008). Cyanide exposure will lead to a chronic neurological disease called Tropical ataxic neuropathy (TAN) (Burns *et al.*, 2010; Nzwalo and Cliff, 2011).

2.3.7 Processing of cassava leaves

Several processing techniques are used for the efficient intake of cassava leaves as feed. Cassava leaves are pounded for about 15 minutes, will results in loss of cyanogens content by 63-73% and pounding/crushing followed by boiling will

eliminates cyanogen content upto 97%. According to Bradbury and Denton (2011), 92% of total cyanide can be removed by pounding for 10 min followed by two washings at ambient temperature with double amount of water and can be reduced to a level of 97% by further washings. Bradbury and Denton (2014) found that three consecutive steps 1) pounding, 2) standing for 2 h in sun or 5 h in the shade in the tropics and 3) three times washing with water can reduce total cyanide content to 72%, 88% and 99%, respectively.

Traditional processing methods do not completely remove the cyanogen content as there is a limited contact between the enzyme linamarase and linamarin due to insufficient breakage or disruption of cells. Sun drying alone can eliminates 90% of HCN and it is an economical method for preserving micronutrient rich foods. For cassava leaf processing techniques solar drying coupled with any suitable pre-treatments can be introduced. It is reported that the linamarin content of leaves can be reduced upto a certain extent after 24 hours of treatment with lactic acid bacteria, moulds and yeast, but this process is time consuming and could not completely remove linamarin (Sornyotha *et al.*, 2010).

Introduction of processed cassava leaves as a vegetable or ready-to-eat snack in human diets can help to reduce malnutrition, such as anaemia, vitamin A and protein deficiencies in millions of people (Aregheore, 2012).

2.4 NUTRITIONAL COMPOSITION OF CASSAVA LEAVES

Nutritional composition of cassava leaves varies on both quality and quantity and depends on the age of the plant, variety of cassava, and the proportional size of the leaves. Cassava leaves are rich sources of proteins, minerals, vitamins B1, B2, and C, and carotenoids.

2.4.1 Proteins and carbohydrates

For both humans and animals cassava leaves acts as a potential alternative protein resource (Fasuyi, 2005). The leaves has a high protein content which is 5 to 10 times higher than that of roots and is comparable to the protein content of egg and also similar to sweet potato and peanut leaves however the leaves and roots are deficient in sulphur containing aminoacids cysteine and methionine. Ravindran and Ravindran (1988) found that the crude protein content decreased from 38.1% in very young leaves to 19.7% in mature leaves.

The cassava leaves have a lower carbohydrate content compared to roots and it decreases with maturity. The carbohydrate content in cassava leaves (7 to 18 g/100 g) is comparable to that of green-snap beans (7.1 g/100 g), carrots (9.6 g/100 g), or green soybeans (11.1 g/100 g), and it is higher than those of leafy vegetables such as green leaf lettuce (2.8 g/100 g) and New Zealand spinach (2.5 g/100 g). The carbohydrate content of cassava leaves are mainly starch, with amylase content ranges from 19% to 24% (Gil and Buitrago, 2002).

2.4.2 Minerals and Vitamins

The mineral content of roots is lesser when compared to leaves and is 2 to 5 times higher than that of the roots. Cassava leaves are rich in iron, zinc, manganese, magnesium, and calcium (Wobeto *et al.*, 2006). The contents of potassium, magnesium, phosphorous, zinc and manganese decreased with leaf maturity, while calcium, sodium and iron increased with leaf maturity. The calcium content in the leaves is 100 times higher than in roots that is 0-43% in very young leaves and it increased to 1.14% in mature leaves and the phosphorus content is 2 to 3times higher in roots than in the leaves. The leaves preferentially accumulate potassium than sodium. Iron and zinc content in cassava leaf meal are comparable to that of sweet potato leaves and peanut leaves while calcium content is comparable to that of peanut and broccoli.

Vitamins are more concentrated in cassava leaves than the roots. In leaves Thiamin and Niacin contents are 4 to 5 times higher while riboflavin and vitamin C are 10 to 12 times higher in the leaves than in roots. High quantity of vitamin A in the form of a pro vitamin carotenoids are observed in the leaves. Vitamin E is lower in both leaves and roots. Bradbury and Holloway (1988), Woot-Tsuen *et al.*, (1968) reported that except for soyabeans the vitamin content of cassava leaves is richer in thiamin (vitamin B1, 0.25 mg/100 g) than legumes and leafy legumes, except for soybeans (0.435 mg/100 g). Comparing to several animal foods including fresh egg, cheese, the cassava leaves has the more thiamine content. Bradbury and Holloway (1988), Woot-Tsuen *et al.*, 1968 also reported the riboflavin (vitamin B2) content of cassava leaves (0.60 mg/100 g) surpasses that of legumes, leafy legumes, soybean, cereal, egg, milk, and cheese.

The niacin content (2.4 mg/100 g) of cassava leaves is comparable to that of maize (2 mg/100 g), and surpasses those reported for legumes and leafy legumes, egg, and milk. The vitamin A content of cassava leaves is comparable with that of carrots and the vitamin C content (60 to 370 mg/100 g) of the leaves are found to be higher when compared with values reported for other vegetables.

2.4.3 Amino acid composition and digestibility of cassava leaves

The leaf protein of cassava has similar amino acid patterns but considerable variability is observed for individual aminoacids. Cassava leaf protein (CLP) is deficient in methionine, possibly marginal in tryptophan, but rich in lysine. Methionine, which is a sulphur containing aminoacid is deficient in cassava leaf protein and it has an ability to detoxify the cyanide content. Supplementation of the aminoacid methionine in diets can reduce the cyanide toxicity in humans as well as animals. The variation in the leaves amino acid content can be attributed to the stage of leaf maturity and the sampling procedures. When the leaves mature the aminoacid concentration declines only the non-essential aminoacids, glutamic acid,

proline and glycine increased while valine and phenyl alanine were unaffected. The essential aminoacids lysine and histidine shows the most decrease when the leaf matures. The lysine content is higher in very young leaves which has a value of 75 g per kg protein and has a decreased content of 38 g per kg protein in mature leaves (Ravindran and Ravindran, 1988). The tryptophan concentrations of leaves is comparable to those found in eggs, but the cysteine content in leaves is only about half that of the egg (Jacquot, 1957). The arginine has a relatively lower content in leaves and is comparable to that of eggs. Due to the limitation in the sulphur containing aminoacids methionine and cysteine in the leaves it should be added to the diet.

It is observed that the diet containing cassava leaves has a higher nitrogen retention and digestibility coefficients for both dry matter and organic matter compared to the diet without cassava leaves. Borin *et al.*, (2006) observed that the weight of the small intestine, caeca, gizzard and pancreas in Cambodian ducks and White Pekin ducks increased with increasing dietary cassava leaf meal content.

2.5 CASSAVA LEAF PROTEIN CONCENTRATE

The anti nutritional factors of cassava leaves such as high cyanide content, high fibre and tannin content limits the use of cassava leaves as a major source of protein and these limitations could be overcome by separating the proteins from the fibre content of leaves by a juice extraction step followed by steam coagulation (Pirie, 1971). The chopped, grinded cassava leaves are pressed out for the extraction of juices and the soluble fraction is then concentrated to 50% by the process of evaporation (Muller, 1977). Tupynamba and Vieira (1979) reported that cassava LPC, on average, contained 461 g kg^{-1}crude protein, 35 g kg^{-1} crude fibre, 29 g kg^{-1} ash and 198 g kg^{-1}ether extract. The LPC is deficient in sulphur containing aminoacids and this kind of deficiencies can be balanced by synthetic methionine, thus the amino acid content is comparable or quite higher to that of animal protein

supplements and oil meals (Oke, 1984; Tupynamba and Vieira, 1979). However, LPC showed poor animal performance due to its low digestibility. But it is a satisfactory source of protein for chicks and a more effective protein source compared to fish meal (Adegbola and Oke 1973).

2.6 CASSAVA LEAF SILAGE

For dry season feeding ensiling of cassava leaves is an appropriate method and also helps for feed conservation (Limon and Bui Van Chinh, 1992; Du Thanh Hang 1998; Ly and Rodríguez 2001). The process ensiling is completely dependent on the natural lactic acid fermentation under anaerobic conditions. Ensiling of cassava leaves is an effective way to reduce the cyanogen content and can be effectively used as feed purposes. The silage obtained after 56 days of ensiling resulted in a stable minimum HCN content of 20 mg/kg of fresh cassava foliage (Chhay Ty al, 2001). The feed intake, digestibility and productive performance of the ruminants can be improved by treating the low quality forages with silage additives such as chemicals, enzymes, bacterial agents, carbon and nitrogen sources and other protein rich products (McGuire *et al.*, 2013; Kholif *et al.*, 2014 ; Liu *et al.*, 2015). Addition of urea and molasses is a common and cheap method which increases the nitrogen and sugar content. Different combinations of urea is added into the silage as the increased quantity of urea resulted in decrease in fermentation quality and an increase in pH of the silage. A minimal amount of fermentable carbohydrates in plant materials are necessary for fermentation processes which results in reduction in pH of the silage which provides it as a good quality silage. Cassava leaf silage has the potential to contribute a greater proportion of aminoacids for milk synthesis in lactating cross breed dairy cows other than maize bran. It has similar effect to that of feeding the cows with cassava hay and resulted in an increase in milk fat percentage (Wanapat *et al.*, 2000). In comparison with sun drying ensiling the cassava leaves was found to results in higher Coefficient of total tract apparent digestibility (CTTAD) of dietary components and it is found to

be due to the hydrolysis of fibrous components during the ensiling processes (McDonald *et al.*, 1995). According to the study conducted by Phuc *et al.*, (2000) the cassava leaf silage has higher N retention and has better digestive property compared to sun dried leaf meal. During the ensiling processes minimal degradation of aminoacids occurs due to microbial activities (McDonald *et al.*, 1991). In west African goats the ruminal fluid parameters has improved when treated ensiled cassava leaves with 5% molasses. The quantity and quality of the cssava top silages can be improved by the addition of urea and molasses (Wanapat *et al.*, 2013, 2014). The feed intake, nutrient digestion and milk yield depends on the fermentation processes of silages (Huhtanen *et al.*, 2007)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "Investigations on nutritional aspects of cassava (*Manihot esculenta* Crantz) leaf and its enrichment" was carried out at the Division of Crop Utilization, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during the year 2018-2019. Details regarding the experimental materials used and the procedures followed in the study are elaborated in this chapter

3.1 SOURCE OF GERMPLASM

Ten genotypes of cassava namely Sree Jaya, Sree Vijaya, Sree Athulya, Ci-848, Quintal, Sree Swarna, M4, Me 833, H 1687, and H 226 were selected and raised in the fields of ICAR – CTCRI for the present study. The leaves were collected from these genotypes at maturity for evaluating the nutritional quality.

3.2 PHYSIOLOGICAL STUDY

3.2.1 Leaf fresh weight

The fresh leaves of all ten genotypes of cassava that was having a maturity of 90 days were collected from the field of ICAR – CTCRI. The fresh weight of the leaves per plant of each genotype was taken using a weighing balance and the values were noted.

3.2.2 Leaf dry weight

The fresh leaves collected per plant from the field was oven dried for three to four days at 90 - 95°C. The oven dried samples were weighed using a weighing balance. The dry weight of the leaves per plant was weighed and the values were noted.

3.2.3 Number of leaves per plant

The number of leaves per plant of all ten genotypes were counted and noted at a stage of maturity of 90 days.

3.3 CRUDE FIBRE CONTENT

Crude fibre content of the samples were determined by acid-bases digestion with 1.25% H2S04 (W/V) and 1.25% NaOH (W/V) solutions. Two grams of dried powdered leaf samples were taken. The samples were placed in a beaker and 200 ml of 1.25% $H_2SO_4$was added. Place the beaker on a hot plate and boil for 30 minutes, occasionally rotating the beaker. Cool and filter the residues using a muslin cloth and wash with boiling water until washing are no longer acidic. After filtration the residue were carefully transferred into another beaker and add 200 ml of 1.25% NaOH. Boil for 30 minutes. The residue after digestion were again filtered and washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml 95% alcohol. The residues were then oven dried for 2h at 130°C. Cool it in a desiccator and weigh. The sample residues were again heated for 30 minutes at 600°C in a muffle furnace. Cool it in a desiccator and weigh. Percentage of crude fibre in the ground samples was determined by the loss in weight on ignition.

$$\% \text{ Crude fibre in the ground sample} = \frac{\text{Loss in weight on ignition}}{\text{Weight of the sample}} \times 100$$

.....Equation 3.3

3.4. LIGNIN CONTENT

The lignin content of the leaves was determined using Klason method. Initially the protein content of the samples and other UV absorbing materials were removed to quantify lignin. 0.3 g of dry samples were homogenized in 50mM potassium phosphate buffer (7ml, pH 7.0) using a mortar and pestle and then

transferred into a centrifuge tube. The samples were then centrifuged at 1400 g for 5 minutes. The supernatant was then removed and the pellets were washed sequentially. Initially the pellet was centrifuged two times with 7 ml phosphate buffer with pH – 7.0, then washed three times with 7ml of 1% (v/v) Triton X- 100 in pH 7.0 buffer, then again washed two times with 7 ml 1M NaCl in pH 7.0 buffer, two times with 7ml for 24h and cooled in a vaccum desiccators. The dry matter obtained was defined as the protein free cell fraction. 1 g of the protein free sample was digested with 72% H_2SO_4 at 47°C for 7 minutes. After digestion the sample was then autoclaved at 121°C, 1 atm for 30 minutes. The soluble and insoluble fractions are then separated using a filter paper. The soluble fraction was measured at 280 nm and the acid soluble lignin content was calculated using the formula.

$$S = \frac{\text{UV abs} \times \text{Volume of filtrate} \times \text{Dilution}}{\text{Ɛ} \times \text{Weight of the sample (mg)} \times \text{Pathlength}} \times 100 \quad \text{.....Equation 3.4 a}$$

$$\text{Dilution} = \frac{\text{Volume of the sample} + \text{Volume of the diluting solvent}}{\text{Volume of the sample}} \quad \text{.....Equation 3.4 b}$$

Ɛ = Absorptivity of biomass at specific wavelength

The molar absorptivity of the solution was taken as 30.

3.5 LEAF NITROGEN CONTENT

0.3 grams of powdered leaf sample was weighed and placed in the kjeldahl digestion flask. A mixture of sodium sulphate and copper sulphate in the ratio 5:1 was also added to the flask. The flask was then kept in the digestion rack for 2h at 420°C and it was then digested with 10 ml of concentrated sulphuric acid. After cooling, 30 ml of distilled water was added to the hydrolysates before neutralisation and titration. The digestion was followed by distillation and then titration.

The sample containing flasks are then kept in the distillation unit. The solution in the digestion flask is then made alkaline by addition of 60 ml 40%

NaOH, which converts the ammonium sulfate into ammonia gas. The distilled ammonia was collected in a flask containing 20 ml 4% boric acid. The nitrogen content was then estimated by titration of the ammonium borate against 0.1 N HCl using a suitable indicator to determine the end point of the reaction. Appearance of light pink colour was observed at the end point.

$$\% \text{ N} = \frac{(\text{Tf} - \text{Ti}) \times \text{N} \times 14.007}{\text{Weight of the sample (mg)}} \times 100 \quad \text{.....Equation 3.5}$$

T_f = final titration value

T_i = initial titration value

N = Normality of the acid

3.6 LEAF PROTEIN CONTENT

0.3 grams of powdered leaf sample was weighed and placed in the kjeldahl digestion flask. A mixture of sodium sulphate and copper sulphate in the ratio 5:1 was also added to the flask. The flask was then kept in the digestion rack for 2h at 420°C and it was then digested with 10 ml of concentrated sulphuric acid. After cooling, 30 ml of distilled water was added to the hydrolysates before neutralisation and titration. The sample containing flasks are then kept in the distillation unit. The solution in the digestion flask is then made alkaline by addition of 60 ml 40% NaOH, which converts the ammonium sulfate into ammonia gas. The distilled ammonia was collected in a flask containing 20 ml 4% boric acid. The nitrogen content was then estimated by titration of the ammonium borate against 0.1N HCl using a suitable indicator to determine the end point of the reaction. Appearance of light pink colour was observed at the end point. The nitrogen value obtained from the titration is multiplied with 6.25 for determining the percentage of crude protein.

3.7 DETERMINATION OF LINAMARASE ENZYME ACTIVITY

3.7.1 Isolation of the enzyme Linamarase from cassava latex

The enzyme linamarase was isolated from the cassava latex and it was collected from the cut end of cassava petioles. The isolation was done using acetone and ammonium sulphate precipitation. The activity of the crude enzyme, and the enzyme isolated by the above two methods were calculated. Two grams of cassava latex was taken and stirred with 50 ml phosphate buffer for 30 min (pH-6.0). The solution was centrifuged at 10,000 g for 30 minutes. The clear supernatant obtained was collected and divided into two equal fractions. To one fraction thrice the volume of chilled acetone was added. The solution was kept at 4°C overnight. The precipitated proteins were collected by centrifugation at 10,000g for 15 minutes and dissolved in 10 ml phosphate buffer (0.1M, pH-6.0). The enzyme was then stored in refrigerator at 4°C and is used for determining the enzyme activity. The centrifuged second fraction after the removal of cell debris was brought to 60% saturation ammonium sulphate and kept at 4°C overnight. The solution was then centrifuged at 10000g for 30 minutes and the precipitate obtained was dissolved in 10 ml of phosphate buffer and dialyzed extensively against ten fold diluted buffer and was used for the assay of enzyme activity.

3.7.2 Determination of total protein by Lowry's method

A standard protein solution with BSA (1 mg/ml) was prepared. Different dilutions of BSA solutions was prepared from the stock by mixing it with distilled water ranging a concentration between 0.05 to 1 mg/ml. Pipette out 0.2 ml of protein solution from these different dilutions to different tubes. 2 ml of alkaline copper sulphate reagent was prepared by mixing 50 ml of 2% sodium carbonate mixed with 50 ml of 0.1 N NaOH solution (solution A) and 10 ml of 1.56% copper sulphate solution mixed with 10 ml of 2.37% sodium potassium tartarate solution (solution B). The reagent was prepared by mixing 2 ml of solution B with 100 ml

of solution A. Alkaline copper sulphate solution of 2 ml was added to tubes and mixed well. Then the solution was incubated at room temperature for 10 minutes. 0.2 ml of reagent Folin Ciocalteau solution (dilute 2 ml of commercial reagent with 2 ml of distilled water) to each tube and incubated it for 30 minutes. The absorbance of the purple colour generated by the solutions was measured at 660 nm by using UV Visible spectrophotometer and a standard calibration curve was plotted against protein concentrations. The absorbance of 0.2 ml of the unknown sample followed by the addition of these reagents was measured at 660 nm and the protein concentration was determined using the standard calibration curve.

3.7.3 Determination of enzyme activity of the enzyme Linamarase

Linamarase enzyme activity was measured as 1) β – glucosidase activity using p – nitrophenyl β – D – glucoside (PNPG) as substrate by the procedure of Agarwal and Bahl (1972) and 2) activity using linamarin as substrate.

A 100µl of PNPG solution prepared by dissolving 15 mg PNPG in 2 ml phosphate buffer at pH 6.0. The solution was then incubated with 0.85 ml of phosphate buffer and 50 µl of suitably diluted enzyme solution for 15 minutes at 30°C. The reaction was stopped with the addition of 2 ml of 0.2 M sodium carbonate. After 5 minutes the total volume of solution was made upto 10 ml with water. The optical density of the yellow colour formed solution was measured at 420 nm using UV- Vis spectrophotometer. Here P-nitrophenol was used as standard. The activity of the enzyme was expressed in units, where 1 unit is that which releases 1 mg p-nitrophenol per 15 min. A standard curve of p-nitrophenol is prepared in the concentration range of 20-100µg.

0.1 ml of linamarin solution prepared by dissolving 0.1 mg/ml linamarin in phosphate buffer at pH 6.0 was taken in a stoppered test tube. 0.85 ml of phosphate buffer and 50 µl of diluted enzyme solution was added to the tubes. The reaction mixture was incubated at 30°C for 15 minutes and the reaction was stopped by

adding 0.6 ml of 0.2 N NaOH and mixed well. After one min, 2.8 ml of phosphate buffer at pH 6.0 was added and shaken well followed by the addition of 1% Chloramine T solution. After mixing well 0.6 ml of colour reagent prepared by dissolving 0.7 g of 1,3 – dimethyl barbiturate and 0.57 g of iso-nicotinic acid in alkaline NaOH solution with intensive stirring. The absorbance of the pink complex formed was read at 605 nm by using UV-Vis Spectrophotometer after 10 minutes. The enzyme activity is expressed as units where 1 unit is that which releases 10 µg cyanide/15 min at 30°C.

3.8 ESTIMATION OF LINAMARIN CONTENT OF FRESH SAMPLES

Fresh leaf samples are collected and 2 g of the samples were weighed. The samples were homogenised with 25 ml ortho phosphoric acid using mortar and pestle. The homogenised samples were centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. The residue were re-extracted in 25 ml ortho phosphoric acid and the supernatants combined and made upto 50 ml. To the test tubes add 0.1 ml of the supernatant followed by the addition of 0.4 ml phosphate buffer (0.1 M, pH – 7.0) and 0.5 ml phosphate buffer (pH-6.0) and it is made upto 1 ml. 20 µl of the enzyme linamarase was added to the tubes and incubated at 30°C for 15 minutes. The reaction is stopped by adding 0.6 ml 0.2 N NaOH and mixed well. After 1 minute, 2.8 ml phosphate buffer of pH – 6.0 was added and shaken. To this tubes add 0.1 ml chloramine T solution, after mixing well 0.6 ml of iso-nicotinic acid barbiturate colouring reagent was also added and the absorbance of the pink complex formed was read after 10 min of incubation at 605 nm.

3.9 ESTIMATION OF LINAMARIN CONTENT OF DRIED SAMPLES

The leaf samples of ten varieties of cassava were selected, dried and made into powdered form. 2g of the samples were taken for the estimation of linamarin content in dried samples. The samples were homogenised with 25 ml ortho phosphoric acid using mortar and pestle. The homogenised samples were

centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. The residue were re-extracted in 25 ml ortho phosphoric acid and the supernatants combined and made upto 50 ml. To the test tubes add 0.1 ml of the supernatant followed by the addition of 0.4 ml phosphate buffer (0.1 M, pH – 7.0) and 0.5 ml phosphate buffer (pH-6.0) and it is made upto 1 ml. 20µl of the enzyme linamarase was added to the tubes and incubated at 30°C for 15 minutes. The reaction is stopped by adding 0.6 ml 0.2N NaOH and mixed well. After 1 minute, 2.8 ml phosphate buffer of pH – 6.0 was added and shaken. To this tubes add 0.1ml chloramine T solution, after mixing well 0.6 ml of iso-nicotinic acid barbiturate colouring reagent was also added and the absorbance of the pink complex formed was read after 10 min of incubation at 605 nm.

3.10 ESTIMATION OF LINAMARIN CONTENT OF BOILED SAMPLES

The fresh leaf samples of cassava were selected. The samples were boiled in distilled water for 30 minutes. 2 g of the boiled samples of each varieties were taken for the estimation of linamarin. The samples were homogenised with 25 ml ortho phosphoric acid using mortar and pestle. The homogenised samples were centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. The residue were re-extracted in 25 ml ortho phosphoric acid and the supernatants combined and made upto 50 ml. To the test tubes add 0.1 ml of the supernatant followed by the addition of 0.4 ml phosphate buffer (0.1 M, pH – 7.0) and 0.5 ml phosphate buffer (pH-6.0) and it is made upto 1 ml. 20 µl of the enzyme linamarase was added to the tubes and incubated at 30°C for 15 minutes. The reaction is stopped by adding 0.6 ml 0.2 N NaOH and mixed well. After 1 minute, 2.8 ml phosphate buffer of pH – 6.0 was added and shaken. To this tubes add 0.1 ml chloramine T solution, after mixing well 0.6 ml of iso - nicotinic acid barbiturate colouring reagent was also added and the absorbance of the pink complex formed was read after 10 min of incubation at 605 nm.

3.11 DETERMINATION OF PROTEASE INHIBITORS IN LEAF SAMPLES

The quantification of the protease inhibitors is essential to determine the anti-nutritional status of food crops. The dietary effects of inhibitors include impaired digestion of proteins due to complex formation of the inhibitor with digestive proteases. Here the content of trypsin inhibitors is quantified by the following procedure. For the extraction of trypsin inhibitor 2g of dried powdered leaf was taken. The leaf was obtained after the removal of leaf pigments such as chlorophyll etc by treating with chloroform etc. The powdered samples were homogenized with 15 ml of 0.01 M sodium phosphate buffer containing 1% poly vinyl pyrrolidone. The homogenized crude slurry was then kept in refrigerator at 10°C for 2h to improve the extraction with occasional stirring. The suspension was then centrifuged at 10,000 rpm for 20 minutes. The supernatant obtained was treated with 50% TCA (1:10 ratio v/v). The precipitated proteins after the treatment were rapidly removed through centrifugation and the pH of the supernatant was adjusted to 8.0 using 5N NaOH. The supernatant obtained has taken for the assay of trypsin inhibitor activity.

Caseinolytic assay is the easiest one for the assay of trypsin inhibitors. An uninhibited and inhibited system was taken. The uninhibited system consists of 2 ml of 2% Casein, 1.8 ml of 0.01 M Sodium phosphate buffer with pH 8.0 and 0.2 ml of trypsin from 1 mg/10ml stock. In the inhibited system 0.2 ml of trypsin and 0.3 ml of inhibitor extract were pre incubated for 30 minutes for maximum inhibitor response. 2 ml of casein was added followed by the addition of 0.01M sodium phosphate buffer at pH 8.0. The system was then incubated for 20 minutes at 30°C and the reaction was stopped by adding 4 ml of 10% trichloroacetic acid (TCA). TCA was added to the uninhibited system as well and the three systems are kept for one hour to complete protein precipitation. The precipitate was then centrifuged at 10,000 rpm for 10 minutes. The absorbance of the clear supernatant was measured at 280 nm in UV Visible spectrophotometer against appropriate

blanks. The TCA soluble peptide fractions of casein formed by the action of trypsin was quantified in the presence and absence of inhibitor by comparing with tyrosine as standard. One unit of trypsin activity was defined as the amount of enzyme that liberated 1µg of tyrosine per millilitre of the reaction mixture per minute under the assay conditions. One unit of inhibitor activity was defined as the decrease by one unit of absorbance of TCA soluble casein hydrolysis product liberated by trypsin action at 280nm per minute at 37°C in the given assay volume.

The protease inhibitory activity was expressed in terms of percent inhibition and it was calculated as

$$\text{Inhibitory activity (\%)} = \text{Amount of tyrosine released without inhibitor} - \frac{\text{Amount of tyrosine released with inhibitor}}{\text{Amount of tyrosine released without inhibitor}} \times 10$$

.....Equation 3.11

3.12 *IN VITRO* PROTEIN DIGESTIBILITY STUDIES

Based on the method described by Akeson and Stahmann (1964) with slight modifications the *in vitro* protein digestibility studies of the samples were performed. An amount of 0.25 g of the powdered leaf samples were taken and suspended in 15 ml of 0.1 mol equi/L HCl containing 1.5 mg/ml pepsin. The samples were then incubated for 3h at 37°C in a water bath. After the addition of 7.5 ml of 0.5 mol equi/L of NaOH neutralization reaction occurs and the pepsin hydrolysis were ceased.

The pancreatic digestion initiated with the addition of 10 ml of 0.2 mol/L phosphate buffer (pH-8.0) containing 10 mg of pancreatin with 1 ml of 0.005 mol/L sodium azide. Sodium azide was used to prevent microbial growth in samples. The samples were incubated at 37°C overnight. After the pancreatic hydrolysis, 1 ml of 10g per 100 ml of trichloroacetic acid was added. The samples were then

centrifuged at 503g for 20 minutes and the total protein content was estimated using kjeldahl method.

$$\% \text{ Digestibility} = (N_S - N_B) / N_S \times 100 \quad \text{..... Equation 3.12}$$

N_S – Nitrogen content in sample

N_B – Nitrogen content in blank

3.13 DETERMINATION OF MINERAL CONTENT OF CASSAVA LEAF GENOTYPES

3.13.1 Tri-acid digestion of cassava leaf samples

The dried powdered leaf samples were taken for the analysis of mineral content. 0.5 g of samples were taken into the test tubes containing 15 ml of Tri acid mixture. The Tri acid mixture containing Nitric acid (HNO_3), Per chloric acid ($HClO_4$) and sulphuric acid (H_2SO_4) was prepared in the ratio 10:4:1. The tubes were kept overnight in a beaker containing a small volume of sulphuric acid (sulphuric acid bath) for wet digestion. After wet digestion the samples were kept for hot digestion in a hot plate at a temperature of 125° C for about 2 hours for complete digestion. The clear sample digest was taken after cooling and made upto 100 ml with distilled water. This extract was used for the estimation of the minerals Ca, Mg, Zn, P, K, Fe, Mn, and Cu.

3.13.2 Determination of Ca, Mg, Fe, Mn, Zn and Cu

The Tri acid digest was used for the estimation of Ca, Mg, Fe, Mn, Zn and Cu using Atomic Absorption spectrophotometer (AAS). It is based on absorption of light at a specific wavelength by free metallic ions. The Tri acid digest after required dilution was read directly in the equipment. The mineral concentration of the samples are determined by the standard mineral values or can be calibrated to read directly the concentration of minerals in the solution.

Percentage of elements in the plant sample was calculated as

$$\% \text{ of the element in the plant sample} = \frac{\text{Volume made up x Reading in ppmx Dilution}}{\text{Weight of sample x 10,000}}$$

.....Equation 3.13.2

3.13.3 Estimation of Phosphorous (P)

The leaf phosphorous content was estimated calorimetrically using spectrophotometer (PC Based Double Beam Spectrophotometer 2202, Systronics). The tri acid digest with a volume of 5 ml was pipetted out into a 25 ml standard flask. Five ml of Vanadomolybdate reagent was added and made the final solution into 25 ml with distilled water. The sample solution was then kept for one hour for colour development. The yellow colour developed was then read at a wavelength of 420 nm in a spectrophotometer and the absorbance was noted.

The percentage of phosphorous in the plant sample is calculated as follows:

$$\% \text{ P in the plant sample} = \frac{\text{Value in ppm from graph} \times \text{volume made up} \times \text{Dilution}}{\text{Weight of plant sample} \times 10{,}000}$$

.....Equation 3.13.3

3.13.4 Preparation of standard curve

For the estimation of Phosphorous content in the leaf samples a standard curve was made. 0.4394 g of Potassium dihydrogen orthophosphate was dissolved in 800 ml distilled water and made upto 1 litre. The required standards are diluted and the absorbance was taken calorimetrically. A standard curve was prepared in a MS Excel with standard concentration along the X-axis and value in the Y-axis. The unknown sample mineral concentration was determined.

3.13.5 Estimation of Potassium (K)

The Potassium content of the leaf samples were determined using Flame photometer. One ml of the tri acid digest was taken and made into 25 ml in a standard flask. The made-up extract was directly read in the flame photometer (Systronics Flame Photometer Model No. 128) by using the concerned filter for potassium.

Percentage of potassium in the plant sample calculated as follows:

$$\% \text{ K in the sample} = \frac{\text{Volume made up} \times \text{Reading} \times \text{Dilution}}{\text{Weight of sample x 10 x 10,000}} \quad \text{.....Equation 3.13.5}$$

3.14 PREPARATION OF CASSAVA LEAF SILAGE

5 kg fresh cassava leaves of M4 and H-1687 varieties were collected from the fields of ICAR – CTCRI. The leaves were spread out and wilted for 12 hours by maintaining 70% moisture content. The wilted leaves were chopped into pieces into a length of 10mm using a hand operated chopping machine. The chopped leaves were weighed and then thoroughly mixed with 50 grams of encapsulated yeast (*Saccharomyces cerevisiae*) activated in a concentrated sugar solution and 10 grams of urea was added. The rate of the fermentation reaction can be increased by the addition of sugar. The leaves were mixed well and packed into polypropylene plastic bags of 60 cm width by 100 cm length and compacted to expel air from the bag (Plate 1). The bags were then tightened using rubber bands and were kept under anaerobic conditions for 3-4 weeks (Plate 2). The mineral and nutrient content of the silage was checked at first, second and third week. The quality of the silage was compared with the fresh leaves.

Plate 1: Packing of the leaf sample genotypes of A) H1687 and B) M4 at 0^{th} day of enrichment

Plate 2: Enrichment A) at 0^{th} day B) and 20^{th} day of the cassava leaf sample genotype Malayan 4

3.14.1 Determination of quality parameters of Cassava leaf silage

The cassava leaf silage of H 1687 and Malayan 4 varieties that are enriched at Day 7 and Day 20 was taken for the determination of Nitrogen content, Protein content, Linamarin content and the mineral content. The methodology for the determination of Nitrogen content, Protein content, Linamarin content and mineral content was described in the above procedures. The required samples were oven dried and made into powdered form. The powdered samples were taken for the analysis.

RESULTS

4. RESULTS

The present study entitled the "Investigations on nutritional aspects of cassava (*Manihot esculenta* Crantz) leaf and its enrichment" was conducted at the Division of Crop Utilization, Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram. The results of the study are presented below.

4.1 CASSAVA GENOTYPES USED IN THE STUDY

Ten genotypes of cassava namely, Sree Jaya, Sree Vijaya, Sree Athulya, Sree Swarna, CI 848, Me 833, Quintal, Malayan 4, H 1687, H 226 were used in the present study (Plate 3). Cassava stem cuttings were raised in farm of ICAR-CTCRI

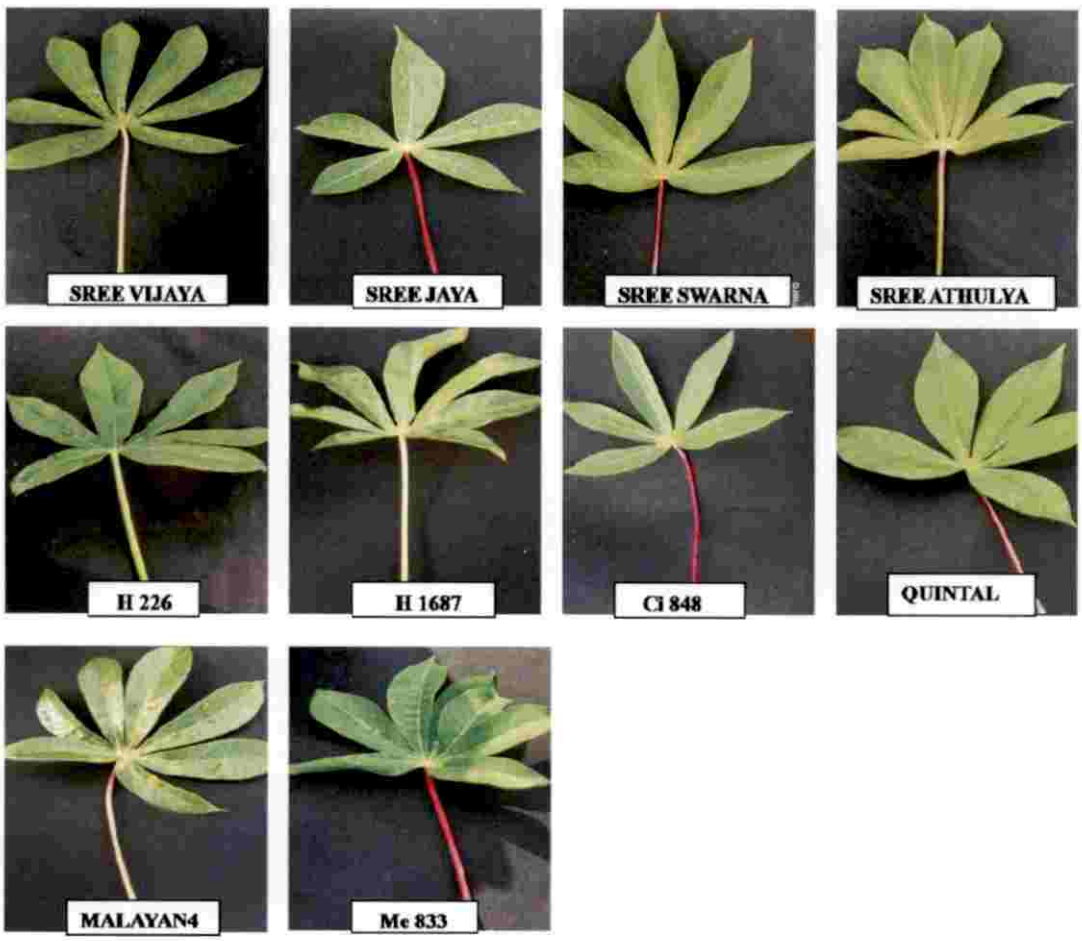


Plate 3: Ten genotypes of Cassava leaf samples for enrichment studies

during 2nd week of November 2018 in the main field. Recommended fertilizer management and intercultural operations were carried out. The leaves were sampled at the third month of sampling and processed for the analysis.

4.2 PHYSIOLOGICAL CHARECTERISTICS

4.2.1 Total Fresh weight of leaves (g plant^{-1})

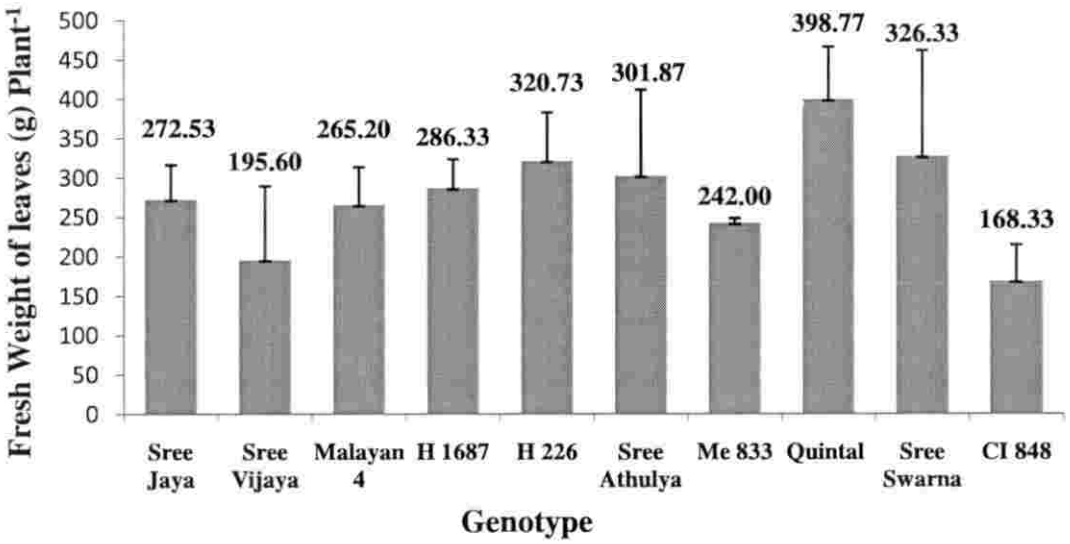


Figure 1: Total Fresh weight of leaves (g plant^{-1}) of ten genotypes of cassava

The leaves of cassava genotypes were harvested carefully and sealed in polythene bags and weighed. The fresh weight data of the leaves of ten genotypes of cassava is presented in Figure 1. The fresh weight of leaves ranged from 168.33 to 398.77 g plant^{-1} in the genotypes studied.

4.2.2 Total Dry weight of leaves (g plant^{-1})

The data on total dry weight of leaves of cassava genotypes are presented in Figure 2. The total dry weight of the leaves per plant did not vary significantly among the genotypes studied. The dry weight of the genotypes ranges from 129.67

to 44.57 g plant^{-1}. The plant to plant variation for fresh weight and dry weight was high and hence they were not statistically uniform for this parameter.

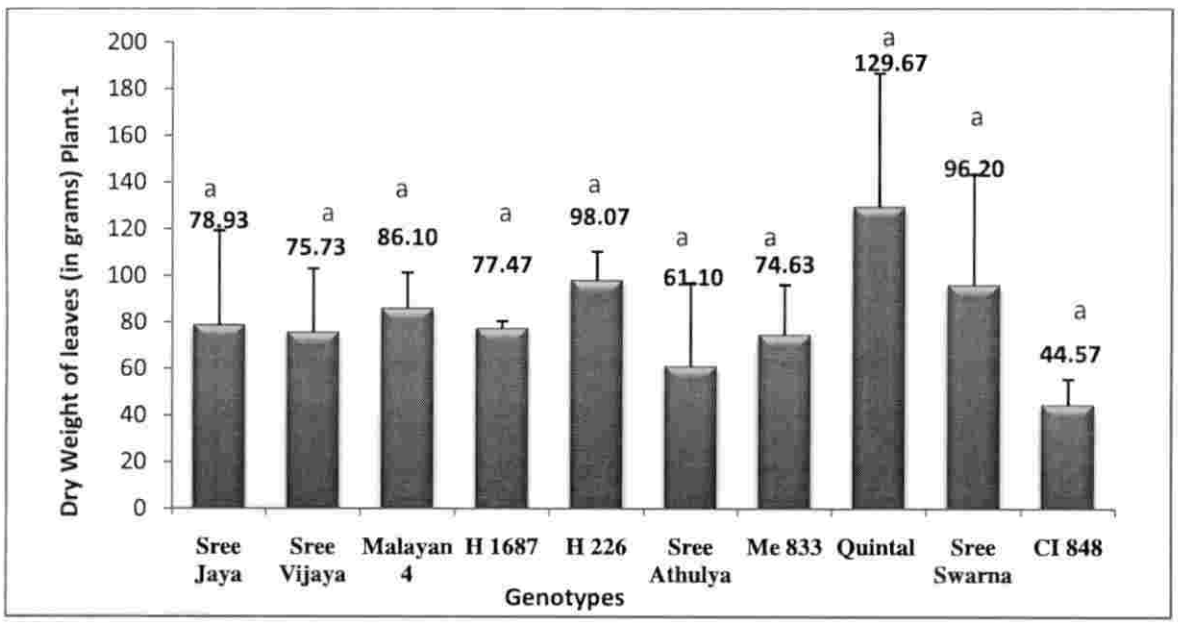


Figure 2: Total Dry weight of leaves (g plant^{-1}) of ten genotypes of cassava

4.2.3 Total number of leaves per plant of cassava genotypes

There was significant difference for total number of leaves plant^{-1} of cassava genotypes studied. The data on total number of leaves per plant is presented in Figure 3. Sree Jaya had the highest number of leaves (121 leaves plant^{-1}) and Sree Athulya (51 leaves plant^{-1}) had the lowest. The genotypes Sree Vijaya, Malayan 4, H 1687, H 226, Me 833, Quintal, Sree Swarna and CI 848 had similar number of leaves

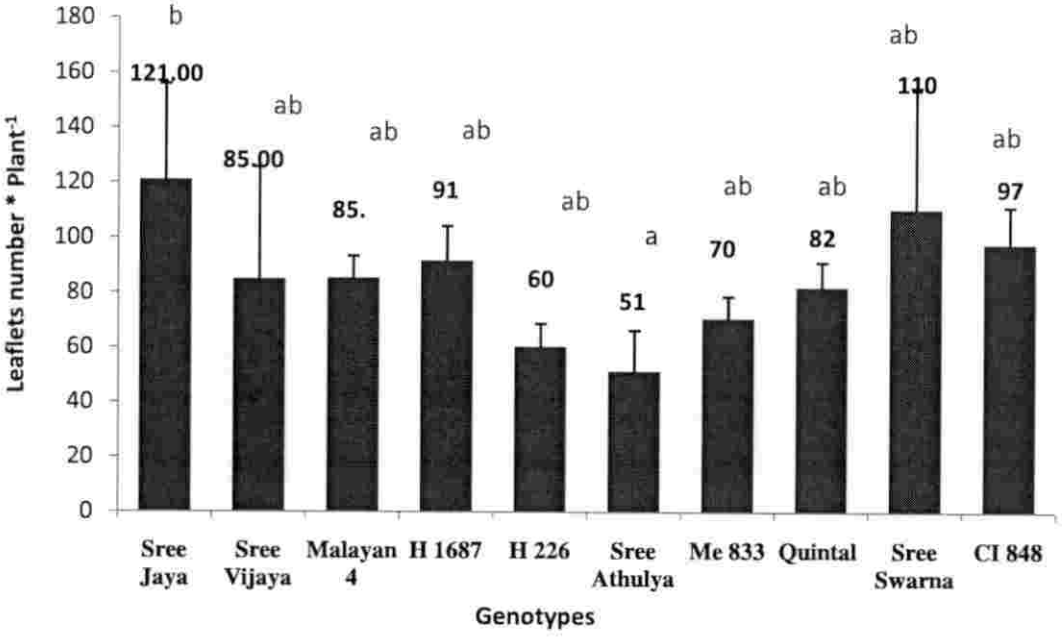


Figure 3: Number of leaflets per plant of cassava genotypes

4.3 CRUDE FIBRE CONTENT OF CASSAVA LEAF SAMPLES

The genotype H 226 had the highest fibre content of 48.67% (0.49 gg^{-1}) while Sree Swarna had the lowest 18.73% (0.19 gg^{-1}). The genotypes CI 848, H 1687, Me 833, and Sree Jaya were not significantly different while the genotypes Me 833 and Quintal were significantly different. Not much difference was observed between the genotypes Sree Jaya and Malayan 4. The mature leaves has the highest fibre content compared to younger leaves. The data on leaf crude fibre content is presented in Figure 4. The oven dried fibre residues of the leaf samples after acid base hydrolysis is presented in Plate 4.

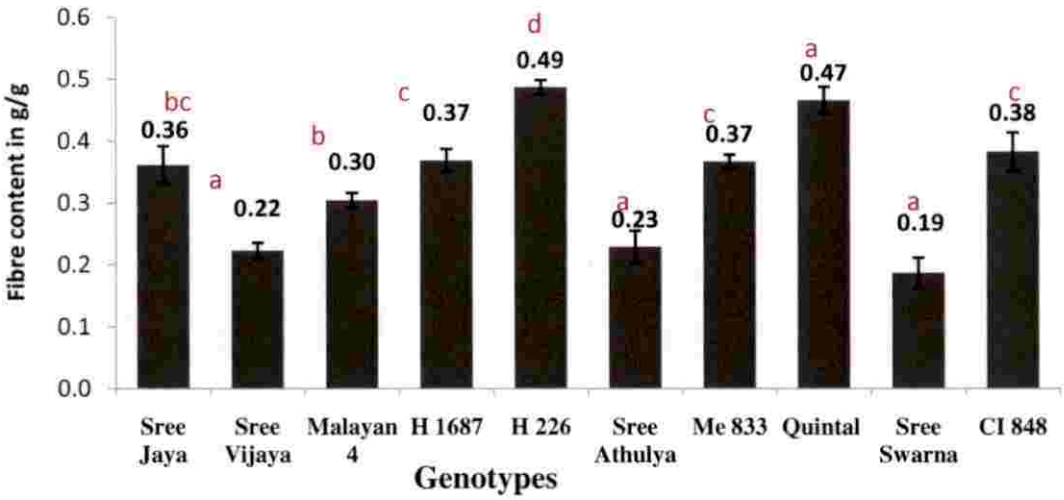


Figure 4: Crude fibre content (gg^{-1}) of cassava genotypes

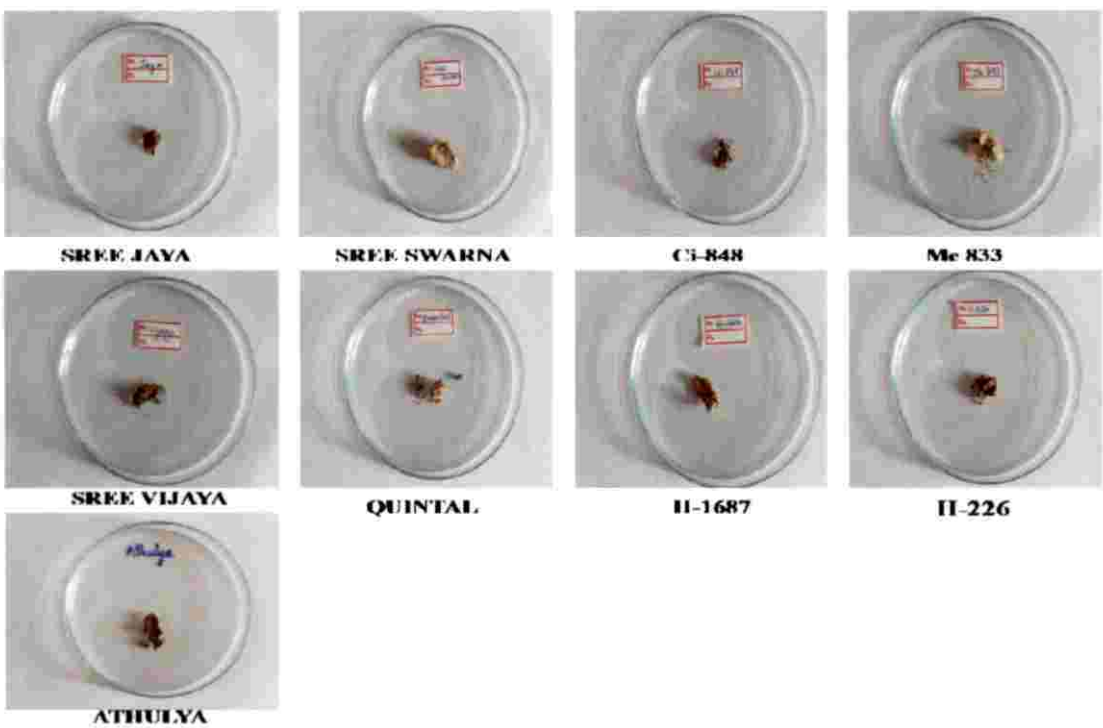


Plate 4: Crude fibre of cassava leaf genotypes after acid – base digestion

4.4 LIGNIN CONTENT (g g^{-1})

The lignin content of cassava leaves were determined for the selected genotypes and the data is presented in Figure 5. Lignin content of the leaves varied significantly. The highest lignin content was recorded in genotype Malayan 4 with a crude fibre content of 0.15 (g g^{-1}) while Sree Athulya and Sree Swarna had the lowest lignin content of 0.06 (g g^{-1}) among the genotypes studied. The genotypes Sree Jaya, Quintal, CI 848 and H 1687, H226. showed similar level of fibre content. The genotype Sree Athulya had nearly 9% lower fibre content compared to M4. Lignin content of cassava leaves was found to be relatively lower when compared to the fibre content.

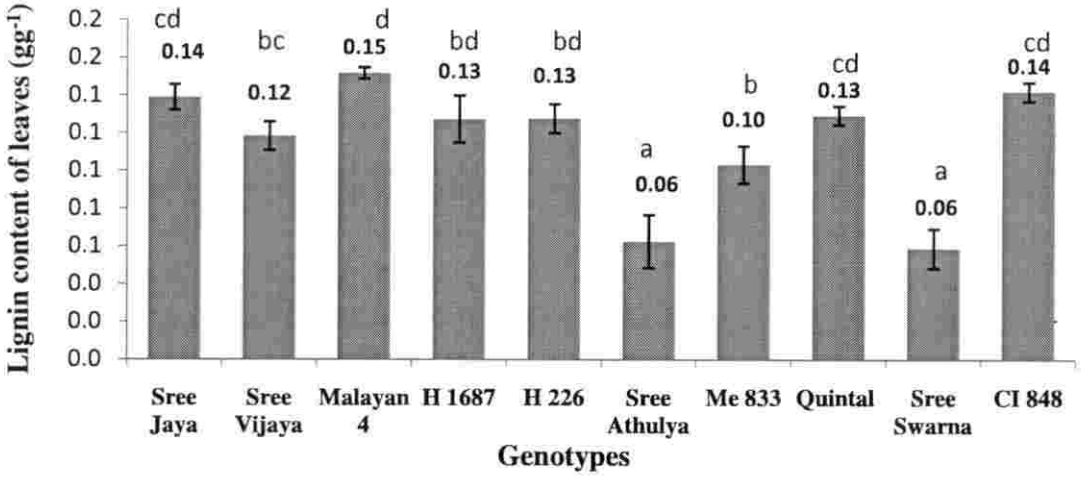


Figure 5: Lignin content (gg^{-1}) of cassava leaf genotypes

4.5 LEAF NITROGEN CONTENT (%)

The leaf nitrogen content was estimated by Kjeldahl method. The data on nitrogen content of cassava genotypes is presented in Figure 6. Significant difference for the leaf nitrogen content of leaves was observed for the genotypes

studied. The genotype CI 848 (4.92%) had the highest N content. Whereas Sree-Jaya had the lowest nitrogen content (2.93%). The percentage nitrogen content ranged between 2.93 – 4.93% on dry weight basis in the leaves. Sree Vijaya, Sree Swarna, Malayan 4 and H 1687 showed similar N content (nearly 4 to 4.5%). The results showed that cassava leaf are a good source for Nitrogen content.

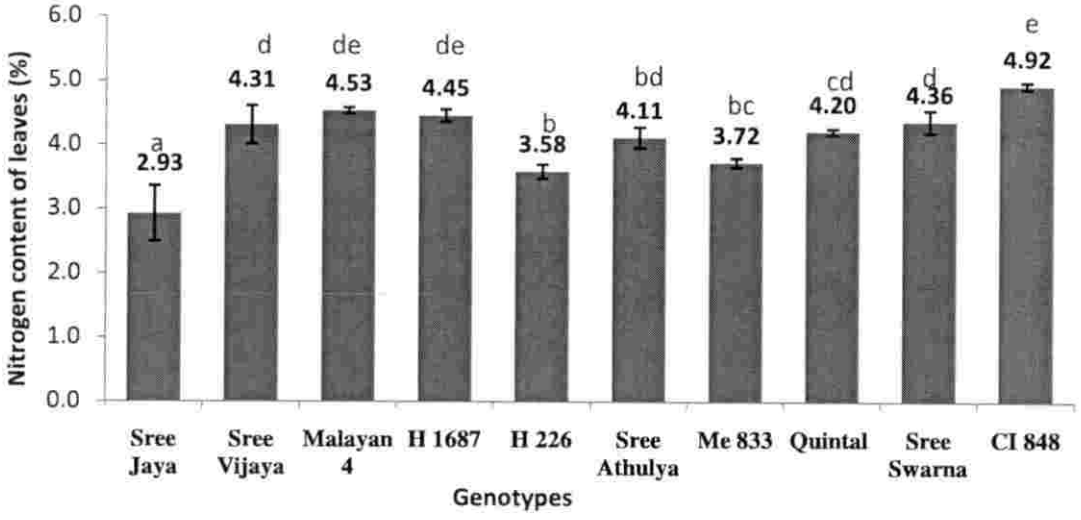


Figure 6: Leaf nitrogen content (%) estimated by Kjeldahl method

4.6 LEAF PROTEIN CONTENT (g g^{-1})

Significant difference for the leaf protein content of leaves was observed for the genotypes studied. The crude protein content of cassava leaves ranged from 0.18 – 0.3 (g g^{-1}) on dry weight basis. Highest protein content was obtained for the genotype CI 848 and the lowest for the genotype Sree Jaya (0.18 g g^{-1}). The genotype with the highest nitrogen content had the highest protein content. Similarly protein content was observed for the genotype Sree Vijaya and Sree Swarna (0.27 g g^{-1}). The genotypes Malayan 4 and H 1687 showed similar protein

content. The genotypes Sree Vijaya and M4, H 226 , Sree Athulya and Me 833, Quintal and Sree Swarna showed similar protein content.

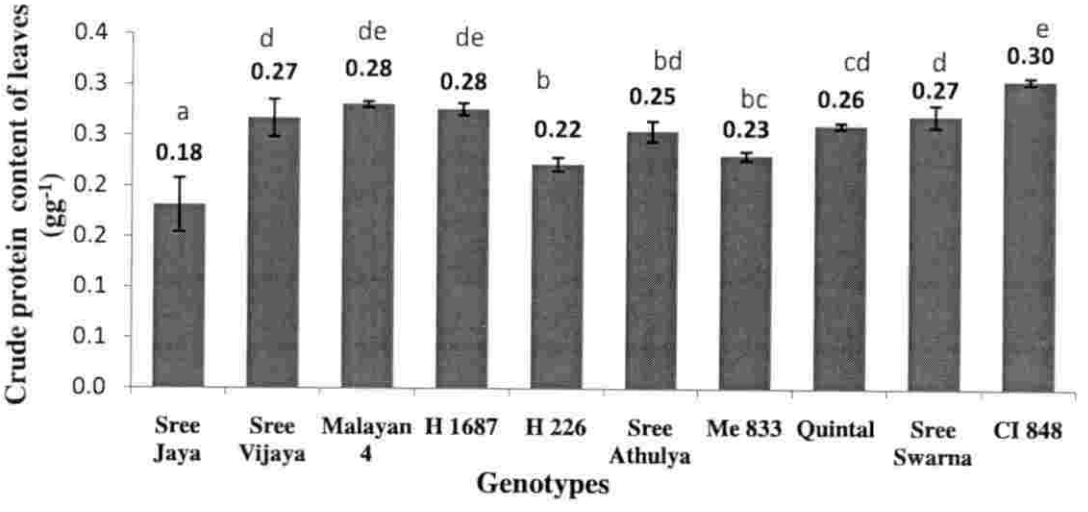


Figure 7: Leaf crude protein content (g/g)

4.7 LINAMARIN CONTENT OF FRESH, BOILED AND DRIED SAMPLES

The linamarin content of fresh, boiled and dried leaf samples of cassava were compared. From figure 8 it is clear that the linamarin content was highest for fresh leaf samples compared to boiled and dried samples. The linamarin content of fresh samples ranges between 0.75 – 6.04 mg/g. The content was found to be highest when compared with boiled and dried samples. The genotype Quintal had the highest content of linamarin on fresh weight basis and it was lowest for the genotype Sree Jaya. There was no significant difference observed between the genotypes Sree Jaya, Sree Vijaya, M4, H 226, Sree Swarna, and CI 848. For the boiled samples the content ranges from 0.34 – 1.2 mg/g on dry weight basis. The content was highest for the sample Quintal and lowest for CI 848. No significant difference was observed between the genotypes Sree Jaya, Sree Vijaya, M4, H 226, Sree Athulya, Sree Swarna and CI 848. In the case of dried leaf samples the

linamarin content ranges between 0.34 – 0.5 mg/g. The highest content was found to be 0.5 mg/g for the genotype H 1687 and the lowest was 0.34 mg/g for the genotype Sree Swarna. For dried samples the genotype Me 833 had a significant difference with all the other genotypes. A decrease in 4.84 mg/g and 5.7 mg/g of linamarin content was observed between the fresh sample - boiled sample, and Fresh – dried sample of genotype with highest linamarin content.

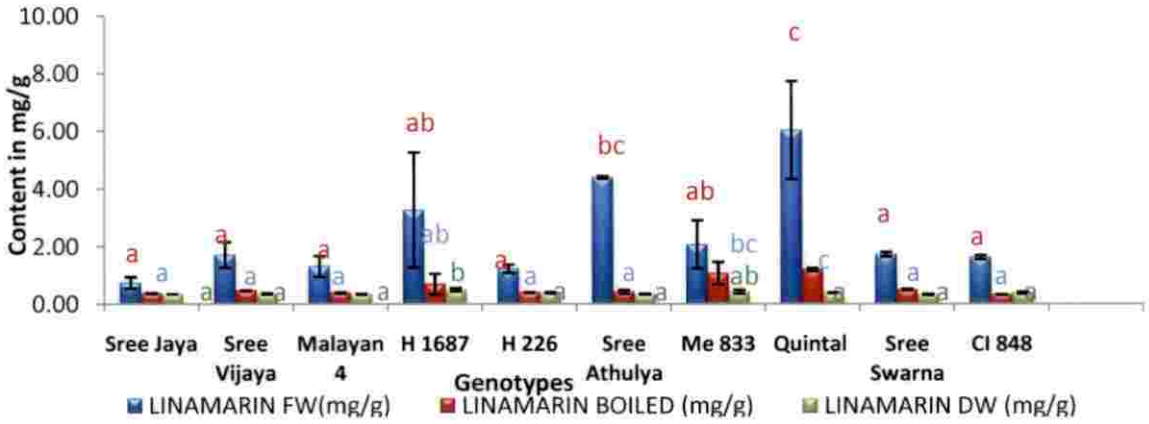


Figure 8: Linamarin content of Fresh, Boiled and Dried samples

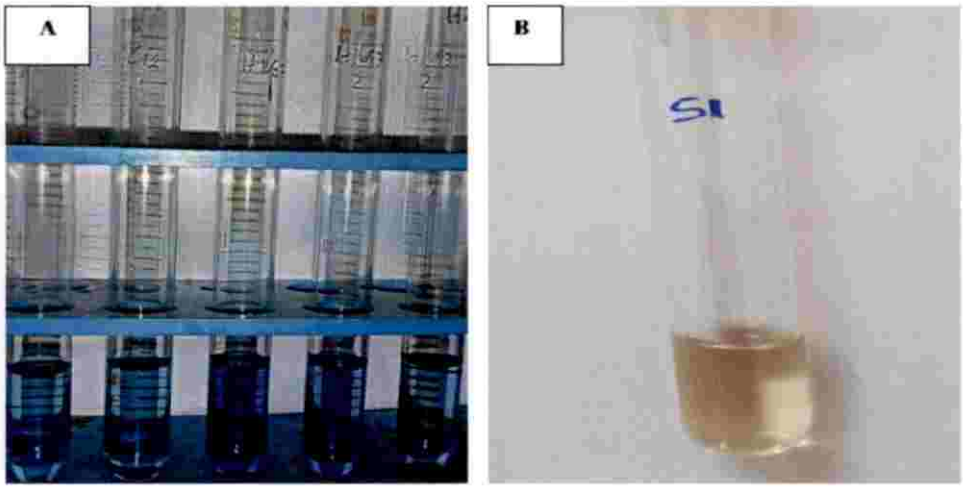


Plate 5: Linamarin content of A) Fresh leaf samples and B) Dried leaf samples

4.8 DETERMINATION OF LINAMARASE ENZYME ACTIVITY

The activity of the enzyme linamarase isolated from cassava latex were determined and the data is presented (Table 1). The activity of the enzyme was found to be lower when it is in a crude form. Pure linamarase enzyme was isolated by precipitation with ammonium sulphate and acetone fraction. The activity of the enzyme was found to be highest in acetone purified fraction when compared to crude and ammonium sulphate fraction. The activity of the enzyme was found to be lower in leaf and rind. More activity was observed for the enzyme isolated from cassava latex.

Table 1: Determination of Linamarase enzyme activity

Tissue		Total activity/g tissue	
		Linamarase[a] (units)	β glucosidase[b] (units)
Latex	Crude	5130	6213
	Ammonium sulphate	3014	5240
	Acetone	3110	5650

a - 1 unit = amount of enzyme which liberates 10µg CN/15min

b - 1unit = amount of enzyme which liberates 1 mg p-nitrophenol /15 min

4.9 *IN VITRO* DIGESTIBILITY OF LEAF SAMPLES

4.9.1 Nitrogen content (%) of leaf samples after enzymatic digestion

The results for the nitrogen content of leaf samples before and after digestion with the enzymes Trypsin and Pancreatin is represented in Figure 9. The nitrogen content of the samples was found to be reduced after the enzymatic digestion. For the genotype Quintal which had the highest amount of nitrogen content was reduced by 97 percent after enzymatic digestion. The genotype H 1687 had the highest rate of digestibility 98.74% while Malayan 4 had the lowest ie, 95.67%. The digestibility rate of all the ten genotypes was found to be relatively higher having a percentage rate of above 90.

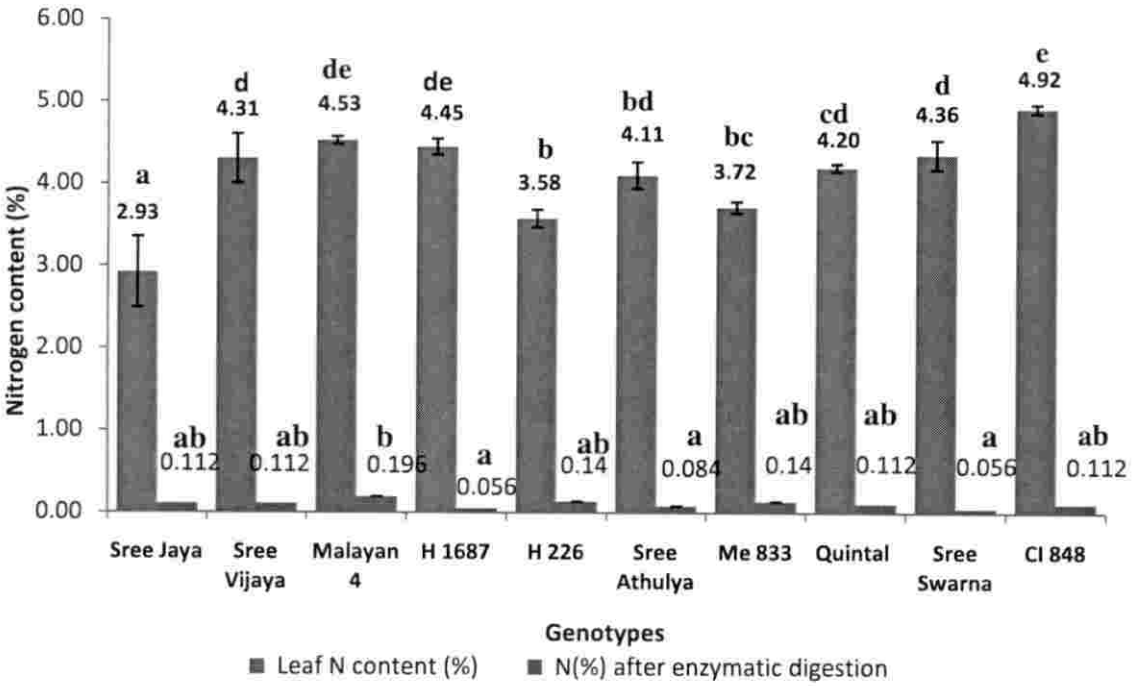


Figure 9: Nitrogen content (%) after enzymatic digestion

4.9.2 *In-vitro* protein digestibility (Content of leaf protein (µg) after enzymatic digestion)

A high rate of protein digestibility was observed for cassava leaf samples when the samples were subjected to enzymatic digestion. Figure 10 represents the protein content of the leaf samples of cassava genotypes. The protein content after digestion of the samples was found to be highest in Malayan 4 (12.152 µg/g) and it was found to be lowest for the genotype Sree Swarna (3.472 µg/g). From the ten genotypes selected the genotype H 1687 had the highest rate of protein digestibility (98.76%) and Sree Athulya had the lowest (94.15%). The percentage range of digestibility varies from 94.15% to 98.76%.

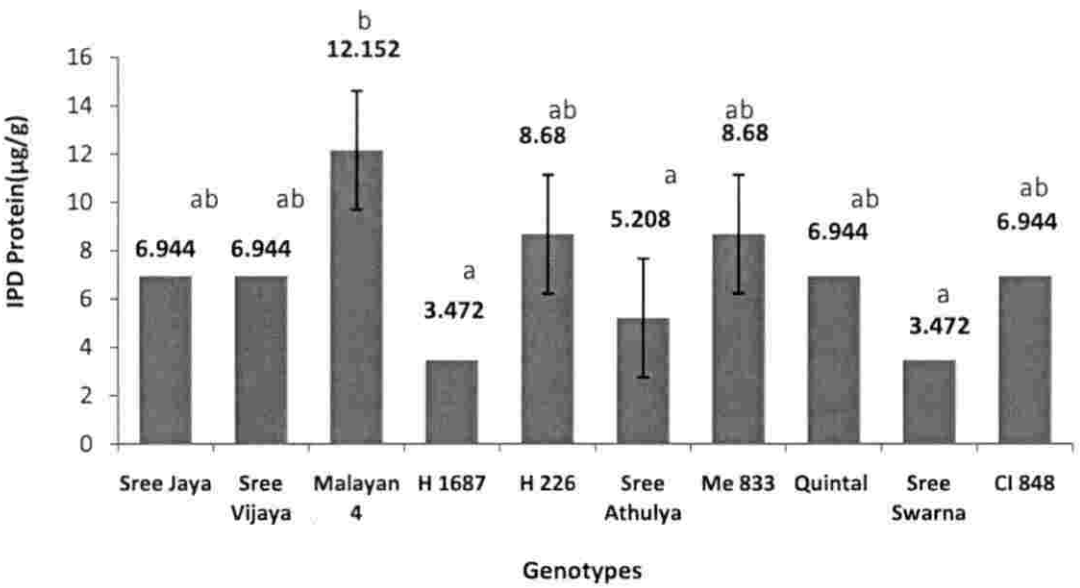


Figure 10: Content of Protein (µg/g) after enzymatic digestion

4.10 CONTENT OF PROTEASE INHIBITORS (%)

The Figure 11 below shows the content of protease (Trypsin) inhibitors in cassava leaf samples. The highest content was observed for the genotype Sree Jaya (33.41%) and the lowest for the genotype H 226 (3.74%). The inhibitor content ranges between 3.74% to 33.41%. No significant difference was observed among the genotypes Sree Vijaya, Quintal and Malayan 4. A significant difference was observed for the genotypes Sree Jaya, H 1687, H 226, Sree Athulya, Me 833, Sree Swarna and CI 848.

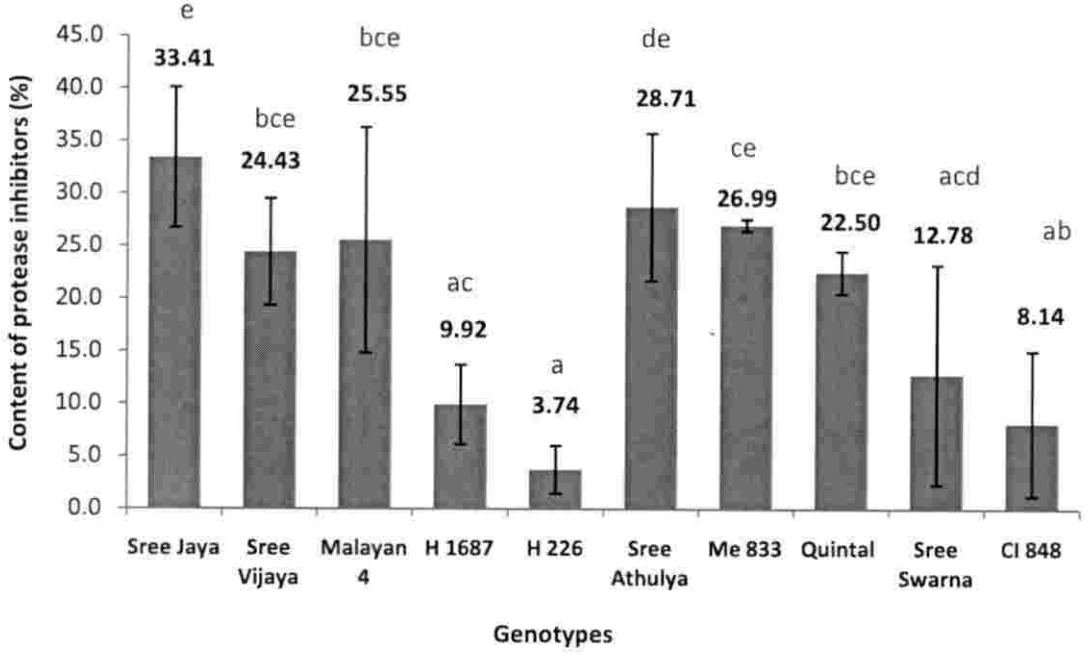


Figure 11: Content of Protease inhibitors (%) in cassava leaf samples

4.11 MINERAL CONTENT OF CASSAVA LEAF GENOTYPES

4.11.1 Copper (Cu) content (mg/L)

The Copper content of cassava leaf genotypes are shown in Figure 12. The content ranged between 24.7 to 29.7 (mg/L). No significant difference was observed for the copper content of cassava leaves of selected genotypes.

4.11.2 Zinc (Zn) content (mg/L)

Significant difference was observed for the zinc content of cassava leaves of selected genotypes. The leaf samples of H 1687 (230.9 mg/L) had the highest Zn and Me 833 (123.2 mg/L) had lowest Zn content. Similar Zn content was observed for genotypes Sree Athulya, Sree Swarna, H 226 and Malayan 4. The data is presented in Figure 13.

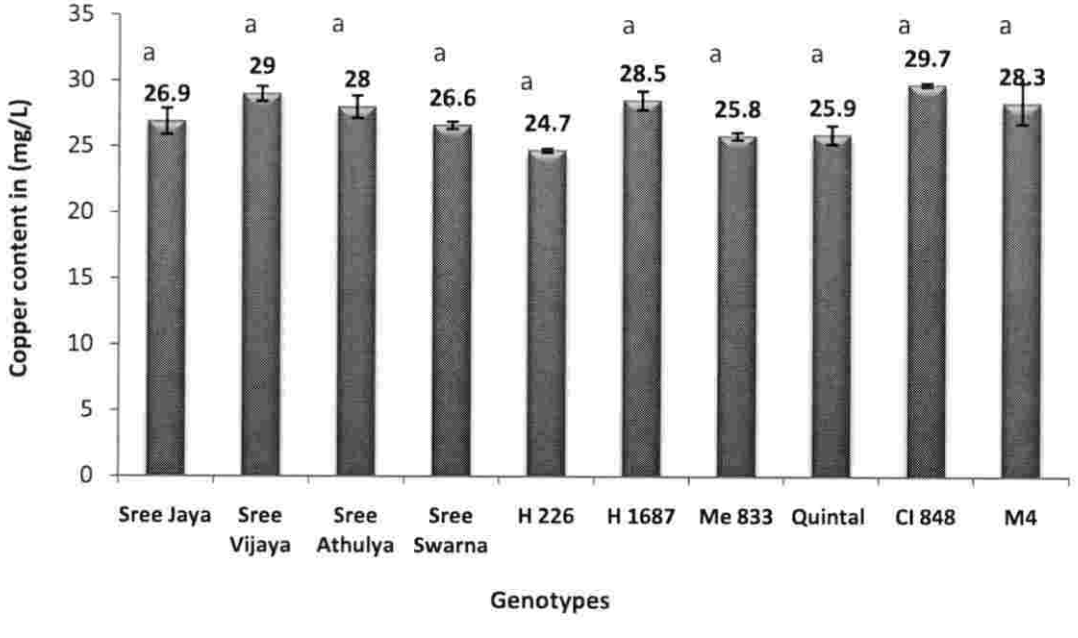


Figure 12: Copper (Cu) content (mg/L)

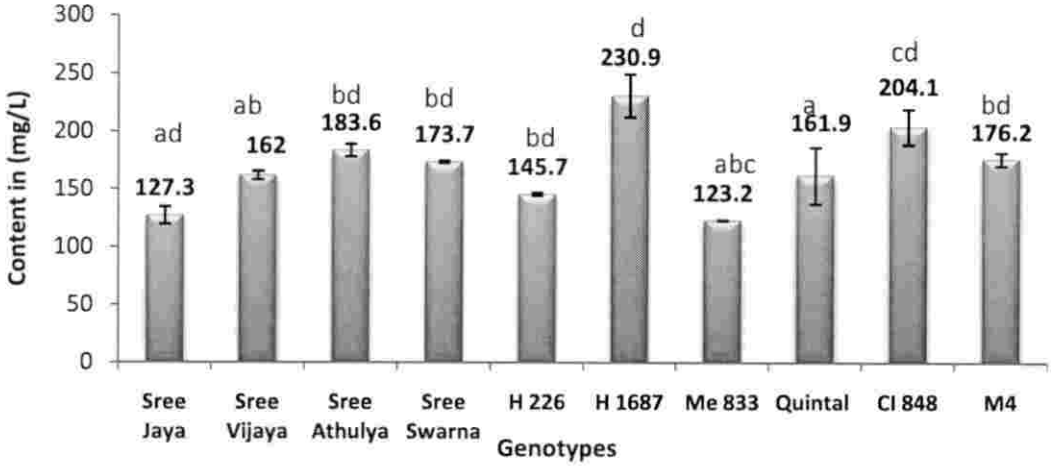


Figure 13: Zinc (Zn) content (mg/L)

4.11.3 Iron (Fe) content (mg/L)

The Fe content of cassava leaf samples ranged between 420 (mg/L) and 476.7 (mg/L). The genotype Me 833 had a lowest content (420mg/L) while Malayan 4 had the highest content (476.7 mg/L). The genotypes H 1687 and Malayan 4 had similar Fe content of 475 and 476 mg/L.

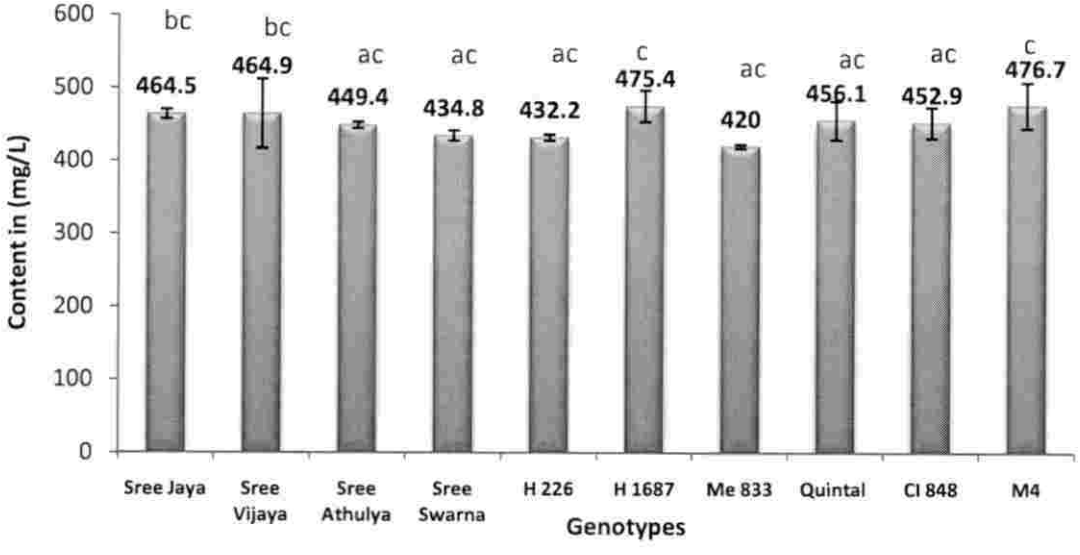


Figure 14: Iron (Fe) content (mg/L)

4.11.4 Manganese (Mn) content (mg/L)

Significant difference was observed for Mn content of cassava leaves of selected genotypes. The Mn content ranged from 151.3 (mg/L) to 385.9 (mg/L). The manganese content was observed to be lowest for the genotype Sree Jaya having a content of 151.3(mg/L), whereas, CI 848 had the highest content of 385.9 (mg/L).

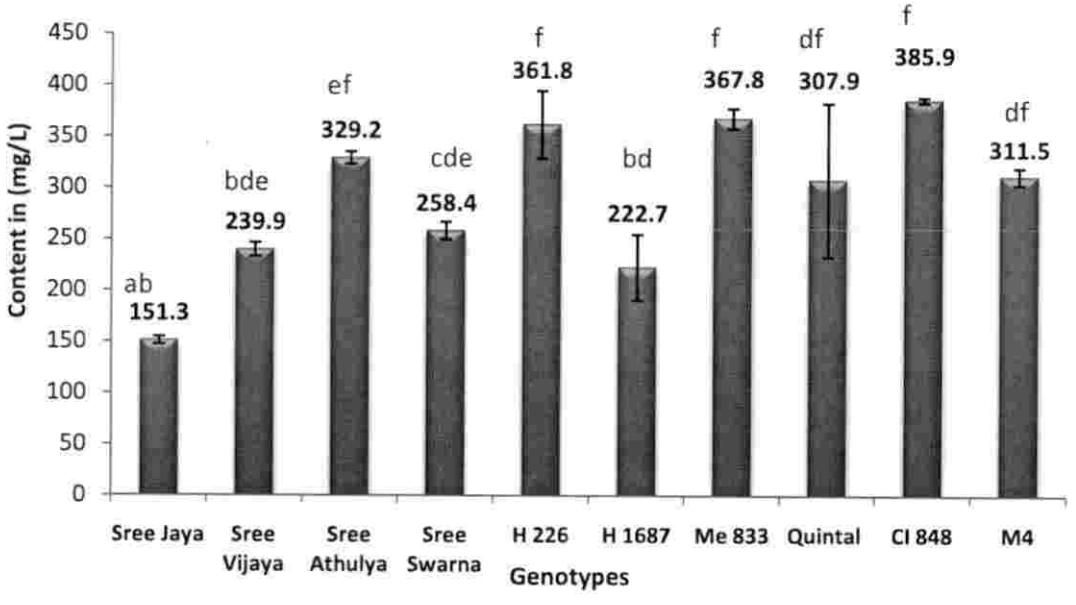


Figure 15: Manganese (Mn) content (mg/L)

4.11.5 Phosphorous (P) content (mg/L)

The phosphorous content in cassava leaf samples were found to be lower when compared to all the other minerals (Figure 16). Significant difference was observed for the P content of cassava leaves of selected genotypes. The P content ranged from 1.53 (mg/L) to 2.95 (mg/L). The highest content was observed for the genotype CI 848 (2.9466 mg/L) and the lowest was observed for the genotype Sree jaya (1.533795 mg/L). Genotypes Sree Jaya, H 226, Me 833 and H 1687 and Malayan 4 had similar P content.

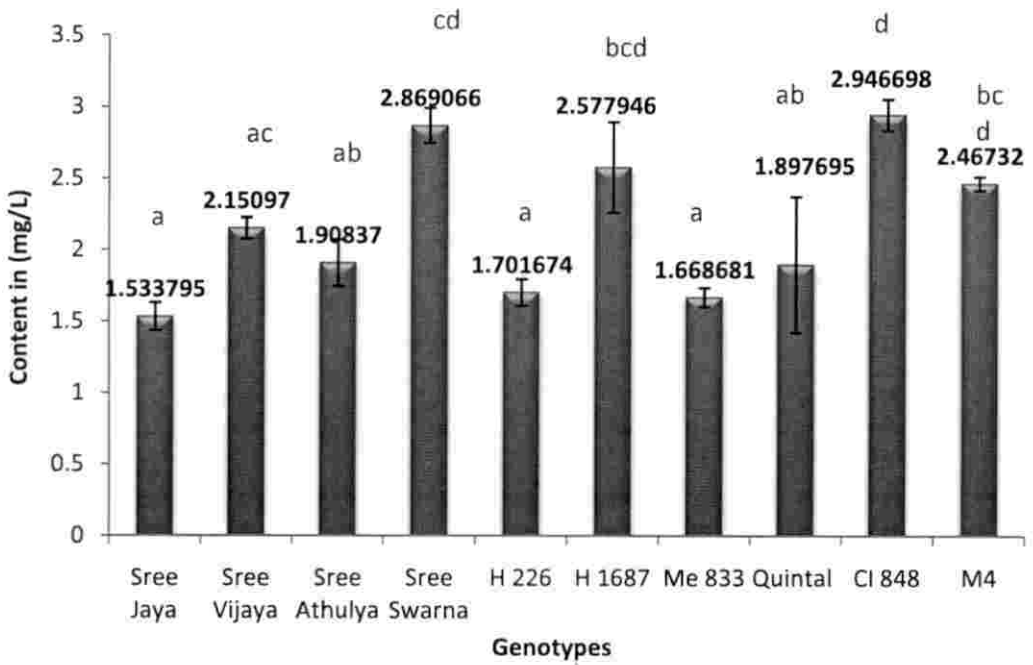


Figure 16: Phosphorous (P) content (mg/L)

4.11.6 Potassium (K) content (mg/L)

Significant difference was observed for the K content of cassava leaves of selected genotypes. The potassium content of cassava leaf samples ranged from 10900 (mg/L) to 19275 (mg/L) (Figure 17). The genotype H 226 and Me 833 had the lowest potassium content (10900 mg/L) and H 1687 had the highest content of 19275 (mg/L). Sree Jaya and Quintal, Sree Vijaya, Sree Athulya and M4, H 226 and Me 833 contained similar quantity of K in the leaves.

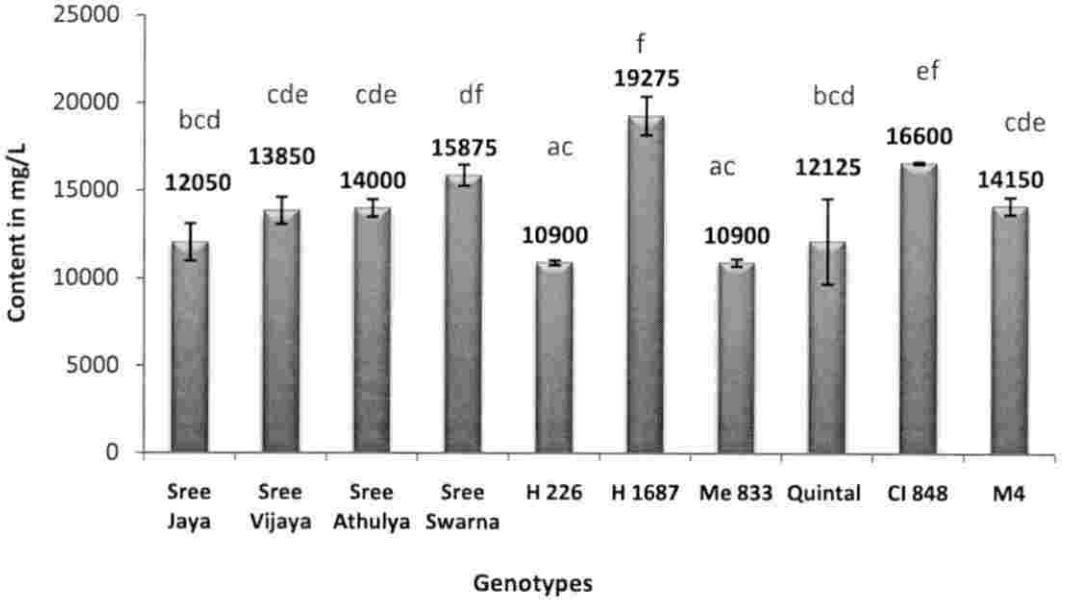


Figure 17: Potassium (K) content (mg/L)

4.11.7 Calcium (Ca) content (mg/L)

Significant difference was observed for the Ca content of cassava leaves of selected genotypes. The content ranged from 6880 mg/L to 10792.5 mg/L (Figure 18). The content was found to be highest for the genotype CI 848 (10792.5 mg/L) and lowest for the genotype Sree Vijaya (6880 mg/L). The genotype Malayan 4, Me 833, Quintal and Sree Athulya were found to have similar Ca content in the leaves.

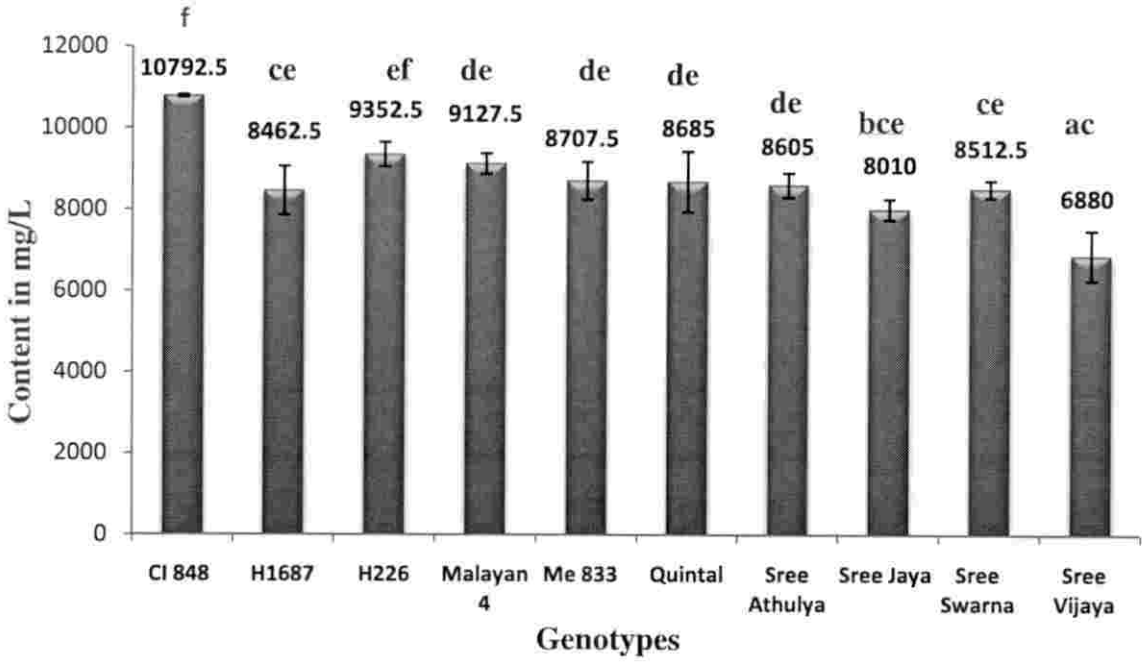


Figure 18: Calcium (Ca) content (mg/L)

4.11.8 Magnesium (Mg) content (mg/L)

Significant difference was observed for the zinc content of cassava leaves of selected genotypes. The highest magnesium content was found in the genotype Sree Vijaya (8630 mg/L) and lowest for the variety H 1687 (6037.5 mg/L). Mg content was at par in genotypes Sree Vijaya, Sree Athulya, H 226, Quintal and Sree Swarna.

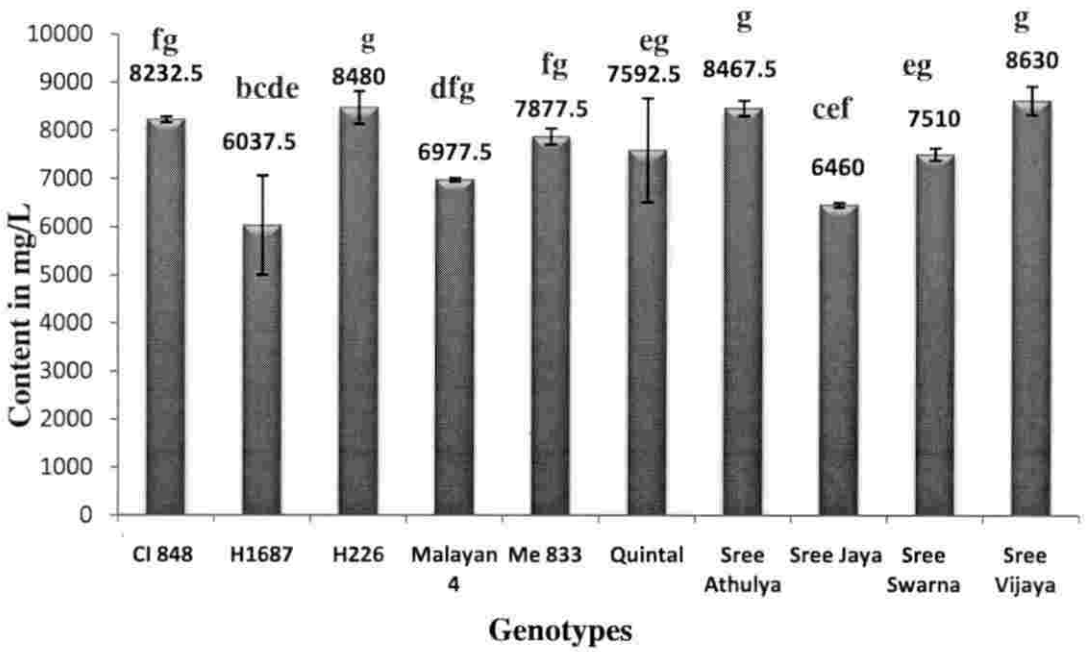


Figure 19: Magnesium (Mg) content (mg/L)

4.12 ENRICHMENT OF THE CASSAVA LEAF SAMPLES

Based on the nutrient analysis two genotypes H 1687 and Malayan 4 were selected for the enrichment studies. The linamarin, protein and the nitrogen content were also analysed for the enriched samples. Figure 20 represents the linamarin content of the enriched samples. The linamarin content at the start of the treatment was found to be 3.27 mg/g for the genotype H 1687 on a fresh weight basis and it was reduced at 7th day (0.3459 mg/g) and the content did not vary afterwards. At 20th day the content was similar to previous sampling (0.3504 mg/g). Similarly for the genotype Malayan 4 the content was found to be 1.32 mg/g on a fresh weight basis and reduced at 7th (0.3631 mg/g) and 20th day (0.3558 mg/g) of enrichment.

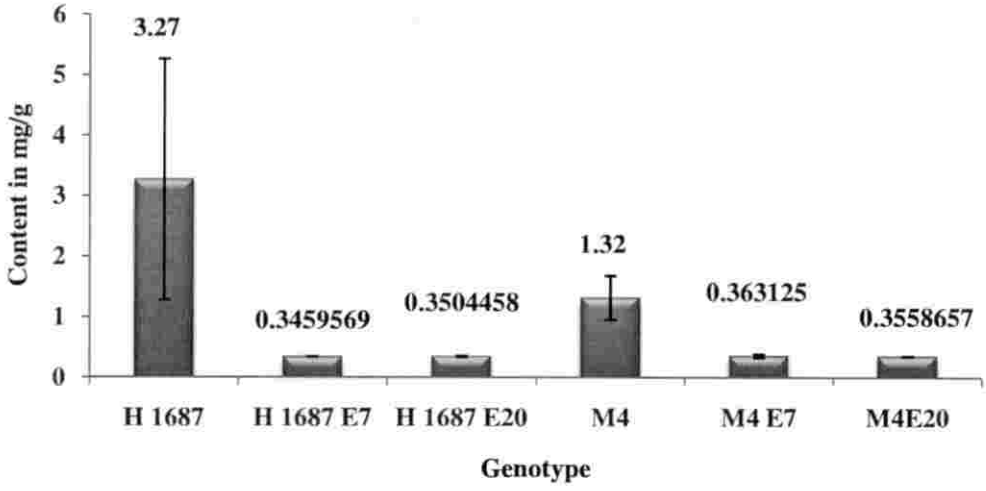


Figure 20: Linamarin content of enriched leaf samples of genotypes H 1687 and Malayan 4 at 7th day and 20th day of enrichment

The protein content of the enriched samples was found to be reduced at 7th day and 20th day of enrichment. The protein content of the genotype H 1687 was found to be 0.238 gg^{-1} and 0.186 gg^{-1} at 7th and 20th day of enrichment, respectively. Similarly for the genotype Malayan 4 the content was found to be reduced to 0.215 gg^{-1} and 0.193 gg^{-1} at 7th and 20th day of enrichment.

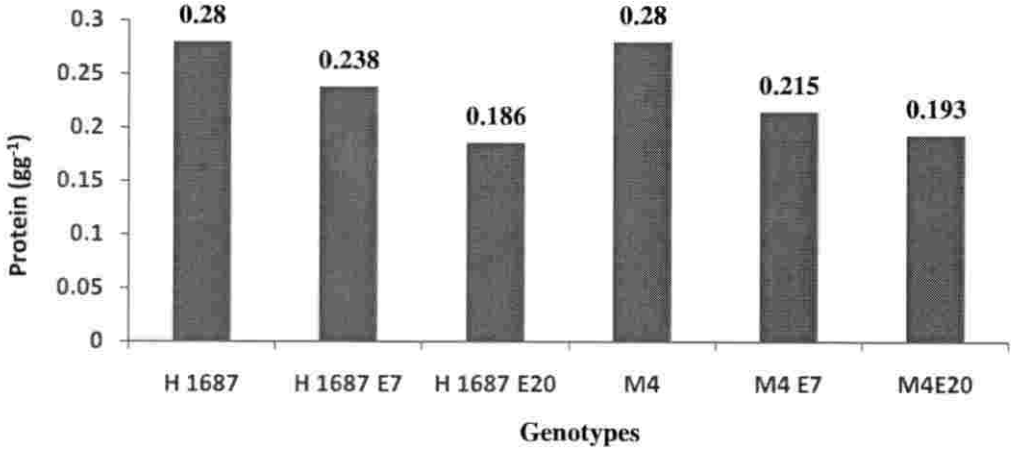


Figure 21: Protein content of the enriched samples

The mineral analysis of these genotypes were carried out at 0^{th}, 7^{th} and 20^{th} day of enrichment. Figure 22, 23 below shows the mineral content of the enriched samples in comparison with the samples that are not enriched. The copper content of the genotype Malayan 4 at 0^{th} day was observed to be 28.3 mg/L. Unlike other elements it was observed that the content got increased to 34.3 mg/L and 44.6 mg/L on the 7^{th} and 20^{th} day of enrichment. Similarly for the genotype H 1687 at 0^{th} day the copper content was found to be 28.5 mg/L. The content got increased to 46.2 mg/L and decreased to 33.2 mg/L on the 7^{th} and 20^{th} day of enrichment.

For the genotypes Malayan 4 and H 1687 the Zinc content at 0^{th} day was observed to be 176.2 mg/L and 230.9 mg/L. But the content got reduced for both of the genotypes M4 and H 1687 on enrichment at 7^{th} and 20^{th} day. The zinc content of Malayan 4 at 7^{th} and 20^{th} day was found to be 167.2 mg/L and 127 mg/L and for the genotype H 1687 the content got reduced to 166.1 mg/L and 144.3 mg/L respectively.

The Fe content of the leaf samples were comparatively higher for both enriched and non enriched samples. For the genotype Malayan 4 and H 1687 at 0^{th} day the Fe content was observed to be 476.7 mg/L and 475.4 mg/L. For both the genotypes the content got reduced at the 7^{th} day of enrichment. The content for both the genotypes was observed to be 397.1 mg/L and 379.7 mg/L . At the 20^{th} day of enrichment there was a slight increase in the iron content reaching a value 433.4 mg/L and 394 mg/L for the genotypes Malayan 4 and H 1687.

The Mn content was found to be 311.5 mg/L and 222.7 mg/L for both M4 and H 1687 genotypes. At the 7^{th} and 20^{th} day of enrichment the content was reduced to 117.2 mg/L and then increased to 160 mg/L for M4 genotype. In the case of H 1687 the content was reduced to 162.8 mg/L and again decreased to 104.2 mg/L at the 7^{th} and 20^{th} day of enrichment.

The Phosphorous content in leaf samples were found to be lower compared to other minerals. M4 and H 1687 were found to have 2.467 and 2.577 mg/L of phosphorous content respectively. At the 7th day of enrichment the content was 2.913 and 2.2412 mg/L for M4 and H 1687 respectively. At the 20th day of enrichment the P content slightly increased reaching a value of 2.86 and 2.66 mg/L respectively.

The K content of the samples were analysed (Figure 22). The potassium has a higher content in the leaf genotypes when compared to other minerals. M4 (14150 mg/L) and H 1687 (19275 mg/L) has a higher content of potassium at 0th day. The content got reduced for M4 genotype at the 7th day of enrichment (9075 mg/L) and an increase at the 20th day of enrichment (10600 mg/L). For H1687 the content got reduced at the 7th (12025 mg/L) and 20th day (7650 mg/L) of enrichment.

The Calcium content for the M4 and H 1687 at 0th day was found to be 9127.5 and 8462.5 mg/L. The calcium content at the 7th and 20th day of enrichment for M4 genotype was found to be reduced and then increased reaching a value of 6630 mg/L at 20th day. For the genotype the content got reduced to 7th day (7532.5 mg/L) and then to 20th day (6465 mg/L) of enrichment.

The magnesium content of the samples Malayan 4 and H 1687 was found to be 6977.5 mg/L and 6037.5 mg/L. The content was reduced at 7th day (3952.5 mg/L) and increased at 20th day (5290 mg/L) of enrichment. But for the genotype H 1687 the content was reduced at 7th day (5135 mg/L) and 20th day (4472.5 mg/L) of enrichment.

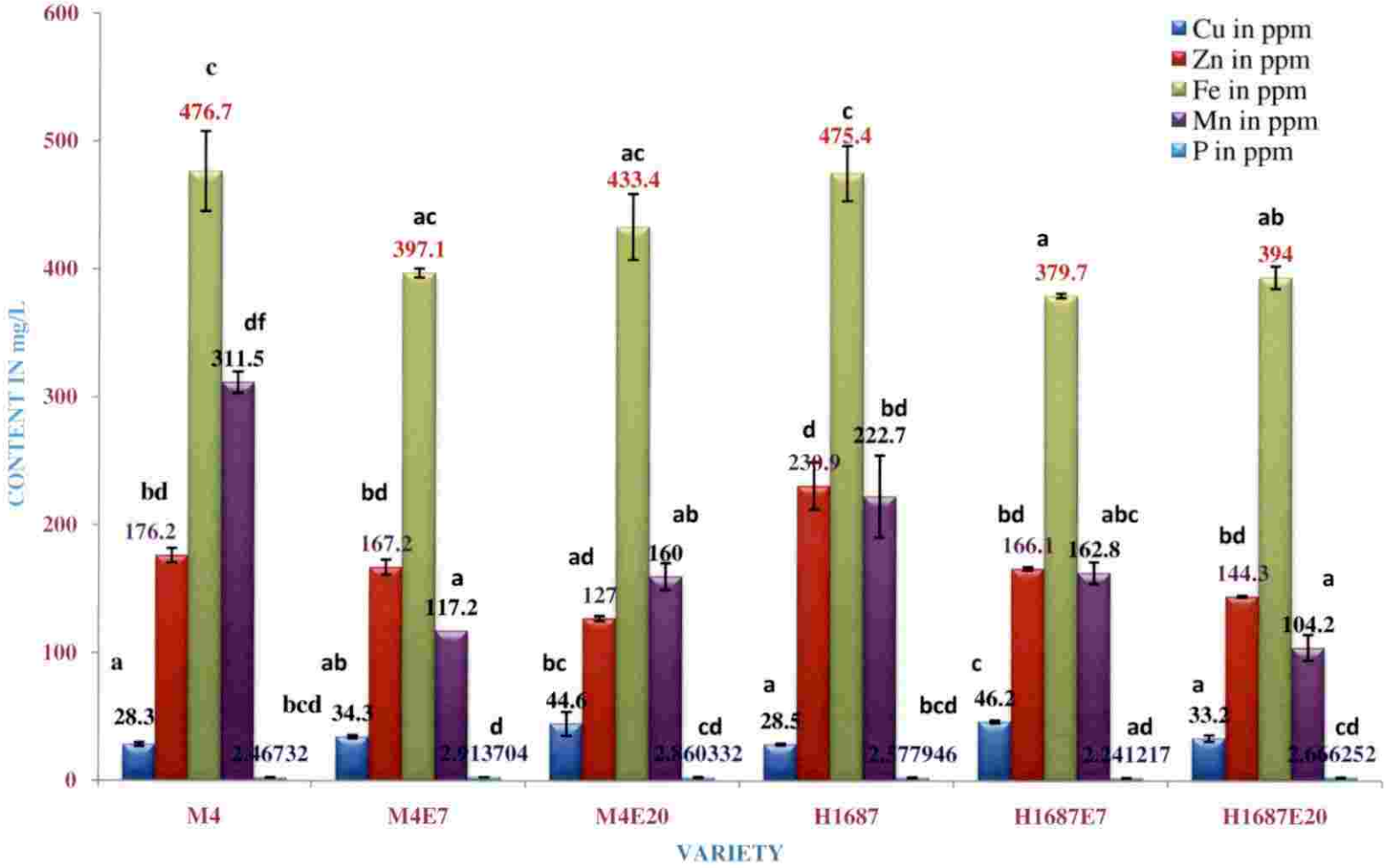


Figure 22: Mineral content of enriched cassava leaf samples at 7th and 20th day of enrichment

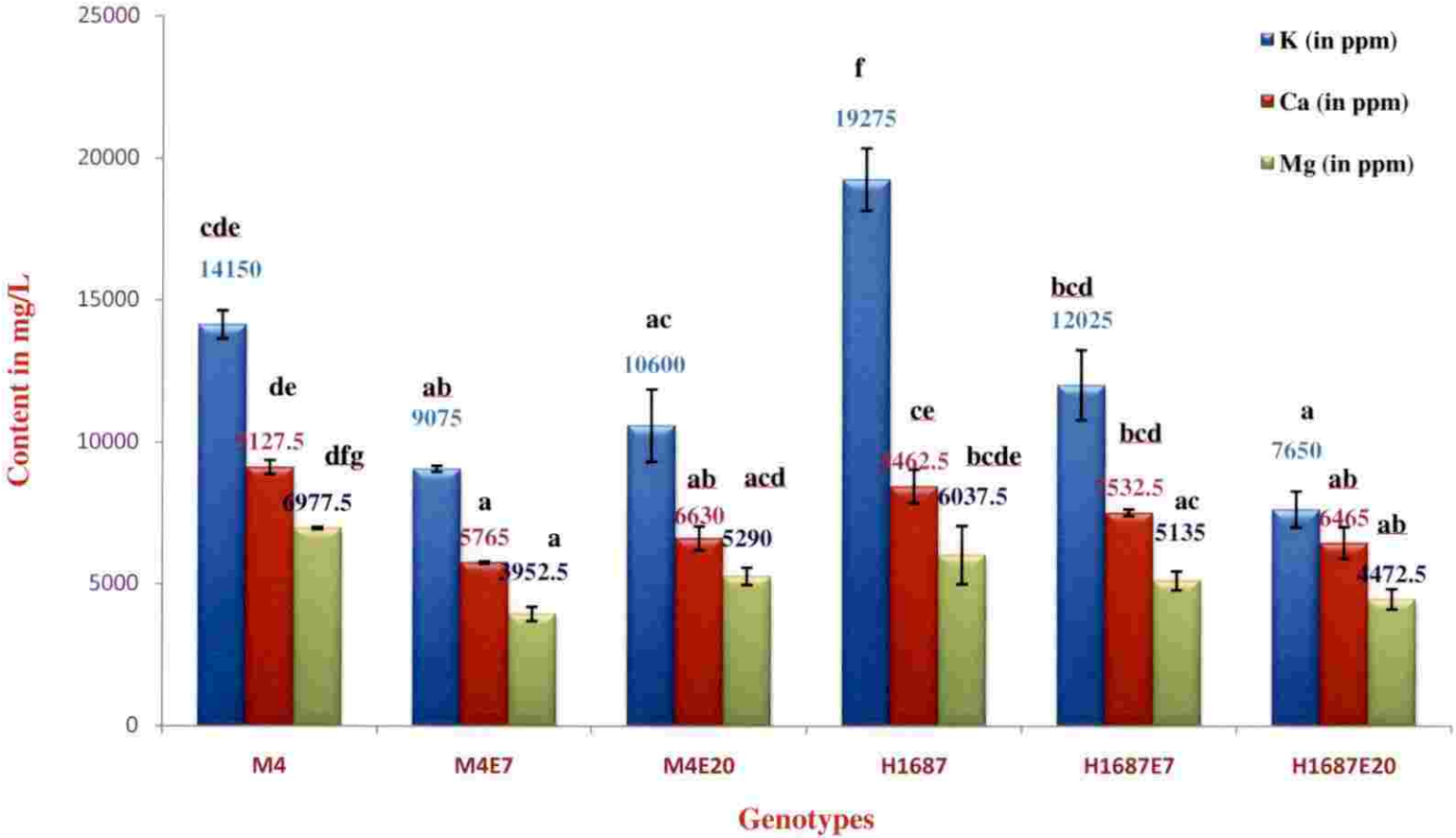


Figure 23: Mineral content of enriched cassava leaf sample at 7th and 20th day of enrichment

DISCUSSION

5. DISCUSSION

Cassava (*Manihot esculenta* Crantz) an important root crop and is widely cultivated in a tropical climatic conditions around the globe, Starchy tuberous roots and leaves are the main parts of cassava that are commonly used for food consumption. In Africa, cassava leaves are incorporated in food as a major source of protein, vitamins and minerals (Bokanga, 1994; Adjebeng-Danquah, 2013). With no decline in performance, cassava crop can be cultivated and harvested to feed ruminants and pigs (Preston and Rodriguez, 2004). This implies that there is the potential for cassava foliage to be utilized in human and animal consumption to provide cheap sources of feed for livestock and thus fresh cassava foliage containing the young stems, leaves can be directly fed to ruminants after sun-drying and can be incorporated in diets of non-ruminants such as pigs and poultry. High quality silages fed to animals resulted in sustainable animal health and thus reduces the cost of production, so the efforts to minimize the quality losses during preservation are still made (Bartzanas *et al.,* 2013). Ensiling is a method based on natural lactic acid fermentation under anaerobic conditions (Ki *et al.,* 2009) and to improve these processes, silage additives such as chemicals, enzymes and bacterial agents can be used while addition of carbon and nitrogen sources may positively affect the silage quality and thus consequently, improves the rumen degradation and animal production.

In this investigation, the physiological and biochemical studies of ten genotypes of cassava leaves were done with major emphasis on their nutritional aspects. Leaf characteristics of selected genotypes were carried out. The total fresh weight and dry weight (in grams), and number of leaves (per plant) were studied. For the ten different genotypes the fresh weight and dry weight of the leaves ranged from 168.33 g – 398.77 g plant^{-1} and 129.67 to 44.57 g plant^{-1}. The number of leaves per plant also varied greatly in number per plant of each genotypes. Tung *et al.*, (2001) reported that that some cassava varieties/genotypes are better foliage producers than others. Cassava foliage

yield and foliage productivity depends on genotype, soil fertility, frequency of foliage harvesting and climate. (Ravindran, 1991). Different genotypes of cassava has variability in the foliage production due to the ability exhibited by different genotypes to tolerate periodic pruning (Tung *et al.*, 2001).

The crude fibre content of the leaves ranged from 6.4 to 10.3% (Oke, 1966). In the present study the fibre content of leaves was obtained ranging from 18.73 to 48.67% for different genotypes. The major factor that determines the fibre content of leaves is the stage of maturity. Young leaves has lower content of fibre (8.3%) while for matured leaves the fibre content increases and for meal preparation the fibre content can be reduced to about 17% by discarding the petioles during meal preparation (Ravindran, 1985). Highest protein content was obtained for the genotype CI 848 (0.3 g g^{-1}) and the lowest for the genotype Sree Jaya (0.18 g g^{-1}). The lignin content in cassava leaves determines its anti nutritional quality. Higher content of lignin in feed limits its consumption. The lignin content obtained from the study ranges from 0.06 – 0.15 gg^{-1} for different genotypes. The lignin content was found to be higher in matured leaves than the younger leaves. According to Ravindran *et al.*, (1988) the lignin content of very young leaves, young leaves and matured leaves was found to be 0.9, 4.1 and 8.4 g/100g dry matter.

The linamarin content present in the leaves of the ten cassava genotypes were analysed and it ranged from 0.75 to 6.04 mg g^{-1} on a fresh weight basis. On a dry weight basis the content ranges from 0.34 to 1.2 mg g^{-1}. As maturity of the leaves increases the cyanogenic glucoside content decreases and the content of the leaves are six times higher than that in the roots in very young leaves. (Ngiki *et al.*, 2014). According to Ravindran *et al.*, (1987) the average cyanide content of fresh cassava leaves was found to be 1,436 mg/kg which has a higher content, but when they were sun dried it was reduced to 173 mg/kg. Ensiling of cassava leaves was found to be an effective method for the reduction of cyanide content. Stable minimum HCN content (20 mg kg^{-1} of fresh cassava foliage) occurred after 56 days of ensiling (Chhay Ty al, 2001). In this study, the leaves

were first wilted for 12 hours and was made into silage. The linamarin content at 7th day and 20th day of enrichment of the genotypes H1687 was found to be 0.3549 and 0.3504 mg g^{-1} while for the genotype Malayan 4 it was found to be 0.3631 and 0.355 mg g^{-1} respectively. The content can be reduced greatly as the duration of fermentation increases. The rapid reduction in the level of cyanide after ensiling implies that ensiling is an effective way of reducing HCN in cassava leaves and roots before feeding them to dairy animals (Ly and Rodríguez, 2001). According to Huhtanen *et al.*, 2007 fermentation of cassava leaves has a critical influence on feed intake, nutrient digestion, and milk yield in the ruminants.

In the present study conducted in different genotypes of cassava leaves showed that the content of trypsin inhibitors reaches upto a maximum value of 33.41% for the genotype Sree Jaya (three month old plants). The presence of protease inhibitors in the diet will results in the inhibition of the activity of the proteolytic enzymes within the gastrointestinal tract of animals. Wobeto *et al.*, (2007) reported that antinutrients such as trypsin inhibitors are found lower in 12 months old plants.

The protein content of the cassava leaves was found to be higher in all genotypes. The leaves are highly nutritious and has a high protein content ranging from 16.6 % to 39.9% (Khieu *et al.*, 2005). The protein content of all ten genotypes in the present study was found to be ranging from 18.275% - 30.721%. The protein content of the enriched samples was found to be reduced at 7th day and 20th day when compared to the samples at 0th day. Addition of additives such as molasses, carbohydrates and other feed materials reduces the concentration of protein in the leaf meal. According to Khang *et al.*, (2005) the crude protein content of the cassava top silage was found to be slightly reduced after ensiling when compared to fresh cassava leaf or hay. The variations in cassava protein digestibility were reported in many studies. The young and old leaves of cassava leaf protein has a digestibility of 80% and 67% respectively (Bokanga, 1994). In the present study the digestibility rate of the ten genotypes

of cassava leaves was found to be higher. The highest rate of digestibility was observed for the genotype H 1687 (98.76 %) and lowest for Sree Athulya (94.15%).

The presence of high content of mineral elements particularly Ca, Mg, K elements in cassava leaves confirms its importance as a rich source of mineral elements. Calcium was found to be essential for a healthy diet for building strong and healthy bones. In the study conducted ten genotypes of cassava leaves were taken for the mineral analysis. The mineral elements Cu, Zn, Fe, Mn, P present in leaves ranges between 24.7 to 29.7 (mg/L), 123.2 to 230.9 (mg/L), 420 (mg/L) to 476.7 (mg/L), 151.3 (mg/L) – 385.9 (mg/L) and 1.53395 (mg/L) -2.9466 (mg/L) respectively. The phosphorous content was found to be lower when compared with other mineral elements (Oke, 1966). It is essential for the body's utilization of carbohydrates and fats, for the synthesis of proteins, maintenance and repair of cells. According to Guil-Guerrero *et al.*, (1998) for efficient calcium and phosphorus intestinal utilization, Ca : P must be close to unity. For the minerals K, Ca and Mg highest content was observed for which the values ranges from 10900 (mg/L) – 19275 (mg/L), 6880 mg/L – 10792.5 mg/L, and 6037.5 mg/L -8630 mg/L respectively. The mineral elements was found to have a slight decrease in the content on enrichment at 7^{th} and 20^{th} day when compared to initial days. The level of Potassium, Magnesium, Phosphorous, Zinc and Manganese decreases while Calcium, Sodium and Iron increases with leaf maturity (Ravindran and Ravindran, 1988).

Addition of carbon and nitrogen sources may improve the silage quality and hence the rumen degradation of the food material and a considerable amount of fermentable carbohydrates in plant material is necessary for lactic acid production which reduces fermentation pH and guarantees the good quality silage (McDonald *et al.,* 2011). For the present study urea was added as N source, yeast and molasses are also added into the silage for the enrichment purposes which enhance the fermentation rate of the silage that resulted in a characteristic odour, flavour and colour of the silage.

SUMMARY

6. SUMMARY

The study entitled "Investigations on nutritional aspects of cassava (*Manihot esculenta* Crantz) leaf and its enrichment" was carried out at the Division of Crop Utilization, ICAR – Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during the year 2018 – 2019. The objective of the study was to screen the cassava genotypes for suitability of leaf meal and characterization of leaf protein, nutrient content and its enrichment.

In the present study ten genotypes of cassava were raised in the fields of ICAR- CTCRI. The ten genotypes of cassava selected were Sree Jaya, Sree Vijaya, H 226, CI 848, H 1687, Malayan 4, Sree Athulya, Quintal, Me 833 and Sree Swarna. The physiological and biochemical studies were conducted in the leaf samples. The physiological parameters such as leaf fresh weight, dry weight and the number of leaves per plant was done. The fresh weight and dry weight of the leaves ranged from 168.33 g – 398.77 g plant^{-1} and 129.67 to 44.57 g plant^{-1}. The number of leaves per plant also varied greatly in these genotypes. Biochemical parameters such as Leaf protein content, anti nutritional factors such as crude fibre content, Lignin content, content of protease inhibitors and cyanogenic glucosides were done. The fibre content, lignin content, protein content and linamarin content ranges from 18.73 - 48.67%, 0.06 – 0.15 gg^{-1}, 18.275% - 30.721%, 0.75 to 6.04 mg g^{-1} (fresh weight basis) and 0.34 to 1.2 mg g^{-1} (dry weight basis) for different genotypes. The content was found to be reduced at 7th day and 20th day of enrichment for the genotypes H1687 (0.3549 and 0.3504 mg g^{-1}) and Malayan 4 (0.3631 and 0.355 mg g^{-1}). The content can be reduced greatly as the duration of fermentation of the silage increases.

The cassava leaves are considered as a good source of minerals. The mineral elements Cu, Zn, Fe, Mn, P present in leaves ranges between 24.7 to 29.7 (mg/L), 123.2 to 230.9 (mg/L), 420 (mg/L) to 476.7 (mg/L), 151.3 (mg/L) – 385.9 (mg/L) and 1.53395 (mg/L) -2.9466 (mg/L) respectively. For the minerals K, Ca

and Mg highest content was observed for which the values ranges from 10900 (mg/L) – 19275 (mg/L), 6880 mg/L – 10792.5 mg/L, and 6037.5 mg/L -8630 mg/L respectively.

Based on the nutritional status of the leaf genotypes two best genotypes Malayan 4 and H 1687 were selected based on quality for enrichment studies. The leaves were then processed to remove the cyanogen content and supplemented with additives such as Carbohydrates, urea. yeast and molasses. The digestibility studies were carried out using enzymes such as Pepsin and Pancreatin. The ensiling of the cassava leaf silage resulted in a rapid reduction in the cyanogenic content which can be then supplemented as animal feed. The mineral elements was found to have a slight decrease in the content on enrichment at 7^{th} and 20^{th} day when compared to initial days. A slight reduction in the protein content was also observed for the enriched samples.

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APPENDICES

APPENDIX I

Potassium Phosphate buffer	(pH - 7, 0.05 M)
K_2HPO_4	4.672 g
KH_2PO_4	3.154 g
Make up to 1 litre with distilled water	

APPENDIX II

70% Ethanol

100% Ethanol 70 mL

Distilled water 30 mL

APPENDIX III

1.4 gram 1,3 dimethyl barbiturate

1.14 gram isonicotinic acid

Dissolve it in 0.74 gram NaOH in 40 ml

APPENDIX IV

Sodium phosphate buffer pH – 6.0 of 0.1 M

12.3 ml Na_2HPO_4 (35.61 g/L) and 87.7 ml 0.2 M NaH_2PO_4 (31.21g/L)

Mix the solution and make upto 200 ml

INVESTIGATIONS ON NUTRITIONAL ASPECTS OF CASSAVA (*Manihot esculenta* Crantz) LEAF AND ITS ENRICHMENT

By

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Abstract of Thesis

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9. ABSTRACT

The study entitled "Investigations on nutritional aspects of cassava (*Manihot esculenta* Crantz) leaf and its enrichment" was carried out at the Division of Crop Utilization, ICAR – Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during the year 2018 – 2019. Cassava (*Manihot esculenta* Crantz) is a major stable food crop for sustainable agricultural practice and food security in both developing and under developed countries. Cassava leaves are rich in protein, carotenes, vitamins B_1, B_2, and C, and minerals such as calcium, magnesium and also rich in anti-nutritional factors such as cyanogens. Efficient use of cassava products resulted in reduced feed costs. With proper processing the inclusion levels of cassava leaves in diets can be increased and can be used as an alternative energy source.

The total fresh weight and dry weight (in grams), and number of leaves (per plant) were studied. For the ten different genotypes the fresh weight and dry weight of the leaves ranged from 168.33 g – 398.77 g plant^{-1} and 129.67 to 44.57 g plant^{-1}. The number of leaves per plant also varied greatly in number per plant of each genotypes. The crude fibre content of the leaves ranged from 6.4 to 10.3% (Oke, 1966). In the present study the fibre content of leaves was obtained ranging from 18.73 to 48.67% for different genotypes. The lignin content in cassava leaves determines its anti nutritional quality. Higher content of lignin in feed limits its consumption. The lignin content obtained from the study ranges from 0.06 – 0.15 g/g for different genotypes.

The linamarin content present in the leaves of the ten cassava genotypes were analysed and it ranged from 0.75 to 6.04 mg g^{-1} on a fresh weight basis. On a dry weight basis the content ranges from 0.34 to 1.2 mg g^{-1}. The linamarin content at 7th day and 20th day of enrichment of the genotypes H1687 was found to be 0.3549 and 0.3504 mg g^{-1} while for the genotype Malayan 4 it was found to be 0.3631 and 0.355 mgg^{-1} respectively. The content can be reduced greatly as the duration of fermentation increases. The rapid reduction in the level of cyanide

after ensiling implies that ensiling is an effective way of reducing HCN in cassava leaves and roots before feeding them to dairy animals.

In the present study, different genotypes of cassava leaves showed that the content of trypsin inhibitors reaches up to a maximum value of 33.41% for the genotype Sree Jaya (three month old plants). The protein content of the cassava leaves was found to be higher in all genotypes. The protein content of the enriched samples was found to be reduced at 7^{th} day and 20^{th} day when compared to the samples at 0^{th} day. Addition of additives such as molasses resulted in reduction of protein content in the silage,

The presence of high content of mineral elements particularly Ca, Mg, K elements in cassava leaves confirms its importance as a rich source of mineral elements. The mineral elements Cu, Zn, Fe, Mn, P present in leaves ranges between 24.7 to 29.7 (mg/L), 123.2 to 230.9 (mg/L), 420 (mg/L) to 476.7 (mg/L), 151.3 (mg/L) – 385.9 (mg/L) and 1.53395 (mg/L) -2.9466 (mg/L) respectively. The mineral elements was found to have a slight decrease in the content on enrichment at 7^{th} and 20^{th} day when compared to initial days. For the present study urea was added as N source, yeast and molasses are also added into the silage for the enrichment purposes which enhance the fermentation rate of the silage that resulted in a characteristic odour, flavour and colour of the silage.

The H1687 and Malayan 4 (M4) genotypes having high dry matter and protein content is suitable for use as leaf meal and preparation of silage from cassava leaves.