BIOCHEMICAL AND NUTRITIONAL EVALUATION OF CHAYOTE (*SECHIUM EDULE*) AND LUNGRU (*DIPLAZIUM ESCULENTUM*) OF PALAM VALLEY OF HIMACHAL PRADESH

THESIS

BY

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IN

Partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE IN BIOCHEMISTRY
(MINOR: PLANT PHYSIOLOGY)

CERTIFICATE - I

This is to certify that the thesis entitled "**BIOCHEMICAL AND NUTRITIONAL EVALUATION OF CHAYOTE (*SECHIUM EDULE*) AND LUNGRU (*DIPLAZIUM ESCULENTUM*) OF PALAM VALLEY OF HIMACHAL PRADESH**" submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (BASIC SCIENCES)** in the subject of **Biochemistry** (Minor: **Plant Physiology**) of the Himachal Pradesh Krishi Vishvavidyalaya, Palampur, is a bonafide research work carried out by Ms Sudha Prasad (S-96-30-04) under my supervision and that no part of the thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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Dated: 5th August, 1998

CERTIFICATE II

This is to certify that the thesis entitled, **"BIOCHEMICAL AND NUTRITIONAL EVALUATION OF CHAYOTE (*SECHIUM EDULE*) AND LUNGRU (*DIPLAZIUM ESCULENTUM*) OF PALAM VALLEY OF HIMACHAL PRADESH"**, submitted by Ms. Sudha Prasad to the Himachal Pradesh Krishi Vishvavidyalaya, Palampur, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (BASIC SCIENCES)** in the subject of **Biochemistry** has been approved by the Examination Committee after an Oral Examination of the same in collaboration with the External Examiner.

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Sudha Prasad

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ABBREVIATIONS

$BaCl_2$	:	Barium Chloride
$Ca\ Cl_2$	:	Calcium Chloride
cm	:	Centimeter
$C_2H_3O_2\ Na$	:	Sodium acetate
D.W. /d.w.	:	distilled water
d.w.b.	:	dry weight basis
f.w.b.	:	fresh weight basis
g	:	gram
HCl	:	Hydrochloric acid
H_2HO_4	:	Sulphuric acid
HNO_3	:	Nitric acid
$H\ ClO_4$	:	Perchloric acid
Hr.	:	Hour
HPLC/ h.p.l.c.	:	High Pressure Liquid Chromatography
K_2SO_4	:	Potassium sulphate
KCal	:	Kilocalorie
$KMnO_4$	:	Potassium permangnate
m	:	meter
mg	:	milligram

ml	:	millilitre
MSL	:	Mean Sea Level
NO_3	:	Nitrate
$Na\cdot H_2PO_4$	:	Sodium dihydrogen phosphate
Na_2HPO_4	:	Disodium hydrogen phosphate
NaOH	:	Sodium hydroxide
nm	:	Nanometer
$NaHCO_3$	:	Sodium bicarbonate
O.D.	:	Optical density
ppm	:	Parts per million
R H	:	Relative Humidity
TLC	:	Thin Layer Chromatography
μ	:	micro
%	:	Per cent
λ	:	Lambda

CHAPTER 1

INTRODUCTION

Himachal Pradesh is an abode of wide variety of flora especially in the Himalayas, many of which are yet to be brought to limelight for their rational uses. Chayote (*Sechium edule*) and Lungru (*Diplazium esculentum*) constitute an important part of flora and are consumed predominantly as seasonal vegetable in Himachal Pradesh. Chayote is a cucurbit and lungru is a pteridophyte.

Though cucurbits are unpreferable for their use for caloric, mineral and vitamin value, it forms an exception by having fairly high amount of calcium content. Chayote has tremendous economic value as food plant. Chayote bears single seeded fruit on a perennial rooted vine that bears large crops. It is considered as vegetable of secondary importance and is known by various names such as chow -chow, chayotli, chouchoute; cho-cho, chuchu or ruxu, choko, chaiota, mirliton, Christophine and Pipinella. In Tamil, it is called Katrikkai. In Himachal Pradesh, locally it is called "Launku".

Chayote is a crop of tropical and subtropical regions of the world. It can be grown in temperate regions with certain change in its cultivation practices. It grows exceptionally in the semi temperate climate of Palam Valley in North Western Himalayas. Chayote owes its origin in Mexico and Gutemala. In India, it is found to be a crop of Karnataka, Tamil Nadu, Maharashtra (Shanmugavelu, 1985) and shows great variation in fruit shape and colour (Engels, 1983).

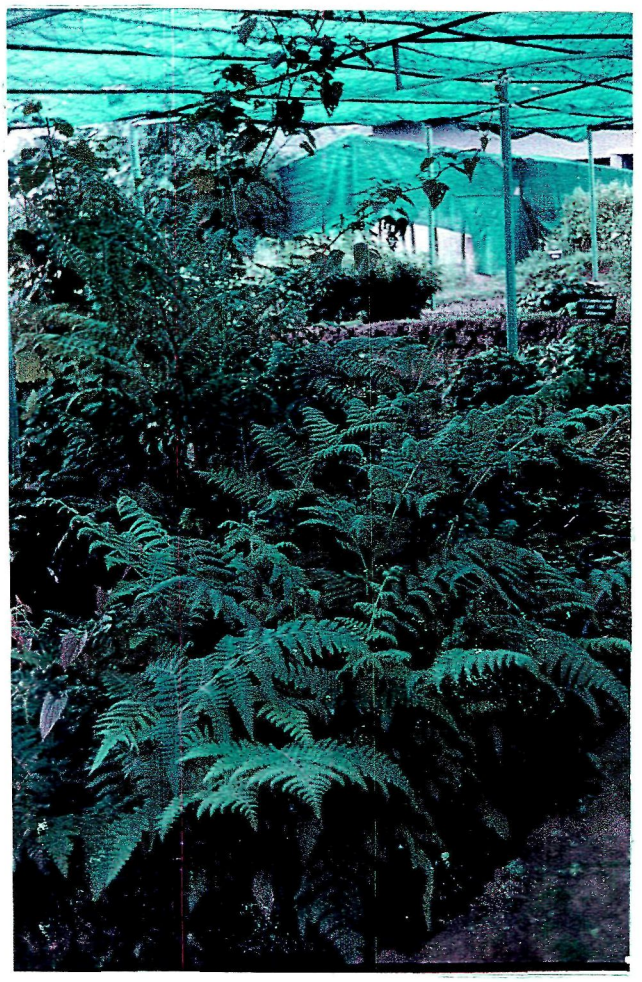
Roots of chayote are of tap root type with lateral roots . At some places, tubers are formed. It is a climbing shrub and produces

PLATE - 1 (A) : SHOWING CHAYOTE VINES BEARING FRUITS

PLATE - 1 (B) & (C) : SHOWING MATURE PLANTS OF LUNGRU

(A)

(B)



(C)

shoots upto 15 meters. Leaves are three to five lobed. Inflorescence is axillary, monoecious but staminate and pistillate flowers are borne on the same node. Male flowers are racemes, while the female flowers are solitary. Fruits bear single, monocarpellary seed. The fruits of chayote are harvested for human consumption 28 -36 days after fertilization and before viviparous germination starts. Chayote is propagated by means of sprouted fruits which are planted entirely. It can be propagated by tender stem cuttings. The yield of 300 to 500 fruits per plant has been observed. One accession produced 45 kg of fruit in Nepal in 9 months. Each fruit weighs from 200 to 450 g . Fruit is slightly bigger in the North-East India (Sheshadri, 1993).

Chayote is found in Palam Valley of Himachal Pradesh during the months of September to November at the altitude of 1300 m above M.S.L. The fruits are grown in local kitchen gardens and consumed as only fresh produce. Because of its exceptional nutritional qualties and tolerance to varied environmental stresses, chayote needs to be exploited further. In addition, chayote leaves are reported to show medicinal property and for this reason, its biochemical and nutritional evaluation would render further information for its rational use and benefit to the mankind. Additionally, the studies on the effect of storage of fruit of chayote would provide in-depth information for its useful longevity. Accordingly, the storage effect on its quality in the semi temperate climatic conditions of Palampur has been undertaken in the present study.

Lungru (*Diplazium esculentum*) belongs to Phylum Pteridophyta including horse tails and club mosses which are also called ferns. The ferns, forming the largest group of pteridophyta, are flowerless

plants with well developed vascular and tegumentary system and complete differentiation into stem, root and leaf. The ferns are distributed in the Northern, Southern and Gangetic peninisular regions of India. It is a large fern with erect, stout, subarborescent rhizome of two pinnate fronds, which are slightly scaly or hairy, grooved, erect and tufted. Scales are dense near rhizome. It produces spores which in turn germinate to give the gametophytic generation. It also gives rise to runners which produce the same plant. It occurs in moist climate and the young curly fronds of this fern are used as popular seasonal vegetable. Though preliminary work has been done in respect of its biochemical and nutritional status indicating it a fairly rich source of dietary fibre. But inadequate information seems to be available about biochemical and nutritional status of *Diplazium esculentum*. It inhibits the micro organisms when applied as an extract in combination with other ferns. Some species of genus *Diplazium* are used for ornamental as well as medicinal purposes.

In countries like Malaysia and Thailand, it forms one of the important and common leafy vegetable consumed. Additionally lungru has been reported to be of medicinal significance.

Literature revealed that apparently little systematic work has been done especially in India with regard to its detailed biochemical composition and level of anti-nutritional factors.

Considering the above facts regarding these underutilized and under exploited vegetables in hills, the study was planned with the following objectives:

I. To study biochemical constituents of chayote (*Sechium edule*) and lungru (*Diplazium esculentum*) of Palam Valley of H.P.

II. a) To assess the level of anti nutritional factors in Lungru (*Diplazium esculentum*) and

b) To evaluate the effect of storage fruits of chayote after harvest on their nutritional quality.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Chayote (*Sechium edule*) and lungru (*Diplazium esculentum*) are important seasonal vegetable crops in the Palam valley of Himachal Pradesh . Chayote has been reported as a perennial vegetable of kitchen garden (Muthukrishnan and Ramdas, 1974). It forms a traditional vegetable in Nepal (Daral *et al.*, 1994). Accordingly efforts are being made for exploiting its potential to maximum. Though chayote is a lesser known member of the gourd family but is gaining popularity and importance world wide for its unique developmental and nutritional aspects. Its hardy nature and adaptability to various climatic conditions are its assets. (Aung *et al.*,1990). Besides, it is fairly good source of energy, minerals among gourds in rainy or off-season when other vegetables are not available locally.

2.1 Chayote (*Sechium edule*)

Enough literature is available covering taxonomy, production technology and other crop management aspects of Chayote, however limtied information is available on its biochemical and nutritional status that too grown under mid hill conditions of Himachal Pradesh.

Nath (1971) opined chayote as capable of being popular vegetable describing its cultivation, harvesting, uses and chemical composition. He reported bio-chemical composition of chayote containing 0.9 g protein, 0.3 g fat, 4 g total sugar, 1.3 g starch, 19 g calcium, 20 mg of phosphorus and 11 mg of vitamin C per 100 g of edible portion.

Flick *et al.* (1977) analysed the samples of flesh of chayote for various biochemical and nutritional attributes. Fresh fruits contained 94.71 % moisture in total, while 94.70% in flesh and 94.08% in skin. The per centage of total solids and pectin in the whole fruit were 5.29 and 1.47 %, respectively. On dry weight basis, chayote flesh contained 2.87% nitrogen, 0.62% ether extractable material, 7.56 % crude fibre and 3.65 % ash. It contained minerals like Calcium (Ca) 0.1790; Iron (Fe) 77.9; Potassium (K) 12879.0; Magnesium 1540; Sodium 177.0; Chlorine 1464.0 and Aluminium 85.3 ppm; forming the higher concentrations. Neither carotene nor the lipoxygenase or polyphenol oxidase activity was detectable.

Flick *et al.* (1978) reported amino acid concentrations in the seeds to be approximately twice those present in the flesh of chayote. Methionine was detected only in the seeds.

Zinsou *et al.* (1983) studied the carbohydrate content at various levels of fruit development of Chayote and found glucose, fructose and sucrose were the major sugars in carbohydrates fraction solubilised by ethanol / water. After 15 days of anthesis, glucose and fructose contents were maximum, however, at the time of picking the starch content increased to 27% of dry matter whereas fructose and glucose level decreased.

Angels (1983) conducted quality studies on *Sechium edule* (Chayote) collected in Central America and reported the proximate composition of Chayote fruits which included 90.8% moisture, 0.4% ash, 0.9 % crude protein, 0.2 g crude fat content 0.6 g fibre, 20 mg ascorbic acid, 12 mg calcium, 30 mg phosphorus, 0.6 mg iron per 100 g on fresh fruit basis.

Salvo *et al.* (1984) determined the fructose and glucose levels in chayote alongwith other members of cucurbitaceae using h.p.l.c. The quantities of fructose and of glucose were below 1%.

Van Suyt and Zinssu (1986) reported fairly high concentration of agmatine (an immediate decarboxylation product of L-arginine amino acid) in the young leaves of Chayote than the mature leaves which contained much higher concentration of arginine than agmatine at all stages of development.

Macleod (1990) analysed sixty one volatile components positively in fruits of Chayote which formed 97% of isolate. Most of the volatile products have been derived from specific lipid oxidation or degradation. The names of two components - contributing the green odour of the fruit have been listed.

Rao *et al.* (1990) analysed the edible leaves of five plants including Chayote . The analysis showed 2.32 % lipids, 2.69 % protein and 2.16 % ash. The lipid classes separated by silicic acid column chromatography were linolenic (18: 3) and palmitic . Proteins were found to contain most of essential amino acids except for those containing sulphur. The availability of Calcium and Magnesium was high, of Iron and Zinc - moderate, while of Copper (Cu), it was low.

Silva *et al.* (1990) determined the cellulose, hemi cellulose and lignin contents of low energy hospital diets. Among vegetables analysed , chayote represented with highest contents of neutral detergent fibre, cellulose and hemicellulose. Lignin content was also high in chayote.

Aung *et al.* (1991) studied the quantitative and qualitative aspects of carbohydrates in the organs of chayote by HPLC, TLC and

colorimetric procedures. The immature fruit showed the starch content of 75 ug /mg DW in flesh. The roots and the tubers had 51% and 72% starch, respectively on dry weight basis. The major sugars in Chayote were glucose, fructose, sucrose, sorbitol and to a less extent raffinose and stacchyose. Two unidentifiable sugars were also detected.

Gopalan *et al.* (1995) reported the proximate composition of chayote on fresh weight basis. The edible fruits were reported to contain 92.5% moisture, 0.7 g % protein, 0.1 g fat; 0.4 g minerals, 0.6 g fibre, 5.7 g carbodydrates and contributing to 27 K cal of energy. They also reported presence of various dietary important minerals, pigments and vitamins in substantial quantities. These were in order of calcium 140 mg, phosphorus 30 mg; iron 0.6 mg, riboflavin 0.40 mg, Niacin 0.4 mg and vitamin C 4 mg per 100 g of fresh fruit. The carotene and thiamine were found to be absent in Chayote fruit.

Moudgil (1997) carried out the comparative nutritional evaluation of chayote (*Sechium edule*) and bottle gourd (*Lagenaria siceraria*). The whole fruit of chayote was reported to contain 93.13 % moisture; 4.90 % total ash, 1.50 % crude fat, 6.54 % crude protein, 5.59 % crude fibre, 4.00 mg /100 g ascorbic acid, 1.22 % starch, 8.09 % total sugars. Variability in the amino acid composition and mineral status were also reported. Lysine and methionine were 102.8 and 19.18 mg /100 g protein respectively. The major minerals consisted of 814 mg Calcium, 12.33 mg Iron, 289.67 mg Phosphorus and 3350.00 mg Potassium. Presence of cellulose, hemicellulose, lignin was also reported while lectin was observed to be absent.

2.1.1 Storage Effects in Chayote

Chayote is an important off season vegetable crop with quite high yields. The yield upto 126 t/ha was reported in the Black Sea Littoral of Georiga (Rossinskii *et al.*, 1986). The full potential could be exploited if the effect of storage following the post harvest was resolved . The work done in this respect has been reviewed as under -

Littamann (1973) reported about the development of the new storage techniques for Chayote (*Sechium edule*) fruits. It was suggested that the supplies of this seasonable crop during off peak periods could attain prices five to six times as high as those during peak periods.

Further studies conducted by Littamann *et al.* (1981) revealed that chilling disorders had occurred at temperature upto 12.5^0 C. Three distinct disorders which developed within two weeks of storage were reported which consisted of diffuse browning of the surface at 2.5^0 C; brown pitting of surface at 5 to 7.5^{0}C and internal browning of the parenchyma was observed at 12.5^0 C.

Alvarado *et al.* (1989) evaluated the post-harvest treatments for improving keeping quality in Chayote (*Sechium edule*) fruits. There was no deterioration of quality in the fruits, stored at ambient condition i.e at 19 to 23^0 C and 80 to 85 % relative humidity or at 12 to 14^0 C with 85 - 90% relative humidity. The fruits treated with fungicides and then stored in low density polythene bags (25 fruits per bag) having peduncle, decayed more than those without peduncle on the same treatment. The use of low density polythene bags (25 fruits per bag) at low temperature of 12 to 14^0 C was best for storage in all cases.

2.2 Lungru (*Diplazium esculentum*)

Lungru (*Diplazium esculentum*) sw Athyriaceae is the most commonly known fern which is quite large, erect, stout and with subarborescent rhizome. It grows usually in shady and moist environments. In northern India, lungru starts growing in April / May, reaches to its full bloom in June/ July and dies by September. The young fronds of this fern are used as a popular seasonal vegetable. It occurs from 1200 to 2500 m (M.S.L.) altitude. There are many ferns which are stated as edible, but lungru (*Diplazium esculentum*) was reported to be the most important edible fern used as greens throughout the Malayan and adjacent regions. These were collected from wild plants and marketted (Copeland 1942). Very limited work seems to have been done with respect to its biochemical, nutritional and anti-nutritional status particularly in Palam valley of Himachal Pradesh. The literature available on these aspects in respect of the genus Diplazium has been reviewed as under.

Copeland (1942) reported that *Diplazium esculentum* had large clumps and fronds and each plant bore a number of fronds measuring about 2.0 to 2.5 cm in length.

Bir (1968) found that fronds of *Diplazium latifolium* were about one meter long with stipes 30 to 40 cm long. The rhizome was about 2.5 cm in diameter.

Caldwell (1972) analysed various green leafy vegetables consumed in Malaysia, collected over a period of several months, for ascorbic acid content. A wide variation was found in the ascorbic acid values obtained from different samples of the same vegetable. The average

ascorbic acid content of *Diplazium esculentum* was reported to be 9 mg / 100 g.

Prasad *et al.* (1977) conducted studies on the role of fern *Diplazium esculentum* in the genesis of chronic bovine haematuria in calves. The calves when fed with mature fern, biochemically showed decreased haemoglobin, blood glucose, plasma ascrobic acid, serum thiamine and calcium. A marked increase in blood pyruvic acid and serum phosphatase were also reported, whereas levels of creatinine and phosphorus were not much affected proved fatal finally.

Revina Gureeva (1985) found *Diplazium sibiricum*, as the richest source of ecdysterone (0.3 %) and the levels were highet at the start of growth.

Zanariah *et al.* (1986) evaluated the protein and amino acid composition of Malaysian vegetables by the Technicon Sequential Multisample amino -acid Analyser System. The shoots of *Diplazium esculentum* were found to contain the protein content of 2.5 % .

Mead (1989) listed *Diplazium esculentum* as new host for *Aphetenchoides fragariae* according to the Division of Plant Industry files Florida, USA.

Das (1989) gave description of *Cercospora diplaziicola* from the leaves of *Diplazium esculentum.*

Yasmeen and Saxena (1990) reported that the extracts of the ferns *Adiantum caudatum, Diplazium esculentum* and *Pteris vittata* reduced the growth and germination of *Alternaria brassicicola* and *Aspergillus niger.* The rhizome extracts were found to be more toxic than leaf extracts.

Jones (1991) described botany and the cultural requirements of ornamental fern *Diplazium proliferum.*

Siddiqui and Husain (1991), in the medico botanical survey conducted amongst herbalists and villagers reported *Diplazium esculentum* among the medicinal ferns in Hardoi district of Central Uttar Pradesh. Uses and application methods were also described.

Gupta (1991) conducted preliminary studies on lungru collected in Palam Valley of Kangra district in relation to its nutritional quality and to standardize dehyrdration and product development technology for use in various food preparations. Based on the findings of this study, it was stated that lungru could be an important edible fern to be used for vegetable purpose in this region containing 90.09% moisture, 1.29 % protein, 0.16 % fat, 1.17 % ash, 4.68 % crude fibre and 2.61 % total carbohydrate in fresh edible tissue. The variability in tannins (which was reported to be carcinogenic if given orally or subcutaneously) in shade dried, sun dried , vaccum dehydrated and oven dehydrated lungru samples was observed as 1.28, 1.27, 0.92 and 0.38 % respectively. The study suggested that lungru found in Palam valley of Himachal Pradesh had fibrous value along with some anti nutritional factors but was safe for human consumption.

Yasmeen and Saxena (1992) observed the inhibitory effect of extracts from the leaves of *Diplazium esculentum* and *Microsorum punctatum* on the growth of mycoflora (*Rhizoctonia solani* and *Fusarium oxysporum*) on chickpea seeds.

Taungbodhitham (1995) determined the thiamine content and activity of anti thiamine factor (ATF) in vegetables of Southern Thailand. Among the fourteen types of vegetables commonly consumed in Southern

Thailand, Phak kuad (*Diplazium esculentum*) showed mean of endogenous free form thiamine content to be zero. The activity of heat stable ATF, iuncubated for 3 h (per cent thiamine destroyed /g vegetable) was 100 for the same.

Nakanishi *et al.* (1995) reported the use of Diplazium subsinatum in chinese folk medicine for its diuretic and other properties. Two new hopane - triterpene glycosides from it were isolated.

Gould and Lee (1996) studied blue leaf iridescence in *Diplazium tomentosum*, one among the four Malaysian understorey plants. The ultra structural basis for this in *D. tomentosum* was multiple layers of cellulose microfibrils in the upper most cell walls of the adaxial epidermis.

In view of the limited information about the biochemical and nutritional composition of lungru (*Diplazium esculentum*), literature on the same aspect was reviewed with some related ferns, which is as under-

Hendrick (1919) reported 69.79 to 79. 59 per cent moisture in bracken (*Pteridium equilinum*) with an average of 73.89 per cent. Values for ether extract, ash, crude fibre and carbohydrates in original matter and dry matter were found to be 0.30 and 1.12 , 2.38 and 9.11; 6.36 and 24.30, 15.82 and 60.53 per cent respectively.

Hendrick (1921) recorded moisture and crude fibre in rhizome and fronds of bracken from two different location. Rhizome showed 71.10 and 70.19 per cent moisture; 21.59 and 20.68 per cent crude fibre in two locations; while the fronds contained 19.20 to 19.39 and 14.42 to 90.23 per cent moisture and 3.17 to 44.34 and 15.86 to 42.88 per cent crude fibre. The two different samples were reported to show 6.92 per cent and 8.65 per cent

ash; 66.49 and 56.51 per cent soluble carbodydrates and an average value of 0.82 per cent for ether extract.

Ferguson and Armitage (1944) noted wide range of crude and true protein for bracken from two different localities being 3.87 to 21.42 per cent and 3.0 to 16.06 per cent for crude protein; 3.71 to 18.11 per cent and 2.84 to 14.59 per cent for true protein respectively. Ether extract, ash and starch content for whole plant, leaves and stem were analysed. The whole plant contained 0.93 to 2.25 per cent ether extract, 4.35 to 8.23 per cent ash and 2.31 to 3.02 per cent starch while the leaves and stem were reported to have 1.10 to 2.84 and 0.22 to 1.38 per cent ether extract , 4.98 to 8.37 and 3.21 to 7.09 per cent ash, 2.80 to 3.15 and 1.88 to 2.81 per cent starch content respectively. The values for total and reducing sugars in two different samples ranged from 0.88 to 10.09 per cent and 0.79 to 9.53 per cent respectively. They determined values for specific rotation, saccharic acid, glucose, mucic acid, ketose and osazone from sample solution having 11.30 per cent total sugars and 10.30 per cent reducing sugars. They also concluded that the reducing sugars in the extract of bracken consisted almost entirely of a mixture of glucose and fructose. Non reducing sugars amounted to less than 10.00 per cent of total sugars present.

Ferguson and Naeve (1944) found 12.78 to 14.96 per cent crude protein and 11.41 to 13.48 per cent true protein in dry matter of bracken silages. They reported 28.81 to 31.42 per cent crude fibre in bracken.

Shearer (1945) suggested that a decomposition product of catechol tannins which occur in bracken may be the factor responsible for bracken poisoning. In support, he had reported figures for tannin content ranging from 0.44 to 7.00 per cent at various stages of bracken maturity.

Moon and Pal (1949) determined the range of various biochemical constituents in leaf and stem of bracken such as 6.29 to 17.39 and 1.03 to 8.69 per cent crude protein, 1.05 to 2.75 and 0.53 to 1.21 per cent ether extract and 16.48 to 33.21 and 49.36 to 60.02 per cent crude fibre in leaf and stem respectively. The ash content was reported to range from 5.10 to 6.35 per cent in different parts of bracken. The range of tannins content from 1.47 to 4.65 per cent in leaf and 0.50 to 1.00 per cent in stem of bracken was also reported.

Hou (1950) reported 3.07 to 12.20 per cent ash content and range of different minerals in dry matter of various ferns and fern allies. The amount of aluminium, manganese, phosphorus, calcium and potassium ranged from 0.0047 to 0.7925 per cent; 0.0032 to 0.451 per cent, 0.0903 to 0.3821 per cent, 0.0503 to 1.8615 per cent and 0.5903 to 3.3097 per cent resepectively.

Smith and Agiza (1957) analysed bracken (*Pteris aquilina*) to find 1.70 to 4.30 per cent nitrogen in dry matter and good composition of amino acids but the value for tryptophan was found to be negligible.

Bianki (1959) stated that the amount of carotenoids in ferns increases with maturation of reproductive organs.

Kirby (1960) brought the fact in the light that condensed tannins evoked sarcomas.

Rangaswamy and Iyer (1967) identified a carotene mono-epoxide in *Adiantum venustum*.

Berti and Bottari (1968) concluded that the free amino acid fraction of ferns was frequently characterised by presence of unusual amino acids which included homoserine, 3 -4- dihydroxy - glutamic acid, 4-

methyl glutamine and 4- hydroxy, 4-methyl glutamic acid which constituted 90 per cent of free amino acids of *Adiantum pedatum*.

Rakhimov and Abdullaen (1971) attempted to determine carotene level in *Salvinia natans*.

Bottari *et al.* (1972) obtained waxes, fatty acids, hydrocarbons, alcohols, sterols in various species of ferns.

Bremmer and Wilkie (1972) while working on hemicelluloses of brackens, isolated galacto glucomannan from bracken which on hydrolysis yielded mannose, glucose and galactose in molar ratios of 60:15:1. The rhizomes of bracken have been reported to contain 21.3, 35.00 and even 45.00 per cent starch.

Wang *et al.* (1976) demonstrated that condensed tannins evoked urinary bladder carcinogenecity in mice.

Gemmrich (1977) reported 5.6 per cent lipids of total weight of dry spores of *Anemia Phyllitidis*.

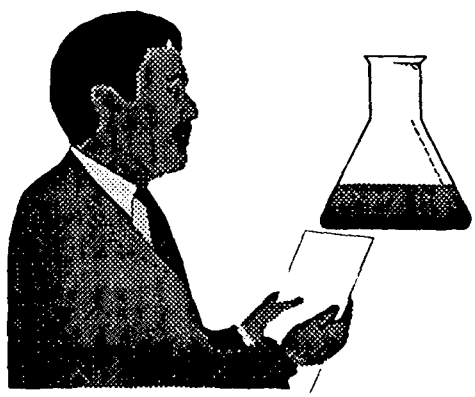
Brahman and Saxena (1978) found 3.30 to 5.60 per cent tannins in frond and rhizome of *Acrostichum aureum* respectively.

Czeczuga (1980) reported the presence of 27 carotenoids from pteridophyta. In fern species, carotenoids present were β cryoto xanthum, lutein expoxide, violoxanthin and rhodoxanthin.

Pamucku *et al.* (1980) isolated 2.45 g pure tannin and 100 g tannin free fraction from one kg of bracken fern.

Randi and Felippe (1989) reported 43.00 per cent lipids in *Cyathea delgaddi*. The major fatty acids were oleic acid followed by palmitic and linolenic acid.

MATERIALS AND METHODS



CHAPTER III

MATERIALS AND METHODS

The present investigation was conducted in the department of Chemistry and Biochemistry, College of Basic Sciences, Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 1997 -98 to find out the biochemical and nutritional status of Chayote (*Sechium edule*) and Lungru (*Diplazium esculentum*) grown in mid hills of Himalayan terrain. Besides, some anti-nutritional factors were also evaluated in Lungru. Effect of storage on the variations of biochemical composition of Chayote fruits was studied at length on retention of dietary nutrients.

The chapter has been dealt in following subheads -

3.1 Procurement of materials

3.2 Preparation of samples

3.3 Biochemical analyses

3.4 Statistical analysis

3.1 Procurement of Materials

Chayote (*Sechium edule*) fruit samples were procured from the local growers /kitchen gardens around Palampur (1300 -1400 m M. S. L.) at ten days interval during October-November, 1997.

Freshly harvested lungru (*Diplazium esculentum*) fronds were procured from the local market having supply from Bandla in Palam Valley during its growing season, that is, from May to August, 1997 at an interval

of one month. The collections for the study at various intervals were considered as various test samples as depicted in table 1 and 2.

Table - 1: Collection dates of Chayote (*Sechium edule*)

Test Samples	Dates of Harvest
T_1	8.10.97
T_2	18.10.97
T_3	28.10.97
T_4	8.11.97
T_5	19.11.97

Test Samples T_1 to T_5 represent dates of harvest of Chayote fruits (i.e. at interval of 10 days during October - November, 97

Table 2 Collection Dates of Lungru (*Diplazium esculentum*)

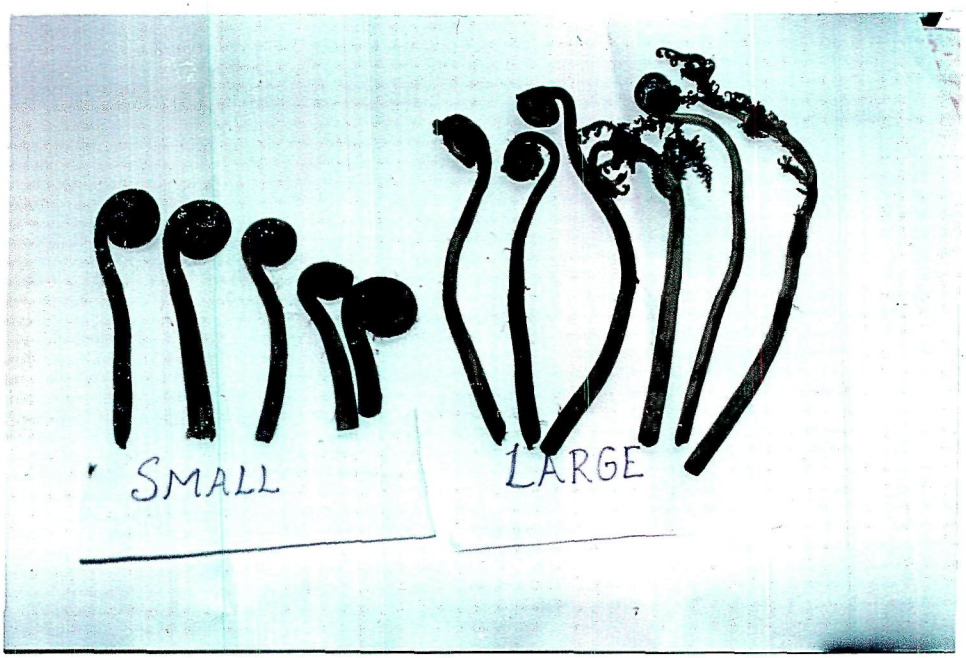
Test Samples	Dates of Harvest
T_1, T_5	19.5. 97
T_2, T_6	16.6.97
T_3, T_7	16.7.97
T_4, T_8	16.8.97

PLATE - 2 (A) : SHOWING SAMPLES OF CHAYOTE FRUITS

PLATE -2 (B) : SHOWING SAMPLES OF FERN LUNGRU

PLATE - 2

(A)



(B)

Test samples T_1 to T_4 and T_5 to T_8 represent dates of picking of lungru (i.e. at interval of one month) for large and small sized, respectively.

3.2 Preparation of Samples

Freshly harvested Chayote fruits were carefully sorted out to remove the damaged or rotten fruits. After proper washings and clearing with clean cloth, it was left for further drying.

Lungru samples were separated into two groups (after removal /discard of rotten ones), the one having small, curled fronds and the other group having large uncurled ones. The hairs were removed by dry cloth and washed to remove the stuck - hairs and soil. The fronds were cleaned with cloth and allowed to dry on the table in air. Chayote and lungru were cut to small pieces, weighed and dried in an electric oven at $60^0 \pm 5\ ^0C$. The dried samples were again weighed and ground in a willy mill to pass through 40 mesh sieve and stored in air tight plastic bags. These were properly labelled and kept at room temperature for further analysis. However, ascorbic acid,carotenoids, polyphenol oxidase and total free amino acids in chayote and lungru were estimated on fresh weight basis in respective samples.

3.3 Biochemical Analyses

Proximate principles e.g. moisture, crude protein, ash, crude fibre, crude fat and carbohydrates were estimated by following standard procedures.

3.3.1 Moisture (AOAC, 1970)

Moisture content in edible parts of the Lungru and Chayote was determined following the oven drying method. 5 g of each sample was taken in a previously weighed, dried aluminium cups. These cups were kept in a hot air oven at 100 ^{0} C for 8 hrs. The aluminium cups were taken out from oven and kept in dessicator for cooling for 30 minutes, for attaining a constant weight. After cooling, samples were weighed with aluminium cups. The loss in weight represented the moisture content of the sample:

Weight of empty aluminium cup = W_1 (g)

Weight of sample = W_2 (g)

Weight of aluminium cup + sample before drying = X (g)

Weight of aluminium cup + sample after drying = Y (g)

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

$$= \frac{(X - W_1) - (Y - W_1)}{W_2} \times 100$$

3.3.2 Crude Protein (AOAC, 1970)

Semi- micro - Kjeldahl method was adopted to determine per centage of nitrogen content and a conversion factor of 6.25 was used to calculate crude protein content. Pre-weighed dried sample (0.5 g) was digested with concentrated Sulphuric acid (20 ml) and digestion mixture (5 g) containing potassium sulphate and copper sulphate in the ratio of 10: 1 in a Kjeldahl digestion flask till the contents of the flask becacme free from organic carbon. The contents were cooled, diluted with small amount of distilled water and transferred into a 50 ml volumetric flask. The volume

was made upto the mark with addition of distilled water. 5 ml of aliquot was taken and transferred in a distillation assembly followed by addition of 10 ml of 40% Sodium hydroxide. On distillation, ammonia liberated was collected in a 250 ml conical flask containing 10 ml of N/10 HCl to which methyl red indicator (2 to 3 drops) was priorly added. It was then titrated against N/10 NaOH solution. One blank containing concentrated sulphuric acid and digestion mixture was also run alongwith the experimental samples.

Calculations:

Weight of the sample taken = W (g)

Sample titre value = a

Blank titre value = b

Volume made up = 50 ml

Aliquot taken = 5 ml

$$\text{Per cent Nitrogen} = \frac{(\text{Sample titre} - \text{blank titre}) \times 0.0014 \times \text{volume of digested sample}}{\text{Aliquot of digest taken} \times \text{Weight of the sample taken}}$$

Crude protein (%) = Per cent Nitrogen X 6.25

3.3.3 Ash Content (AOAC, 1970)

The weighed amount of dried sample (2 g) was taken and put in previously dried and weighed silica crucibles. The samples were first incinerated over an electric hot plate followed by ashing in muffle furnace at a temperature of 550 ± 25^0 C for 6 hours (until a pale white residue

was obtained). These ashed samples were taken out from the muffle furnace and were kept in the dessicator for two hours for cooling. After cooling, the samples were weighed again and per cent ash content was calculated as follows:

Weight of empty crucible = W (g)

Weight of crucible + sample before ashing = W_1 (g)

Weight of crucible + sample after ashing = W_2 (g)

$$\% \text{ ash content} = \frac{\text{Weight after ashing (g)}}{\text{Weight of sample (g)}} \times 100$$

$$= \frac{W_2 - W}{W_1 - W} \times 100$$

3.3.4 Crude Fibre (AOAC, 1970)

Moisture and fat free sample (2 g) was taken in 500 ml beaker and 200 ml of 1.25 % H_2SO_4 was added. It was boiled for half an hour, cooled and filtered through filter paper. Residue was washed several times with hot distilled water and transferred to a beaker after checking for being acid free. 200 ml of NaOH solution was added. The contents were boiled for half an hour, cooled and filtered through filter paper and washed with hot distilled water for a number of times till it became free from alkali. Residue was transferred to a weighed crucible and dried to constant weight at 100^0 C in a hot air oven. The residue was then ashed in muffle furnace at 550 ± 25^0 C for 3 hrs, cooled and weighed again. The loss in weight was recorded.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of residue before ignition - weight of ash after ignition}}{\text{Weight of sample taken (g)}} \times 100$$

3.3.5 Crude fat content (AOAC, 1970)

Fat content was determined by soxhlet extraction method. 5 g of each dried sample was extracted with petroleum ether (B.P. 40 - 60° C) in soxhlet apparatus for 18 hours. The extract containing fat and petroleum ether was evaporated over boiling water bath and dried in an oven at low temperature and weighed. The difference in weight of the soxhlet receiving flask represented ether extract (fat content) present in the sample. Crude fat content was calculated as follows:

Weight of sample = W (g)

Weight of empty flask = W_1 (g)

Weight of flask + fat/ether extract content = W_2 (g)

$$\text{Per cent crude fat content} = \frac{\text{Amount of ether extract}}{\text{Weight of sample (g)}} \times 100$$

$$= \frac{W2 - W1}{W} \times 100$$

3.3.6 Carbohydrates

Carbohydrates present in dried samples were computed by using the following expression :

Carbohydrates (%) = 100 - (% crude protein + % crude fat + % ash + % crude fibre)

3.3.7 Energy

Total dietary energy available from dried samples of fruit / fern was computed using the following expression:

Energy (Kcal /100 g) = Protein (%) X 4 + Fat (%) X 9 + Carbohydrates (%) X 4

3.3.8 Total Sugars and Starch

Total sugars was extracted in alcohol (80%) by following the procedure of AOAC(1965) and estimated thereof in the test extract by the phenol sulphuric acid method of Dubois *et al.* (1965).

Extraction

One gram of the ground sample was taken in a 100 ml conical flask. The sugars were extracted twice with 50 ml of 80% ethanol followed by complete extraction four times with 70% ethanol by refluxing on a boiling water bath for 30 min each time. The contents in the flasks were stirred occasionally. The combined alcoholic extract of each sample obtained was concentrated to an aquous syrup on a boiling water bath. The last traces of concentrated sugar solution was quantitatively transferred to a 100 ml volumetric flask and the volume raised to 98 ml with distilled water. 1.0 ml of saturated solution of lead acetate was added to remove proteins and the volume was made to 100 ml. The contents were filtered through whatman No.1 filter paper and excess of lead ions were removed from the filtrate by addition of sodium oxalate crystals followed by filteration. The clear extract so obtained was then used for the estimation of free sugars.

The sugar free residue obtained was analysed for the starch content.

3.3.8.1 Estimation of Total Sugars (Dubois *et al.*, 1956)

The total sugars were estimated by the phenol Sulphuric acid method :

Reagents

1. 95.5 % Sulphuric acid

2. 5% phenol in glass distilled water

For the determination of total sugars, 0.2 ml of the test extract was taken in each test tube and the volume made to 1 ml with distilled water. After adding 1 ml of 5% phenol to each tube, the tubes were then placed in ice cold water and 5 ml of 95.5 % sulphuric acid was added swiftly, the stream of acid being directed against the liquid surface rather than against the side of the test tube to obtain good mixing. The contents were mixed and equilibrated to room temperature. The absorbance of pink colour developed was read at 490 nm on Spectronic-20. The concentration of total sugars from the test extracts was then calculated from the standard curve prepared from glucose (0.1 mg/ml).

3.3.8.2 Starch (Dubois *et al.*,1956)

The total sugar free residue was dried and the starch was extracted with 52% perchloric acid at room temperature by using phenol sulphuric acid reagent for the determination of starch.

5 ml of water was added to each tube containing 0.2 g of sugar free residue followed by 6.5 ml of 52% perchloric acid (prepared by adding 270 ml of 72% perchloric acid to 100 ml of distilled water). The contents were stirred continuously for 5 minutes with a stirrer and then occasionally for next 15 min. 20 ml of water was added to each tube and the contents were centrifuged for 15 minutes at 3000 rpm. The supernatent was poured in a 100 ml volumetric flask and the extraction was repeated as above extending the time to 30 minutes this time. The contents of the tubes were washed in their respective flasks and the volume of each flask was

made to 100 ml with distilled water and the content was filtered through whatman No.1 filter paper discarding the first 5 ml of the filtrate.

An aliquot of the filtrate in a total volume of 1 ml was taken in test tubes and in each of these tubes 1 ml of 5% phenol was added with vigorous shaking. The contents were equilibrated to room temperature and the intensity of colour developed was recorded at 490 nm on Spectronic -20. The concentration of glucose released from starch was calculated from the standard curve prepared from glucose (0.1 mg /ml) . The concentration of starch was then calculated by multiplying the glucose concentration values with 0.9. This was done because 0.9 g of starch yields approximately 1 g of glucose on hydrolysis.

3.3.9 Ascorbic Acid (AOAC, 1970)

100 g of fresh fruit/fern of test sample was brought to laboratory after picking and was ground with 100 ml of 2% oxalic acid as extraction medium in order to get a slurry. Weight of slurry was recorded . 20 g of slurry was taken in 100 ml volumetric flask and volume was made to 100 ml by addition of 1 % oxalic acid solution. The content of the flask was then filtered. 5 ml of flitrate was pipetted and titrated against dye solution, 2,6 dichloroindophenol prepared by taking 52 mg of dye in 200 ml volumetric flask, adding distilled water, then shaking it in water bath till it dissolved, cooled and added 42 mg of $NaHCO_3$ and made final volume upto the mark. At the same time, a standard reading was recorded by taking 5 ml of prepared standard ascorbic acid solution (100 mg of ascorbic acid in 500 ml 1% oxalic acid). The results were prepared /expressed as mg of ascorbic acid per 100 g of fresh material.

3.3.10 Total Carotenoids (Jayraman, 1981)

500 mg of fresh sample was homogenised in 15 ml of 80 per cent acetone and centrifuged at 4000 rpm for 20 minutes. The residue was re-extracted twice with 5 ml of 80 per cent acetone and centrifuged. The supernatents were pooled and volume was made to 25 ml with 80% acetone. O.D. was measured at 480 nm (using blue filter, 645 and 663 nm (using red filter) on Spectronic-20. 80 per cent acetone of the same volume was used as blank. The amount of carotenoids was calculated as under:

$$\text{Total carotenoids (mg/100 g)} = \frac{(0.D.\ 480) - (0.114)\ (0.D.663) - (0.638)\ (0.\ D.645)\ \text{Volume} \times 100}{1000 \times \text{Wt. of Sample}}$$

3.3.10 Estimation of Calcium, Iron and Sulphur

One gram ground sample was taken in a 150 ml conical flask. To this 25 -30 ml of diacid mixture (HNO_3 - H_2SO_4 in 5:1 v/v) was added and allowed to stand as such for a night. Digestion was performed the next day by heating till clear white precipitate settled down at the bottom. The crystals were dissolved by diluting in double distilled water. The contents were filtered through whatman No.42 filter paper. The filtrate was made to 50 ml with double distilled water and used for determination of various elements such as calcium, and iron using Perkin Elmer atomic absorption spectrophotometer.

3.3.11.1 Total Sulphur (Chesnin and Yein, 1951)

Preparation of mineral solution:

Weighed 5 g of sample in a crucible and ignited in a muffle furnace at 800 ^{0} C for 4 -5 hours. Ash was moistened with small amount of glass distilled water (0.5 to 1.0 ml) and 5 ml of conc. HCl was added. The

mixture was evaporated to dryness on a boiling water bath. Another 5 ml of conc. HCl was added and solution was evaporated. 4 ml of conc. HCl and few drops of water were added and the solution was warmed over boiling water bath and filtered into 250 ml volumetric flask using whatman No.40 filter paper. After cooling, made the volume upto the mark.

Total sulphur was determined by the method given by Chesnin and Yein (1951).

Prepared standard potassium sulphate solution (K_2SO_4. 1.743 g in 100 ml distilled water. Took 10 ml of this solution, and dissolved in 10 ml of distilled water). Took 100 ml of this standard solution . Added 40 ml of water and 0.5 g $BaCl_2$. Stirred with glass rod for 4 to 5 mintues. Observed O.D. at 420 nm. For sample, 10 ml of ash solution is mixed with 40 ml of distilled water, added 5 g of BaCl2 crystals. Allowed to stand for 5 minutes. Stirred with glass rod. Observed the O.D. at 420 nm. Blank contained 50 ml D.W. + 5 g $BaCl_2$. and same procedure was followed as above. The calculation was done as under:

$$\text{Total sulphur (mg/100 g)} = \frac{\text{mg of sulphur in aliquot x Total volume of ash sample}}{\text{Wt of ash solution taken for estimation X Wt. of sample taken for ashing}} \text{ X } 100$$

3.3.12 Amino acids

Hydrolysis (Methods of Enzymology, 1963)

0.5 g of the dried, powdered sample was hydrolysed with 20 ml of 6N HCl in a sealed tube for 24 hours at 110 ^{0} C. 2 g of activated charcoal was added to the hydrolysate, mixed and filtered through Whatman filter paper No.1 The filterate was collected in 25 ml volumetric

flask and volume was made to 25 ml with distilled water. This hydrolysate was used for colorimetric estimation of methionine.

3.3. 12.1. Methionine(Horn *et al.*,1946)

10 ml of hydrolysate was transferred to 100 ml beaker with the addition of 4 ml of distilled water . 2 ml of 5 N NaOH was added to the content of the beaker. Further 0.1 ml of Sodium Nitroprusside (1%) and 2 ml glycine solution (3%) was added. To this 4 ml of orthophosphoric acid was added to develop the colour. The intensity of the colour alongwith the blank was measured in Spectronic 20 at 520 nm (using blue filter). The calculation was done on the basis of standard curve prepared for methionine (standard solution was prepared by dissolving 50 mg methionine /500 ml distilled water i.e. 0.1 mg methionine /ml)

3.3.12.2 Tryptophan (Spies and Chambers, 1949)

0.2 g of homogenous sample was transferred into 100 ml conical flask. 10 ml of 19 N H_2SO_4 was added. The content of the conical flask was kept for 12 hours in dark. After expiry of this period. 1 g of activated charcoal was added and filtered through G1 glass sintered crucible using suction. The decolourised filtrate was collected and 1 ml of distilled water, 1 ml of p-dimethylamino benzaldehyde (30 mg dissolved in 100 ml of 2N H_2SO_4) and 0.1 ml of Sodium nitrite (0.045 % in water) was added. It was kept for 30 minutes for colour development. Then intensity of colour was measured at 620 nm (using red filter) on Spectronic 20. The calculation was done on the basis of standard curve prepared for tryptophan (50 mg tryptophan /100 ml hot distilled water i.e. 0.5 mg tryptophan /ml).

3.3.13 Antinutritional Factors

3.3.13.1 Estimation of Nitrate (Tandon, 1993)

Water soluble nitrate - nitrogen in oven dried samples of lungru was determined by the method given by Tandon, 1993.

Reagents:

1. Phenol disulphonic acid: 25 g pure phenol (White crystal) was taken in a conical flask. To this 225 ml of conc. H_2SO_4 was added. The beaker was covered with watch glass and kept on water bath for 6 hours.
2. Ammonia solution (1:1) with distilled water
3. Potassium nitrate (Analytical grade)

Extraction: 500 mg of dried sample was taken in a 100 ml conical flask. To it 50 ml of deinonised water was added and placed it on a horizontal shaker for 30 minutes . Added a pinch of activated charcoal and filtered the content of flask through Whatman No.1 filter paper.

Preparation of Standard Curve for NO_3^-

1. A stock solution containing 100 ppm NO_3^- N was prepared by dissolving 0.7215 g analytical grade potassium nitrate and volume was made upto 1000 ml.
2. It was diltued 10 fold to give 10 ppm NO_3^- N solution.
3. Aliquots of 2, 5, 10, 15, 20 and 25 ml were taken in the china dishes, evaporated to dryness on water bath and cooled at room temperature.
4. 3 ml of phenol disulphonic acid was added and allowed to react with residue by rotating the china dishes initially and then stirred with glass rod.

5. After 20 minutes, 10 ml deionised water was added and stirred with a glass rod.
6. On cooling, the contents were washed down into a 100 ml volumetric flask.
7. The solution was stirred and 1:1 ammonia and distilled water solution added and volume was made up with deionised water upto 100 ml.
8. The intensity of yellow colour was measured on Spectronic 20 at 420 nm wave length.

Determination of NO_3^- in sample:

2 ml aliquot from water extract was taken in 100 ml beaker and evaporated to dryness. The colour was developed by the same procedure described above for standard curve preparation.

3.3. 13.2 Oxalates:

The oxalates were determined by method described by Abaza (1968).

Reagents:

6 N HCl

Methyl Red Indicator

Concentrated Ammonia

5% $CaCl_2$

Distilled water and sulphuric acid solution (1 : 4)

N/20 $KMnO_4$ solution

Procedure: 2 g of sample was weighed in a 250 ml volumetric flask. To this 190 ml of distilled water and 10 ml of 6 N HCl was added and digested for one hour on boiling water bath. It was cooled and volume was made upto

the point (25 ml) and the supernatent was filtered. To 50 ml filtrate, 20 ml of 6 N HCl was added and evaporated to half of its volume and then filtered. Precipitates were washed several times to make the volume 125 ml. To the filtrate 3-4 drops of methyl red indicator followed by concentrated ammonia was added till the solution turned faint yellow. It was heated at 90 - 100 ^{0}C, cooled and filtered. Then the filtrate was boiled and 10 ml of 5% $CaCl_2$ was added with constant stirring. It was then allowed to stand over night. It was filtered through whatman filter paper No.41 and precipitates were washed several times with hot water to make it free from calcium ions. Precipitates were transferred to original beaker with distilled water and sulphuric acid solution (1:4) was added to it till the precipates were completely dissolved. The filter paper was added to the contents. The contents were warmed (70 ^{0} C) and titrated with N/20 $KMnO_4$ to the near end point.

Calculation:

$$\text{Oxalate (g/100 g)} = \frac{\text{N/20 } KMnO_4 \text{ used (ml) X 0.00225 X volume made}}{\text{Aliquot X Weight of sample}} \text{ X 100}$$

3.3.13. 3 Condensed Tannins (Broadhurst and Jones, 1978)

Tannin content was determined by using vanillin – HCl reagent. For extraction of tannin, dry sample was ground to pass through 60 mesh sieve. To 60 mg of ground material, 4 ml of 50 per cent aquous methanol was added, refluxed at 100^0 C for 10 minutes and filtered, volume was made to 10 ml with 50 per cent aquous methanol.

Catechin was taken as standard and was prepared by dissolving 25 mg of catechin in 100 ml of 50% aquous methanol. For preparing the standard curve 100 μ -l, 200 μ -l, 300 μ -l and 400 μ -l of catechin standard was taken in test tubes and volume was made to 500 μ-l with 50% methanol. For blank 500 μ l of 50% methanol was taken. To each of the test tube, 3 μ-l vanillin (4% vanillin in methanol) and 1.5 ml conc. HCl was added. For the unknown solutions in which tannins were to be determined, 0.5 ml of sample was taken and same quantity of vanillin HCl was added. All the test -tubes were kept for 15 minutes at 20^0 C before taking reading at 500 nm.

For sample analysis, 0.5 g of sample was taken and boiled with 100 ml of distilled water for one hour and made final volume to 100 ml. Centrifuged at 3000 rpm for 5 minutes. After centrifuge, 5 ml of supernatent was used for colour development.

$$\text{Calculation} = \frac{0.1 \times O.D. \times 50 \times 100}{.1111 \times 5 \times 0.5} = \frac{500 \times O.D.}{0.285}$$

3.3. 13. 4 Polyphenol Oxidase (Farkas and Kirlay, 1962)

Fresh samples were analysed for the polyphenol oxidase assay by the method decribed by Farkas and Kirlay (1962)

Reagents

1. 0.2 M Acetate Buffer pH 4.5, prepared by mixing two stock solutions (28 ml of A and 17ml of B and final volume made to 100 ml with D. W.)

   (A) 0.2 M acetic acid, prepared by dissolving 11.55 ml of acetic acid /litre of stock solution.

(B)0.2 M Sodium acetate was prepared by dissolving 27.22 g of $C_2 H_3 O_2 Na_3 H_2O$ in 1 litre.

1) 0.2 M Phosphate Buffer pH 6.0 prepared by mixing two stock solutions (12.3 ml of stock solution A mixed with 87.7 ml of stock solution B and the final volume raised upto 200 ml with distilled water).

(B) 0.2 M $Na_2HPO_4 H_2O$, prepared by dissolving 35.61 g / l.

(C) 0.2 M Na H_2PO_4. H_2O, prepared by dissolving 31.21 g /l.

1) Pryogallol solution (1%).

Procedure: 0.5 g of fresh sample of lungru was homogenised with 10 ml of 0.2 M acetate buffer (pH 4.5). It was then centrifuged at 3000 rpm for 5 mintues. Supernatent was collected and residue was discarded . 2 ml of enzyme extract, 2 ml of 0.2 M phosphate buffer (pH 6.0) and 0.5 ml of pyrogallol solution (1%) were added. The enzyme activity was recorded at the interval of 30s for 3 min at 410 nm using blue filter on Spectronic - 20. The enzyme activity was calculated as unit /min. /g protein.

One unit of polyphenol oxidase activity was defined as the amount of enzyme that caused an increase in /\ 410nm by 0.01 in one minute at 30^0 C.

3.3.14 Effect of Storage on Biochemical Constituents of Chayote

Freshly harvested fruits of Chayote were kept for storage for one month and analysed for retention nutrients on weekly interval. These were kept in ordinary sterile polythene bags and after the expiry of time, the same were powdered (as necessity) for the analysis of proximate composition. Total sugar, starch, ascorbic acid were determined as under

3.3.8 and 3.3.9 respectively. Total free amino acids were determined by the following method of Jayaraman (1981).

3.3. 14.1 Total Free Amino Acids (Jayaraman, 1981)

Total free amino acids in stored undried samples was estimated by method given by Jayraman (1981).

Reagents: 1) Ninhydrin solution: prerpared by dissolving 2 g of ninhydrin in 25 ml of methyl cellosolve (or acetone or water). Add to this solution 25 ml of 0.2 M acetate bfufer (pH 5.5). Store in brown bottle to protect from light.

2) 80% Ethanol in water

Extraction: The samples were powdered in a pestle and mortar into pulp and then extracted with 80% (ethanol in water) solvent . Heated the mixture to 70 - 80 ^{0} C during extraction . The pooled extracts were centrifuged and clear supernatent was concentrated .

Procedure: 1 ml of aliquot was taken and the final volume of 4 ml was made with distilled water. Added 1 ml of ninhydrin reagent and mixed well. The tubes were kept in boiling water bath for 15 mintues. These were cooled and 1 ml of 50% ethanol was added to the tubes . The pink colour developed was measured in a colorimeter at 550 nm (with green filter).

3.3. 15 Rating of various treatments (interval of harvest) for nutritive excellence

Apart from superiority of treatments for specific parameter, the rating was done selecting desirable traits, so as to find the best treatment showing highest nutritive value. In chayote, rating was done for parameters viz. Protein, crude fibre, ash, carbohydrates and calcium. While for lungru,

protein, crude fibre, carbohydrates, ascorbic acid, carotenoids, calcium, iron, sulphur and anti nutrients - nitrates and oxalates were taken for evaluation. Treatments were picked up in descending order for nutrients and in ascending order for anti nutrients.

3.4 Statistical Analysis

Data was analysed statistically by using analysis of variance (Panse and Sukhatme, 1984) as given in Appendix.

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation entitled "Biochemical and Nutritional Evaluation of Chayote (*Sechium edule*) and Lungru (*Diplazium esculentum*) of Palam Valley of Himachal Pradesh" was undertaken to evaluate biochemical and nutritional status of these under utilized vegetables at different intervals of harvest. In addition, proximate composition anti- nutritional factors of lungru viz., the level of nitrates, oxalates, condensed tannins along with poly-phenol oxidase activity were determined. Further in order to assess the keeping quality, freshly harvested chayote fruits were stored at ambient temperature and effect of storage on quality attributes was also studied so as to find out their optimum stage of nutrient retention after harvest for exploring the possibility of maximum produce utilization especially during lean season. Attempts have been made to discuss the fidnings of the experiments in the light of literature cited and work done earlier by the other scientists.

The results of the present study are presented and discussed as under

4.1 Variability in the biochemical constituents of chayote at different Harvest intervals

4.1.1 Moisture content in fresh chayote fruits

The status of moisture content roughly indicates accumulation of different nutrients in food and fibre in produces among most of the vegetable crops. It is an important criterion contributing towards consumer's

acceptability of the harvested crop. Considering this, the variability in the moisture content at different stages of harvest was recorded, pertinent data has been presented in table- 3.

The perusal of the data depicted in table - 3 showed that the variations in moisture at different intervals of harvest was observed from 91.48 to 93.80 per cent. It is evident that the moisture content did not vary significantly over a period of one month after harvest.

Variation in moisture content has been reported earlier to range from 90.8 to 94.71 per cent (Engels, 1983, Flick *et al.*,1977, Gopalan *et al.*, 1989). The results obtained in this study on this aspect are in conformity with the findings of these investigators.

4.1.2 Protein Content

Dietary proteins are of vital importance to living organism as these constitute an improtant component of tissues as well as the body fluids including blood. As such, status of protein content in chayote at different stages of harvest was evaluated and data on this aspect are depicted in table -3. It is apparent that protein content varied from 5.25 to 7.66 per cent with highest value exhibited at T_4 stage of harvest, while the lowest value was observed at T_5 stage. Protein content noticed in T_1 was at par with T_4, where as values in respect of T_2 and T_3 were at par with the lowest value at T_5.

Nevertheless, wide variability in the protein content has been reported from 0.7 to 0.9 per cent (on f.w.b.) by other investigators on this crop.

4.1.3 Fat content

Dietary fat is considered a concentrated source of energy and it supplied per unit weight more than twice the energy furnished by either protein or carbohydrates. It also imparts palatability to a diet and retards stomach emptying time. Presence of fat in the diet is important for the absorption of fat soluble vitamins and also to meet the dietary requirements of essential fatty acids (Gopalan *et al.*, 1989).

As such performance of chayote fruits with regard to fat content was evaluated at different harvest intervals. Variations in the fat content were significant with highest value (1.85 per cent) at T_3 and lowest (1.00 per cent) at T_5 stage of harvesting. T_1 and T_2 stages of fruit harvest showed at par values with T_3 while at T_4 stage fat content was at par with T_5 stage of harvest of fruits. Wide variations in crude fat content have been reported by other workers as well ranging from 0.3 to 0.62 per cent (on dry weight basis) (Nath, 1971 ; Flick *et al.*,1977) and the values obtained in the present investigation are in accordance with the findings of these investigators.

4.1.4 Ash Content

In fact the ash content reflects the sum total of minerals present in any food crop and forms one of the important parameters of assessing nutritional quality traits of food crops.

Data presented in table 3 revealed that ash content at various dates of picking varied from 5.24 to 6.05 per cent. It is evident that ash content in the fruits picked in mid November i.e. T_5 stage showed maximum

Table 3 Variability in proximate composition of chayote *(Sechium edule)* fruit at different intervals of harvest (values on % dry weight basis except moisture)

Test Samples	Moisture (%)	Protein (%)	Crude Fat(%)	Ash (%)	Crude Fibre (%)	Carbohydrates (%)	Energy (Kcal)
T_1	93.80	6.41	1.65	5.28	6.49	80.17	22
T_2	93.29	5.32	1.40	5.69	6.63	80.61	24
T_3	92.36	5.50	1.85	5.24	7.10	80.31	28
T_4	92.12	7.66	1.05	5.35	5.60	80.31	28
T_5	91.48	5.25	1.00	6.05	5.50	82.20	31
SEm ±	0.80	0.44	0.18	0.30	0.30	0.31	
CD at 5%	N.S.	1.46	0.58	N.S.	0.97	0.91	

value with marginal variation at other stages of picking of fruits. The overall status of ash content observed in this study is more or less in agreement with those reported by other workers(Nath, 1971; Flick *et al.* 1977; Rao *et al.*,1990; Gopalan *et al.*,1995)

4.1.5 Crude Fibre

The fibre which remains insoluble on boiling plant material with dilute alkali and dilute acid represents crude fibre. It includes highly insoluble cell wall constituents - cellulose, lignin and hemicellulose and is reported as dietary fibre useful in human nutrition (Gopalan *et al.*, 1989).

The data for crude fibre content revealed significant variation at different intervals of harvest of fruits from 7.10 (T_3) to 5.50 (T_5). The values for T_1, and T_2 stages were at par with T_3 followed by T_4 and T_5 in that order. Thus, study reflected that fibre content was maximum in the fruits picked up in late October.

The observations with regards to crude fibre content among various lots of chayote fruits are more or less in agreement with those reported by Flick *et al.*, 1977; Engels 1983.

4.1 6 Total Carbohydrates

In the Indian diet about 70 to 80 per cent of the energy required by the body is supplied by the carbohydrates and they are considered as readily available source of enrgy apart from forming the structural constituents of the cell wall in the micro organisms and plants (Mudambi and Rajgopal, 1980).

The performance of various vegetable lots in relation to toal carbohydrates at different times of picking is depicted in Table 3. It is

evident from the data that the total carbohydrates ranged significantly from 80.17 to 82.20 per cent. T_5 stage of fruit harvest showed maximum accumulation of carbohydrates indicating marginal increase in the accumulation of carbohydrates with maturity of the plant.

The status of total carbohydrates in chayote was to the extent of 7.5 per cent on fresh weight basis (Hoover, 1923), 7.7 per cent (Engels, 1983) and 5.5 per cent (Gopalan, 1989) and hence, overall performance of this experiment on this aspect is in accordance with the observations of these investigators.

4.1.7 Energy

Quantitative food requirements are usually estimated in terms of energy i.e. calories . The unit of energy hitherto used was physiological caloreis (also called kilo calories - K cal) which is the amount of heat required to raise the temperature of one kilogram of water by 1^0 C from 14.5^0 C to 15.5^0 C (Gopalan *et al.*,1989).

The perusal of table 3 showed that T_5 stage of fruit harvest exhibited maximum value for energy (31 Kcal) followed by T_4 (28 K cal). However, altogether variation in the energy values at different dates of harvest of chayote were noticed from 22 to 31 Kcal. This reflects that like other vegetables it is not rich source of energy. However the findings relating to the energy status of this crop were in agreement with those reported by Engels (1983); Gopalan *et al.* (1989); varying from 27 to 31 Kcal per 100 g on fresh weight basis.

4.1.8 Total sugars

The data regarding variation in total sugars, presented in table 4 reflected a non-significant variation at different stages of harvest. However, it varied from 27.67 to 31.93 per cent with minimum and maximum values exhibtied by T_1 and T_3 stages of harvest. Fruits harvested at T_4 stage with 30.91 per cent sugars secured second place in order of preference.

The results on this aspect are in agreement with those reported by Nath *loc. cit.*. However, Engels (1983) reported 4.0 per cent soluble sugars whereas Aung *et al.* (1990) found 4.2 per cent of soluble sugars (on fresh weight basis). These values after conversion were more or less in conformity with the findings of the present work in this regard.

Table- 4 Variability in Total Sugars and Starch content in chayote (d.w.b.)

Test Samples	Total Sugars (%)	Starch (%)
T_1	27.67	3.42
T_2	28.81	3.29
T_3	31.93	3.17
T_4	30.91	3.18
T_5	28.83	3.05
SEm ±	0.41	0.05
CD at 5 %	NS	0.15

4.1.9 Starch

In our food, major component of carbohydrates forms starch, a polysaccharide synthesized by crop plants during photosynthesis (Conn *et al.*,1992).

As such variability in starch at different stages of harvest was evaluated in chayote fruits. Variation in starch content depicted in Table-4 was statistically significant ranging from 3.02 to 3.42 per cent . The highest value was observed at T_1, which was followed by T_2 stage of fruit harvest.

Earlier, starch content have been reported by some workers in the order of 1.3 per cent (Nath, 1971), 1.9 per cent (Aung *et al.*(1991) on fresh weight basis which corroborates the data of accompanying work.

4.1.10 Total Carotenoids

Carotenoids are widely recognised as nutritional component essentially needed for metabolism regulating processes in human beings. Viewing this total carotenoids status was determined but found to be absent in chayote fruits at all the stages of crop harvest.

Table 5 Variability in total Carotenoids and Ascorbic Acid. Content in chayote (f.w.b.) at different stages of harvest.

Test Samples	Carotenoids (ug /100 g)	Ascorbic Acid (mg/100 g)
T_1	0.00	2.74
T_2	0.00	3.07
T_3	0.00	3.45
T_4	0.00	3.45
T_5	0.00	2.98
SEm ±	-	-
CD at 5 %	N.S.	N.S.

4.1.11 Ascorbic Acid

Ascorbic acid forms a dietary component essentially needed for regulating certain metabolic processes in primates including human beings. It is well known that prolonged deficiency of ascorbic acid causes scurvy and also leads to physiological disorders in the body.

Viewing the importance of this vitamin in human nutrition, the variability in the ascorbic acid content at different stages of harvest of fruit was assesssed and reported in table-5 . Ascorbic acid content showed non-significant variation ranging from 2.74 to 3.45 mg per 100 g (on fresh weight basis). However, T_3, T_4 treatments both were superior in respect of high ascorbic acid content. The results are in conformity with Gopalan *et al.* 1989. However, there have been reports indicating ascorbic acid content as high as 11 mg to 20 mg (Nath, 1971; Engels, 1983).

4.1.12 Mineral Constituents

In view of the dietary significance of minerals both macro and micro elements in human nutrition, it was thought pertinent to evaluate the status of prominent minerals of vegetable chayote. Hence, some important macro and micro elements were estimated. The results on this aspect are presented here with in table 6 and discussed as under -

4.1.12.1 Calcium content

This element is essentially required for various physiological functions such as nerve conduction, muscle contraction and relaxation and it also acts as an activator or stabilizer of some enzymes required for blood clotting. It plays pivotal role in development of skeletal tissue (Sherman, 1955). Therefore, the variability or status of calcium content was evaluated at various harvest intervals of chayote.

The data indicated that T_2 lot emerged to be the superior most with 311.50 mg per 100 g calcium content followed by T_3 for 285 mg per 100 g giving the significant range of variation from 145.5 to 311.5 mg per 100 g.

Table - 6 Variation in calcium, iron and sulphur content in chayote (mg/100 g on d.w.b.) at different stages of harvest.

Test Samples	Calcium (mg /100 g)	Iron (mg/100 g)	Sulphur (mg/100 g)
T_1	145.50	7.03	147
T_2	311.50	9.83	127
T_3	285.00	6.70	137
T_4	157.50	7.83	167
T_5	153.00	18.90	173
SEm ±	4.99	0.07	5.0
CD at 5 %	16.29	0.23	20.0

4.1.12.2 Iron content

In addition to being the constituent of haemoglobin in the blood, iron is a co-factor of certain enzymes in electron transport chain of respiratory system. It is an important dietary element both in animals and human beings as its deficiency in human beings has largely been reported in the form of syndrome of anaemia (Monier and Williams, 1950).

The range of variability in iron content at different intervals of harvest is presented in table -6. It is evident that iron content varied from 18.90 to 6.70 mg per 100 g with significant variation at different stages of

harvest. The maximum value (18.9 mg per 100 g) was showed at T_5 stage of harvest.

4.1.12.3 Total Sulphur

It is predominantly an essential constituent of sulphur containing amino acids, certain vitamins and number of biologically improtant compounds and is associated in the regulation of metabolic processes of animals and plants (Sherman, 1955).

The perusal of data depicted in table- 6 indicated significant range of variation from 127 mg per 100 g to 173 mg per 100 g. of chayote fruit. T_5 stage emerged on the top with maximum concentration of sulphur followed by T_4 stage of harvest. There was an increasing trend of accumulation of sulphur with intervals of fruit harvest. The data obtained with regard to calcium, iron and sulphur content in present study were in conformity with those reported by Nath, (1971) and Flick *et al.*, (1977).

4.2 Variability in the biochemical constituents of lungru (*Diplazium esculentum*) at different stage of picking

4.2.1 Moisture content in fresh lungru

The variation in moisture content at different dates of harvest in two lots i.e. small sized and large sized presented in table 7 was observed from 92.14 to 94.32 per cent with non-significant differences among the stages of picking.

The results are in agreement with those cited by Gupta (1991) reporting 90 per cent moisture in fresh lungru samples. In bracken ferns the range of moisture from 19.20 to 19.39 per cent and 18.42 to 90.23 per cent had been reported in two different samples by Hendrick (1921).

Table 7 Proximate composition of Lungru (Diplazium esculentum) on dry weight basis except moisture

Test Samples	Moisture (%)	Protein (%)	Crude Fat(%)	Ash (%)	Crude Fibre (%)	Carbohydrates (%)	Energy (Kcal)
T_1	94.32	15.04	4.42	10.65	47.37	22.51	11
T_2	93.63	14.57	2.50	13.16	42.67	27.09	12
T_3	93.35	14.29	2.90	15.77	48.67	17.10	10
T_4	94.16	12.55	3.23	15.28	50.00	18.93	9
T_5	92.60	15.65	4.33	16.09	40.67	24.59	15
T_6	92.14	13.97	4.31	14.52	39.33	25.54	16
T_7	92.52	16.38	3.13	13.15	48.00	19.34	13
T_8	94.05	11.70	4.34	16.24	47.67	20.06	10
SEm ±	-	0.79	0.13	1.7	1.02	-	
CD at 5%	N.S.	2.41	0.39	3.66	3.09	N.S.	

4.2.2 Crude Protein

A look at the data presented in table -7 revealed the significant variation in crude protein content from 11.70 (at T_8) to 16.38 per cent (T_7 stage of picking). The values for T_1, T_2, T_3 and T_5 were at par with T_7 with T_5 assuming the second highest rank in protein content.

The variation in crude protein from different locations had been observed from 3.87 to 21.42 per cent and 3.00 to 16.06 per cent in case of bracken (Ferguson and Armitage, 1941), while 14.00 to 20.00 per cent crude protein was reported by Moon and Pal (1949) all being on day weight basis. The protein content of the order of 1.29 to 2.5 per cent have been reported on fresh weight basis by others (Zanariah *et al.*, 1986, Gupta, 1991).

4.2.3 Crude Fat

The highest crude fat content was exhibited at T_1 stage with significant range of variation at different dates of harvest from 2.50 (T_2) to 4.42 (T_1) per cent (table 7). At T_3, T_4 and T_7 stages of picking, values for crude fat content were at par with highest value reported at T_1 stage. Values for crude fat content had been reported to be 0.16 per cent (f.w.b.) by Gupta (1991), which is more or less in agreement with findings of the present investigation. The data indicated that the large lungru fronds were generally better in crude fat content as compared to small ones.

4.2.4 Ash Content

Lungru samples analysed for ash content at various intervals were found to contain as high as 16.24 per cent ash at T_8 period and the lowest value was recorded for T_1, with significant variation among the stages of picking ranging from 10.65 to 16.24 per cent on dry weight basis.

Hou (1950) reported 3.07 to 12.20 per cent ash content for various ferns and fern allies. Gupta (1991) found 1.17 per cent ash in fresh lungru samples. The data obtained in this study on the aspect are in conformity with the findings of these workers.

4.2.5 Crude Fibre

Table 7 showed the degree of variability in crude fibre content in freshly harvested lungru samples at different stages of picking. Statistically significant variation in crude fibre content ranging from 39.33 to 50 per cent was observed . The highest value was recorded for T4 and values in respect of T_1, T_3, T_7 and T_8 were at par with T_4, the second highest rank was exhibited by T_3.

Hendrick (1919) reported 24.30 per cent of crude fibre while Ferguson and Naeve (1944) gave a narrow range of 28.81 to 31.42 per cent in dry matter of bracken. The range from 49.36 to 60.02 per cent in stem of bracken had been reported by Moon and Pal (1949). The values of crude fibre are in parallel with those reported by Moon and Pal (1949).

The study revealed that in general the large as well as small sized ferns at later stages of picking i.e. in the months of July/August possessed maximum fibre content indicating that with maturity the fibre accumulation was increased in lungru.

4.2.6 Carbohydrates

Values for carbohydrates were exhibited highest at T_2 time interval (27.09 %) followed by T_6 showing 25.54 per cent carbohydrates on dry weight basis. However, overall variation in this parameter was observed from 17.10 to 27.09 per cent at different stages of picking of lungru samples.

Gupta (1991) observed 2.61 per cent carbohydrates in fresh lungru. The present data agrees with reported variation.

4.2.7 Energy

Table 7 reflected the energy status of lungru varying from 9 to 16 K cal per 100 g edible portion. In all the small sized fronds imparted fairly good amount of energy. However, data indicated that lungru forms poor source of dietary energy.

4.2.8 Total Sugars

Table-8 revealed total sugar content in lungru at different stages of picking varying from 7.06 to 10.22 per cent exhibited at T_4 and T_1 stages respectively. It is evident that large sized lungru were slightly better in total sugar content.

The values in the range of 0.88 to 10.09 per cent had been observed in bracken by Ferguson and Armitage (1944). In another study 11.30 per cent total sugars and 10.30 per cent reducing sugars have been reported by the same workers.

4.2.9 Starch

Variations in starch content in lungru at different time interval of picking depicted in table 8 were significantly ranging from 3.50 to 5.49 per cent. The maximum amount of starch was found to be at T_2 stage which was at par with all other values for T_3 to T_8.

Studies conducted earlier indicated that starch content varied in the range of 2.31 to 3.02 per cent, 2.50 to 3.15 per cent and 1.88 to 2.81 per cent in whole plant, leaves and stem respectively (Ferguson and Armitage, 1944). The findings of the present study are somewhat in agreement with the co-workers. However, the large sized group of lungru exhibited its superiority over small sized ones in this regard.

Table - 8 Total sugars and starch content of lungru (in % d.w.b.)

Test Samples	Total Sugars (%)	Starch (%)
T_1	10.22	3.50
T_2	9.68	5.49
T_3	8.82	5.41
T_4	7.06	5.00
T_5	8.41	4.36
T_6	8.55	5.08
T_7	7.35	4.67
T_8	7.56	5.37
SEm ±	0.23	0.45
CD at 5 %	0.46	1.40

4.2.10 Carotenoids and Ascorbic Acid

Table 9 showed non-significant difference in total carotenoids content at different intervals of picking. However, variations in total carotenoids was observed form 1.48 to 1.82 mg per 100 g in fresh lungru samples.

Similarly, ascorbic acid content differed non-significantly with regard to various intervals of picking. Nevertheless, values for ascorbic acid content showed a range of variability from 25.33 to 27.83 mg per 100 g of fresh weight of lungru samples

Table - 9 Variation in Total Carotenoids and Ascorbic Acid content in lungru at different stages of picking (mg/100 g on f.w.b.)

Test Samples	Total Sugars (%)	Starch (%)
T_1	1.82	26.24
T_2	1.65	27.05
T_3	1.59	25.33
T_4	1.48	25.85
T_5	1.62	26.70
T_6	1.62	27.83
T_7	1.67	26.99
T_8	1.77	25.77
SEm ±	-	-
CD at 5 %	N.S.	N.S.

The average ascorbic acid content of 29 mg per 100 g had been reported by Caldwell (1972). The values for total carotenoids are in conformity with findings of Gupta *loc cit.* A decreasing trend was seen in the carotenoids content in large sized ferns with advancement of maturity time.

4.2.11 Status of Minerals

Minerals are indispensable dietary constituents in human nutrition. The availability of prominent minerals was worked out at various

intervals of picking of lungru. The data on variability in dietary significant minerals viz., calcium, iron and sulphur are as under

Table - 10 Variability in Calcium, Iron and Sulphur status of Lungru at different stages of picking(mg /100 g)

Test Samples	Calcium	Iron	Sulphur
T_1	157.00	87 30	463.00
T_2	115.00	70 00	493.00
T_3	172.00	94 70	370.00
T_4	97.67	48 00	307.00
T_5	162.67	82 00	493.00
T_6	185.00	135 0	410.00
T_7	162.67	105 0	287.00
T_8	123.33	54 0	383.00
SEm ±	0.96	0.04	10.0
CD at 5 %	2.30	0.11	20.0

4.2.11.1 Calcium

The calcium content in lungru, depicted in table 10, varied significantly at different intervals of collection ranging from 97.67 to 185.00 mg per 100 g for T_4 and T_6 respectively. At T_3 stage of picking lungru showed second position in calcium content.

4.2.11.2 Iron

Like calcium, iron also varied significantly. The highest concentration of iron was 135 mg per 100 g (at T_6) while the lowest value was recorded 48 mg per 100 g at T_4 stage of picking.

The later stages of T_4 and T_8 in both small and large sized, lungru showed a considerable decrease in iron content. So in general there was decrease in iron content with maturity.

4.2.11.3 Sulphur

The sulphur content in lungru at various intervals of picking presented in the table 10 showed statistically significant variation ranging from 287 to 493 mg per 100 g . The highest value was recorded for T_2 and T_5 stages of picking. However, sulphur content appeared to decrease with age of fern, both in small as well as large sized ferns.

Evaluation of minerals variability in edible ferns by Moon and Pal (1949) revealed 0.41 to 1.08 per cent calcium, 0.18 to 0.89 per cent phosphorus and 0.74 to 3.50 per cent potassium content in different parts of bracken. Further studies carried out by Hou (1950) showed substantial amounts of aluminium, manganese, phosphorus, calcium and potassium in different ferns and fern allies in the range of 0.004 to 0.792 per cent, 0.003 to 0.045, 0.090 to 0.382, 0.050 to 1.861 and 0.590 to 3.309 per cent respectively (on f.w.b.). Little higher /lower ranges of trace elements obtained in the study might be the influence of variable agroclimatic conditions.

4.3 Variability in essential amino acid content at different intervals of picking

4.3.4 Methionine

Besides being one of the constituents of proteins, methionine is actively involved in several transmethylation reactions of vital importance in plants and animals (Karlson, 1968).

The data showing the variability in methionine at different stages of picking, presented in table 11 was statistically significant. The methionine content varied from 1.2 to 0.5 mg /16 g N for T_2 and T_4 stages of harvest respectively. The values for T_3, T_5 and T_6 were at par with highest value. However, methionine showed a declining trend with advancement of maturity periods of ferns.

4.3.2 Tryptophan

Tryptophan plays fundamental role in the biosynthesis of nicotinic acid (Vit B_3) as well as in other metabolic processes. This amino acid constitutes one of the essential amino component of dietary protein (Karlson, 1968).

Table 11 depicts the tryptophan content of various dates/intervals of picking varying from 0.80 (T_8) to 1.21 (T_2) mg per 16 g N. The tryptophan content varied significantly and value exhibited by T_4 was at par with highest value.

The studies conducted earlier revealed that bracken samples analysed for different amino acids composition showed negligible amounts of tryptophan (Smith and Agiza, 1951). However, systematic information on the essential amino acid profile is yet to be found out in further investigations.

4. 4 Variability in the anti- nutritional factors of lungru at different time intervals of picking

The foods, particularly from plant origin undoubtedly are rich in dietary nutrients but also possess a vast range of anti nutritional factors which interfere with the assimilation of nutrients contained in the plant. In the green vegetables, important anti nutritional factors are oxalates, tannins, phytates and nitrates which interfere with the utilization of other nutrients like protein and minerals viz. iron, zinc, calcium and iodine. As such quantitative assessment of these anti-nutritional factors was important while evaluating the overall equality of green vegetables (Gopalan *et al.*,1989).

Table - 11 Variability in Methionine and Tryptophan in lungru at various stages of picking (mg /16 g N).

Test Samples	Methionine	Tyrptophan
T_1	0.91	0.96
T_2	1.21	0.80
T_3	1.00	0.92
T_4	0.51	1.12
T_5	0.95	0.86
T_6	1.20	0.87
T_7	0.86	0.82
T_8	0.60	1.21
SEm ±	–	–
CD at 5 %	0.26	0.22

4.4.1 Nitrate

Literature reveals serious health hazards caused by nitrate - nitrite in foods as precursor of carcinogenic nitrosamines (Emerick, 1972 and Wolif and Nasserman , 1972). The knowledge of presence of nitrates to decrease the food value of vegetable crops prompted to investigate nitrate content in lungru.

Significant range of variation from 0.62 to 1.73 per cent was observed amongst various lots at different intervals of harvest. Stage T_3 and T_5 showed lowest and highest values of oxalates, respectively.The second lowest value (0.88 per cent) at T_7 stage was at par with stage T_3 of harvest (Table 12).

4.4.2 Oxalates

The oxalates are found in the free state as well as in the form of salts both in vegetable and animal kingdom. These make the calcium unavailable by interfering in the availability of calcium and other trace elements. Besides excess intake of oxalates may predispose oxalate crystalisation leading to urinary stones (Gopalan *et al.*, 1989).

A close look at the table 12 revealed a range of variation from 8.15 to 9.26 per cent. The lowest value was attributed to T_7 stage of picking followed by T_6. The oxalates content appeared to slightly increase with the period of maturity.

Table - 12 Status of anti-nutritional factors (Nitrates, Oxalates, Condensed Tannins and PPO activity) in Lungru at different stages of picking.

Test Samples	Nitrate (%)	Oxalate (%)	Condensed Tannins (%)	PPO Activity (unit/min/g)
T_1	1.41	8.64	0.37	0.2
T_2	1.50	8.92	0.41	0.2
T_3	0.62	8.81	0.37	0.2
T_4	1.52	8.93	0.36	0.2
T_5	1.73	8.57	0.39	0.2
T_6	1.41	8.24	0.40	0.2
T_7	0.88	8.15	0.36	0.2
T_8	1.57	9.26	0.33	0.2
SEm ±	0.10	-	-	-
CD at 5 %	0.32	N.S.	N.S.	N.S.

4.4.3 Condensed Tannins and Polyphenol oxidase activity

Tannins present in the food are known to cause reduction in the digestibility of dietary protein and to a lesser extent that of available carbohydrates and lipids. Tannins also interfere with dietary iron absorption. In addition, the disclolouration of certain fruits and vegetables is attributed to both enzymatic and non enzymatic browning reactions involving phenolic compounds (Ramaswamy and Rege, 1975). In case of enzymatic browning, the determination of polyphenol oxidase activity in fresh fruits and vegetables is very important to know the extent of

browning. The level of astringency causing compounds, viz., tannins alone cannot determine the extent of discolouration and hence, polyphenol oxidase assay is of great importance in determining the quality attributes of fruits and vegetables.

Polyphenol oxidase (0-Diphenol : oxygen oxido reductase, EC 1.14.18.1) also called as triosinase, polyphenolase, catechol oxidase, cresolase and catecholase at various times, is probably present in all plants which in presence of molecular oxygens brings about the aerobic oxidation of catechols and quinones. Polyphenol oxidase from different sources markedly differ in their specific substrate requirement. The browning of fruits and vegetables caused by polyphenol oxidase is reported to be prevented by exclusion of molecular oxygen (substrate limitation) by addition of reducing agent which prevents the accumulation and polymerisation of O - benzoquinone by metal complexing agents such as sodium or fluoride and azides which inactivate the enzyme by reacting with the essential Cu, or by heat treatment (Whitaker, 1972).

Keeping in view the significance a tannins and polyphenol oxidase activity (PPO activity), it was felt pertinent to evaluate both so as to assess its quality. Table 12 depicting data for condensed tannins showed the highest (0.41 per cent) content for T_2 stage which was succeeded by T_6 for the second highest. However, the range of variability in lungru for condensed tannins was from 0.33 to 0.41 per cent. The minimum level was given by T_8 stage of harvest. The study revealed that in general, the tannin content decreased with maturity, both for small as well as large sized lungru. However, there was no change in PPO activity in different stages of harvest and the enzyme activity was observed to be 0.2 units per minute per gram at all intervals of fern collection.

Apparently, litle information was available with regard to nitrate and oxalate status in fern. Shearer (1945) reported tannin content ranging from 0.44 to 7.0 per cent at various stages of maturity of bracken

and suggested that decompositon - product of catechol tannins is responsible for bracken poisoning. Later Moon and Pal (1949) recorded 1.47 to 4.65 per cent and 0.50 to 1.00 per cent tannins in leaf and stem of bracken respectively, while Brahaman and Saxena (1978) showed 3.30 to 5.60 per cent tannin in frond and rhizome of *Acrostichum avreum* respectively. In the recent past, Gupta (1991) reported tannin content to the extent of 0.38 per cent in oven dried samples of lungru. The tannin content obtained in lungru samples is somewhat within the acceptable limits (Moon and Pal, 1949; Gupta, 1991).

4.5 Effect of storage on constituents of chayote (*Sechium edule*)

Storage of the food items ensures their effective and prolonged use. Also the sustainability or the storage potential allows the transport of vegetables to the markets with such demands. However, upon storage, there is possibility of the change in biochemical constituents which may affect the nutritional quality. Keeping in view the above facts,the present study was conducted to assess change in some of the biochemical constituents at ambient temperature for one month at an interval of one week, the results of which are discussed as under.

4.5.1 Moisture Content

With storage, moisture content decreased, though marginally, as depicted in Table 13 from 94.01 to 91.25 per cent on fresh weight basis. The maximum decrease was observed between T_2 and T_3 from 93.94 to 91.77 per cent, thereby indicating an observable moisture loss during second week of storage.

Table -13 Effect of storage on the biochemical constituents of Chayote (*Sechium edule*).

Test Samples +	Moisture (%) *	Ascorbic Acid (mg/100g)*	Total Free Amino Acids (%)*	Total Sugars (%)	Starch (%)
T_1	94.01	2.95	0.38	30.21	3.21
T_2	93.94	2.85	0.39	30.25	3.18
T_3	91.77	2.84	0.40	30.27	3.16
T_4	91.31	2.77	0.40	30.77	3.08
T_5	91.25	1.39	0.41	30.93	3.07
SEm ±	0.54	-	0.02	-	0.30
CD at 5 %	1.79	N.S.	0.04	N.S.	0.12

* Values are on fresh weight basis

\+ T_1 represents freshly harvested chayote T_2, T_3, T_4, T_5 represent each subseqent week.

4.5.2 Ascorbic Acid

The fresh chayote gave a value of 2.95 mg per 100 g for ascorbic acid content which decreased after fourth week of storage at ambient temperature. Considerable decrease was observed (from 2.77 to 1.39 mg per 100 g) during fourth week of storage.

4.5.3 Total Free Amino Acids

On storage there was a significant increase in total free amino acids in chayote from 0.38 to 0.41 per cent (on fresh weight basis).

4.5.4 Total Sugars and Starch

Freshly harvested chayote, contained 30.21 and 3.21 per cent total sugars and starch respectively on dry weight basis. There was

marginal decrease in starch content ranging from 3.21 to 3.07 per cent and substantial increase in total sugars from 30.21 to 30.93 per cent on storage after one month.

Although specific information with regard to alteration in quality attributes in chayote seems to be lacking. However, Alvarado *et al.* (1989) while evaluating the post harvest treatments for improving the keeping quality of chayote (*Sechium edule*) fruits did not observe deterioration stored at ambient condition. However, the use of low density polythene bags for storage at 12 to 14^0 C temperature was reported to be the best. Hence decline in biochemical constituents of chayote was observed to be marginal after four weeks of storage indicating minimum loss of nutrients for this period. However, in order to exploit its keeping quality potential further studies are warranted in this direction.

4.6 Rating for evaluating the most promising period of harvest for maximum nutritive value

During the course of investigation, it was observed that none of the stage of harvest exhibited excellent performance in each and every quality parameters and question of picking out preferably the best stage of harvest / picking for all characters under investigation seemed baffling. To overcome this problem, the treatments /stages of harvest were graded for their overall performance to strike a balance of maximum superiority among all the characters, although some of the characters had to be sacrificed. In chayote the rating for best harvest interval was done with respect to nutritional quality characters like protein, crude fibre, ash, carbohydrates, and calcium. Similarly, in lungru,rating at different harvest intervals for desired quality characters viz. proteins, crude fibre, carbohydrates, ascorbic acid, carotenoids, calcium, iron, sulphur and those containing least amount of undesirable constituents such as nitrates and

oxalates keeping in view their relative abundance in these vegetables, was done which is given in tables 14 and 15 respectively.

It was obseved that T_2, T_3 / T_4 and T_5 stages of picking secured first, second and third position in order of excellence based on cumulative rating for the chayote fruit. In case of lungru, the harvest interval at T_7 secured first place followed by T_6 and T_1 interval of fern picking. Thus the small sized lungru of June and July and even the large sized lungru of early times of growth i.e. May exhibited their superiority with respect to nutritive value in all. The variation in the accumulation of biochemical constituents might be attributed to environmental conditions.

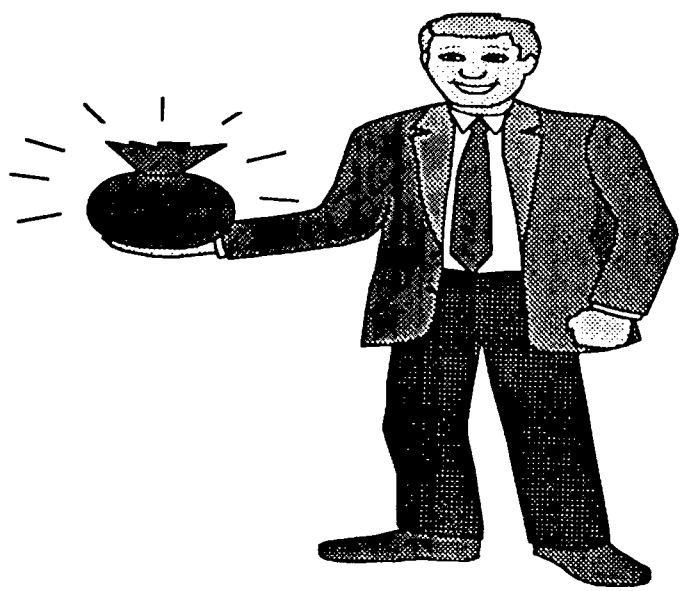
Table 14 Rating for evaluating the most promising period of harvest for maximum nutritive value for chayote(*Sechium edule*).

Biochemical Parameters	Test Samples (Interval of picking)				
	T_1	T_2	T_3	T_4	T_5
Protein	4	2	3	5	1
Crude Fibre	3	4	5	2	1
Ash	2	4	1	3	5
Carbohydrates	2	4	3	3	5
Calcium	1	5	4	3	2
Total	12	19	16	16	14
Cumulative Rating	4	1	2	2	3

Table - 15 Rating for evaluating the most promising period of harvest for maximum nutritive value in lungru (*Diplazium esculentum*).

Biochemical Parameters	Test Samples (Interval of picking)							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Protein	6	5	4	2	7	3	8	1
Crude Fibre	4	3	7	8	2	1	6	5
Carbohydrates	5	8	1	2	6	7	3	4
Ascorbic Acid	4	7	1	3	5	8	6	2
Carotenoids	8	5	2	1	3	4	6	7
Calcium	4	2	7	1	5	8	5	3
Iron	5	3	6	1	4	8	7	2
Sulphur	6	8	3	2	8	5	1	4
Nitrate	6	4	8	3	1	5	7	2
Oxalate	5	3	4	2	6	7	8	1
Total	53	48	43	25	47	56	57	31
Cumulative Rating	3	4	6	8	5	2	1	7

SUMMARY AND CONCLUSIONS



CHAPTER V

SUMMARY AND CONCLUSION

The present investigation entitled "Biochemical and nutritional evaluation of chayote (*Sechium edule*) and lungru (*Diplazium esculentum*) of Palam Valley of Himachal Pradesh" was carried out in the Department of Chemistry and Biochemistry, College of Basic Sciences, Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 1997 - 98 with the following major objectives:

I. To study biochemical constituents of chayote (*Sechium edule*) and lungru (*Diplazium esculentum*) of Palam Valley of H.P.

II. (a) to assess the level of anti-nutritional factors in lungru (*Diplazium esculentum*) and

(b) to evaluate effect of storage of fruits after harvest of chayote on the nutritional quality.

Fresh chayote fruits were collected from local kitchen gardens at Palampur at an interval of ten days, in five lots (denoted as T_1, T_2, T_3, T_4 and T_5) while lungru was collected from local market having supply from Bandla locality at monthly interval four times in two lots each, one containing small sized and the other large sized lungru. Replicate analysis for biochemical parameters of nutritional significance of the above lots, both of chayote and lungru and also for stored chayote was done, following recommended procedures. The salient findings of the study are summarised as follows.

5.1 Variability in biochemical constituents at different stages of picking in chayote

In fresh chayote fruits harvested at various intervals, protein, crude fibre, carbohydrates, calcium, iron and sulphur, content showed significant variability ranging from 5.25 to 7.66 %, 5. 0 to 7.10 %; 80.17 to

82.20 %; 145.5 to 311.5 mg/100 g; 6.7 to 18.90 mg/100 g and 127 to 173 mg /100 g, respectively.

5.2 Variability in biochemical constituents at different stages of harvest in lungru

Analysis of various parameters of biochemical and nutritional significance revealed variation in protein, crude fibre, carbohydrates, carotenoids, ascorbic acid, ash, calcium, iron, sulphur, methionine, tryptophan, nitrate, oxalate and condensed tannin content ranging from 11.70 to 16.38 %; 39.33 to 50.00 % ; 18.93 to 27.09 % ; 1.48 to 1.82 mg /100 g, 25.33 to 27.83 mg /100 g ; 10.65 to 16.24 % ; 97 .67 to 185.50 mg /100 g ; 4.8 to 13.5 mg /100 g ; 287 to 493 mg /100 g ; 0.51 to 1.21 mg/16 g N, 0.80 to 1.21 mg /16gN ; 0.88 to 1.73 % ; 8.15 to 9.26 % and 0.36 to 0.41 % respectively.

5.3 Cumulative rating in respect of both chayote and lungru for evaluating the overall superiority for the time of harvest with respect to nutritional status

Based on over all rating for desirable quality attributes viz. Protein, crude fibre, ash, carbohydrates and calcium in the fruits picked up at T_2 (mid-October), T_3 / T_4 (late October or early November), T_5 (mid November) stages occupied first, second and third place in order of overall excellence in case of chayote. Similarly in lungru at T_6 (small sized lungru of June), T_7 (small sized of July) and T_1 (large sized of May) stages of picking, comparatively better nutrient availablity was observed in that order.

5.4 Effect of storage on nutritional quality of chayote

The study indicated that storage of chayote fruits at ambient temperature for one month has shown marginal variation in biochemical constituents viz., ascorbic acid, total free amino acids, starch and total sugars ranging from 2.95 to 1.39 mg/100 g ; 0.38 to 0.41 % ; 3.21 to 3.07 %,

30.21 to 30.93 % respectively and hence chayote fruit might be safely consumed upto this time frame of storage.

The results of the study, thus clearly indicated that potential does exist for better utilization of these underutilized vegetable crops keeping in view their considerable nutritive value and hardy nature. Further studies directed towards the development of appropriate processing techniques for value added food products and in depth understanding of their medicinal properties, the use of biological trials may add much more recognition to these crops. For chayote, the crop needs to be tested in various agroclimatic conditions of the state to sort out its best habitat for higher yield with optimum nutrition.

Further investigations would also be desirable for studying keeping quality of the chayote fruits over a period of three months that too in varying package materials in order to have a rational idea of its effective utilization in days to come as a subsidiary food item to meet out the challenges of under nutrition.

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APPENDIX

Analysis of variance using randomised block design (RBD)

Summary of data:

Treatments	Replications
1	1,2,3,4, 5.....t
2	
3	
4	
.	
t	

Analysis of Variance

Sourc e of variation	Degre of freedom	Sum of squares	Mean squares	F.Cal
Replications	r-1	RSS	MR = RSS/r-1	MR/Me
Treatments	t-1	TSS	MT = TSS/t-1	MT/Me
Error	(r-1) (t-1)	ESS	Me = ESS(r-1) (t-1)	

Where :

r= Number of replication; t = Number of treatments

RSS, TSS and ESS are the sum of squares due to replications, treatments and errors respectively.

MR, MT, Te are the mean squares due to replications, treatments and errors respectively.

Standard error (SE) = 2 x Me/r

Critical difference (CD) = SE x 't' value at error d.f.