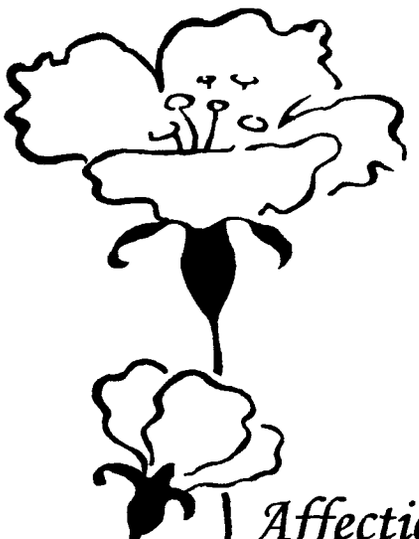

Affectionately with humility and
reverence dedicated to my beloved
parentsAai and Anna
Akka and brothers
Sanjay and Nitin
Late Sister Bharati

.... *Prakash*

STUDIES ON NUTRITIONAL EVALUATION OF SEEDS OF NEWLY DEVELOPED COTTON (*Gossypium hirsutum* L.) CULTIVARS

By

Prakash Kisan Lokhande
(Reg. No.98113)

DEPARTMENT OF BIOCHEMISTRY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722
2000

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A Thesis submitted to the
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of

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POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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2000

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof has not been submitted by me or any other person to any other University or Institute for Degree or Diploma

Place : MPKV,Rahuri
Dated : 26/12/2000

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CERTIFICATE

This is to certify that the thesis entitled, **"Studies on nutritional evaluation of seeds of newly developed cotton (*Gossypium hirsutum* L.) cultivars"**, submitted to the faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** (AGRICULTURE) in **BIOCHEMISTRY**, embodies the results of a piece of *bona fide* research work carried out by **Mr. Prakash Kisan Lokhande**, under my guidance and supervision and no part of the thesis has been submitted for any other degree, diploma or publication in any other form.

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

Place : MPKV, Rahuri
Date : 26/12/2000.

(U.D. Chavan)
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Maharashtra State (INDIA)

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(S.S. Kadam)

Place : MPKV, Rahuri

Date : 26 / 12/2000.

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Place : MPKV,Rahuri (P.K. Lokhande)

Dated :26/ 12 /2000.

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LIST OF ABBREVIATIONS

AAS	:	Atomic absorption spectrophotometer
BSA	:	Bovine serum albumin
C.D.	:	Critical difference
CPFA	:	Cyclopropenoid fatty acids
CRD	:	Completely randomised block design
EBT	:	Erichrome black T
EDTA	:	Ethylene diamine tetra acetic acid
e.g.	:	Exempli gratia (for example)
et al.	:	And other
GABA	:	Gamma amino butyric acid
GLC	:	Gas liquid chromatography
IBA	:	Indole-3-butyric acid
K	:	Potassium
MT	:	Million tonnes
N	:	Nitrogen or normality
NPN	:	Nonprotein nitrogen
PSVs	:	Protein storage vacuoles
RNA	:	Ribose nucleic acid
SDS-PAGE	:	Sodium dodecyl sulfate, poly acrylamide gel electrophorosis
TEA	:	Triethanolamine
viz.,	:	Namely

ABSTRACT

STUDIES ON NUTRITIONAL EVALUATION OF SEEDS OF NEWLY DEVELOPED COTTON (*Gossypium hirsutum* L.) CULTIVARS

By
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A candidate for the degree
of
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The present investigations were undertaken to evaluate the nutritional quality of promising cottonseed cultivars in relation to : seed index, hull to kernel ratio, moisture, oil and oil quality, protein content, soluble protein, free amino acids, limiting amino acids, ash and important minerals. The seeds of eighteen cotton cultivars(*Gossypium hirsutum* L.) newly developed and identified are most promising, were obtained from the Cotton Breeder, Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri and evaluated for various quality parameters using standard analytical procedures.

The seed index (g per 100 seeds) ranged from 5.60 to 8.82 g, the hull to kernel ratio varied from 0.65 to 1.64 and moisture content from 7.4 to 16.10 per cent in the kernels.

The crude oil content ranged from 30.20 to 37.70 per cent in different cultivars, the English red colour were found in most of the cultivars followed by carrot red and brownish orange colour. The iodine

value of extracted oil varied from 95 to 106 and the acid value ranged from 0.36 to 0.69 mg KOH/g oil.

The defatted meal of different cultivars were found to contain 22.30 to 47.6 per cent crude protein, 2.69 to 13.80 per cent soluble protein and 0.05 to 0.88 mg/100 g free amino acids. The content of limiting amino acids such as methionine and tryptophan varied from 1.12 to 2.73 g/16 g N (0.41 to 0.91 g/100 g meal) and 0.97 to 2.06 g/16 g N, (0.41 to 0.65 g/100 g meal), respectively. The protein content in seeds was found to be negatively correlated with the content of oil.

The ash content of defatted kernel meals exhibited a wide variation of 6.6 to 9.8 per cent. The phosphorus content ranged from 1.32 to 2.03 per cent, potassium from 1.61 to 2.33 per cent, calcium from 109 to 215 mg/100 g, magnesium from 80 to 177 mg/100 g and iron from 16 to 42 mg/100 g. The manganese, zinc and copper content of defatted kernel meal of cotton cultivars varied from 1.8 to 3.2 mg/100 g, 1.2 to 2.3 mg/100 g and 4.6 to 17 mg/100 g, respectively.

Among eighteen cotton cultivars studied ERB, LRA-5166, BN, GB-20, RHC-0191 were higher in oil content, while DS-28, RHC-9740, LRA-5166, RHC-1189, G.Cot. 100 and SRT-1 were higher in protein content. Phosphorus content was higher in GB-20, RHC-0688, G.Cot.100, DS-28 and ERB. Potassium was higher in the cultivars GB-20, JLH-168, BN, RHC-3194 and LRA-5166. Calcium content was higher in the cultivars RHC-994, GB-20, RHC-0191, RHC-1088 and RHC-1794, while iron content was higher in the cultivars ERB, G.Cot. 100, SRT-1, RHC-0688, LRA-5166 and RHC-3194.

INTRODUCTION

1. INTRODUCTION

Cotton (*Gossypium spp*) is one of the most important cash crop grown in several countries such as India, USA, USSR, China, Egypt and Africa, mainly for the fibre, which is mostly used for the production of luxury fabrics. Most of the cultivars belong to the species *Gossypium hirsutum* L called as American cotton and *Gossypium barbadense* L. called as Egyptian cotton. Both are referred as new world cotton and these are tetraploids. *Gossypium arboreum* L. and *Gossypium herbaceum* L. both are called as old world cotton.

India is unique in cultivating all the four species of cotton and is the first country to commercialize hybrid cottons. The total production of seed cotton was over 80 lakh tonnes which yields 52 lakh tonnes of cotton seed annually (Anonymous, 1998). Cotton seed is widely distributed as oil seed in tropical and subtropical areas and it plays an important role in the economics of Agricultural Cum Industrial Development.

Maharashtra is a major cotton growing state in the country with 3199 thousand hectares area under cotton and about 576 thousand tonnes production of cotton lint (Anonymous, 1999).

Cotton lint is the main produce obtained after ginning of seed cotton and is used for spinning of fibres for cloth manufacture, while cotton seed is the byproduct. The world cotton seed production

was 35557 thousand M.T. while in India cotton seed production was 5430 thousand M.T. (FAO, 1997).

Although the ratio of cotton lint to cotton seed on an average is 35 : 65, cotton lint is considered to be a main product of cotton crop as it is the important raw material for textile industry. However, the quality of lint depends upon the quality of cotton seed kernel (Rao *et al.*, 1983). The typical composition of cotton seed from various world sources indicates that seeds contain 5 - 12.8 per cent moisture, 15.2 - 28 per cent oil and 17.1 - 21.3 per cent protein. It is also a rich source of minerals, B vitamins and fat soluble vitamins such as A, D and E. The proximate composition of cotton seed varies according to the variety, season and region. Lawhon *et al.* (1977) reported that the oil content of cotton seed ranged from 16.5 to 25.6 per cent and protein from 19.6 to 24.0 per cent. Turner *et al.* (1976) reported average value of 19.6 per cent for oil and 24.8 per cent for protein in cotton seed. Similar oil and protein values from 23.2 to 25.7 per cent and 25.6 to 27.6 per cent, respectively, in cotton seed have been reported by Cherry *et al.* (1981). A number of factors including genetic and growing conditions influence the composition of cotton seed (Wolf, 1988).

In cotton about 9.3 per cent of world oil seed production, 7.2 per cent of global protein meal production, 7.1 per cent of world oil meal export, total oil seed production of 22 MT and oil production of 7 MT. The annual demand supply gap for edible oils is estimated

to be around 12 to 14 lakh tonnes (Anonymous, 1999a). To meet the gap of demand and supply a total import is over 43 lakh tonnes, over three times the short fall which ultimately resulted in unnecessary expenditure of foreign exchange. This situation has arised only because of our dependence on the traditional sources of oil, such as groundnut, safflower, soybean, mustard, sesamum, coconut, etc. and only partial exploitation of the non-conventional, non-leguminous oil sources like cottonseed, rice bran oil, corn oil, palm oil, etc., as against a potential of about 3 M tonnes of oils, only 1.4 M tonnes is being exploited (Anonymous, 1999b). If these sources are exploited on a systematic basis, a sizeable quantity of edible or non edible oils could become available. Therefore, this is foremost task to develop an integrated approach for optimising exploitation of these sources.

Cotton seed oil did not find acceptance as an edible oil till a few decades back due to presence of gossypol. Gossypol, a toxic phenolic compound also contributed to the colour of the oil in unrefined state (Taneja *et al.*, 1991a). Refined cotton seed oil practically lacks gossypol and can be used directly as cooking medium. The content of fatty acid ranged within the limits of 0.4 to 1.3 per cent for myristic acid, 19.8 - 33.7 per cent for palmitic acid, 0.2 - 6.6 per cent for palmitoleic, 1.8 - 4.1 per cent for stearic acid, 13.26 - 26.0 per cent for oleic acid and 41.8 - 61.4 per cent for linoleic acid. The sum of saturated fatty acid ranged from 23.4 per cent for the

Australian small seeded G. *bickii* to 36.4 per cent for the African G. *anomalum* L. (Kyzalakova, 1976).

The production of vanaspati oil has come to depend increasingly on the use of cottonseed oil. The vanaspati industry is required to use a maximum of 30 per cent of cotton seed oil. Only 10 per cent of the cotton seed oil goes for direct consumption and the balance is consumed only by the Vanaspati oil industry. Further, nearly two thirds of the cotton seed oil is obtained from undecorticated cotton seed. This is essentially because of the extremely limited domestic market for decorticated cake. This crushing of decorticated cotton seed is dependent entirely on export demand for oil cake expeller/extraction. Cotton seed protein has been successfully used to rehabilitate malnourished infants (Graham *et al.*, 1969 and 1970 and Scrimshaw *et al.*, 1973). Srikantia and Sahgal (1968) found successful use of gossypol free cotton seed meal in the improvement of protein calore malnutrition. The cotton seed flour has been tried as a supplement in the preparation of bread (Mathews *et al.*, 1970; Tsen *et al.*, 1971; Rooney *et al.*, 1972).

The histidine requirement in children is high whereas that of arginine is high in adult (Pandey, 1984). Both are high in cotton seed protein. Edible cotton seed flour is of extremely good quality containing free gossypol less than 0.5 per cent, protein as high as 60 to 65 per cent, nitrogen solubility 99 per cent and epsilon amino free lysine 3.96 g/16 g nitrogen is present. The degossypolized

cottonseed flour can be used in the preparation of interesting products by the food industry. It can also be the basis for the preparation of other protein products such as isolates and concentrates. Biscuits and reasonably good quality bread loaves have been made with the gossypol free cotton seed flour at level of 5, 10 and 20 per cent.

Twenty children of varying ages were fed balanced diets containing approximately one gram of protein per kilogram of body weight, 30 per cent of which was cottonseed protein. Medical examinations identified the children to be in good health and not currently suffering from nutritional deficiencies. The nutritional status remained adequate throughout the period of investigation. Seventy to 90 per cent of the subjects consuming cotton seed consistently remained in the normal or above normal percentile range for both height and weight measurements (Alford, 1975).

Alford *et al.* (1977) demonstrated that cotton seed protein maintained the nitrogen balance in women when fed at the higher levels of protein intake consumed in the USA. Alford and Onley (1978) found that the minimum cottonseed protein required to maintain the nitrogen balance in woman was 0.106 g of N per kg of body weight. Using a factor of 6.25 for calculating protein from nitrogen results in 0.66 g of protein per kg body weight. This is greater than the FAO safe level of 0.52 g of protein intake for adult woman.

In India during VIIIth five year plan efforts were made to search for the new byproducts and unconventional feeds to improve their quality and formulate complete economic rations for different livestock. The cottonseed are routinely fed to lactating animals, especially the buffaloes by the farmers of Haryana, Punjab, Rajasthan, Andhra Pradesh etc., with the idea that it increases the milk fat.

Cotton stalks are another byproduct of cotton crop which are being used in fuel and shelter making. The possibility of utilizing the cotton stalks as animal feed has been tried by Kumar *et al.* (1993). Cotton stalk is one of the important lignocellulosic wastes materials. Since it is well known that *Pleurotus spp.* grow well on lignocellulosic materials, attempts were made to grow *Pleurotus sajar-caju* an edible mushroom on cotton stalks. Accordingly it has been shown that about 500 g of freshy fruiting bodies could be obtained in one harvest per kg of cotton stalks. Ramanaiah *et al.* (1997) studied the comparative performance of goat and sheep fed cotton seed hulls and poultry waste 30 per cent based concentrate mixture. They observed significantly higher dry matter intake in sheep than in goats, when fed cotton seed hulls based concentrate mixture.

The cottonseed hulls have been used in complete diets by Reddy and Reddy (1998) at 50 and 35 per cent level and these diets were compared with convential diet in crossbred bulls, cottonseed hull contributes to about 37 to 60 per cent of the weight of cotton. Hull is mainly used for cattle feed. Linters are used in the

manufacture of absorbent cotton, medical pads, gauge, twine wicks and carpets yarn, bedding and cushioning for furniture and motor cars (Taneja *et al.*, 1991a).

The levels of the antinutrients gossypol, cyclopropenoid fatty acids and aflotoxin in the seed from the insect protected lines were similar to or lower than the levels present in the parental variety and reported for other commercial varieties (Berberich *et al.*, 1996). Gossypol is toxic to birds and to non-ruminant animals, cottonseed cake can only be fed to cattle. If gossypol can be eliminated from the seed, it would not only improve the oil quality, but it would also increase the value of the cake.

Fortunately, some lines of cotton are available which are glandless and hence, gossypol free. These lines are devoid of the small lysigenous glands on the plant surface and contain gossypol under the admissible limits for the human diets. Therefore, these can be produced and utilized for the sake of cotton seed oil and protein without any purification process. Generally it is reported that glandless types are more susceptible to insects and pests. Bottger *et al.* (1964) and Maxwell *et al.* (1965) noted increased susceptibility in some of the glandless lines than glandular ones to blister beetle and certain other insects. Jenkins *et al.* (1966) reported greater damage caused by *Heliothis zea* on glandular Acala than glandless Acala.

Many programmes have been launched by breeders and biotechnologists to improve the quality of cotton lint as well as that

of industrially important byproduct, like oil and protein in the cotton seed through hybridization (Patel, 1971).

During the recent past, several promising cultivars have been developed in cotton seed. The information on their nutritional composition, particularly proteins, oil and oil quality and mineral content of cotton seed kernels are lacking. Therefore, the present investigation was undertaken with following objectives.

1. To screen eighteen promising cotton cultivars for their oil, protein and proximate composition.
2. To study the protein and oil quality of eighteen cultivars of promising cotton cultivars.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The information on nutritional composition of cottonseed kernel is limited as compared to other major and minor oil seeds. Considerable emphasis is given recently on studies related to the amount of nutritional composition present in cottonseed kernel meal. In this section, a brief account of literature on the nutritional composition of cottonseed is reviewed.

2.1 Nutritional composition

2.1.1 Seed index

The seed indices (g per 100 seeds) range from about 1 g (*G. sturtianum* L.) to 8.5 g (*G. barknessii* L.). These indices are smaller than those reported for the American cultivated species (*G. hirsutum* L.) of 10 g for moisture free seed and 11.1 g for fuzzy seed containing 10 per cent moisture, or the Egyptian cultivated species (*G. barbadense* L.) of 9.1 - 10.5 g (EL-Nockrashy *et al.*, 1969). The seed index values were in the range of 7.6 g to 8.7 g in the samples from Indore, 9.3 to 10.8 g in the samples from Coimbatore and 10.7 to 12.3 g in the samples from Surat of *G. hirsutum* L.. (Pandey and Thejappa, 1976). The low gossypol cotton variety Zhemian-10, evaluated in Zhejiang regional research station showed 9.86 g seed index (Anonymous, 1996).

The seed index of *G. hirsutum* L. was 5.48 to 6.39 g (Singh *et al.*, 1969). Laxmi cultivar of cotton from *G. hirsutum* L. had 2 to 8.15

g seed index (Pandey, 1972). Murthi and Achaya (1975) reported the seed index of *G. hirsutum* L. 6.9 to 11.1 g, *G. barbadense* L. 9.5 to 11.7 g, *G. herbaceum* L. 5.4 to 7.1 g and *G. arboreum* L. 4.3 to 6.9 g. Chakravorty and Singh (1979) reported seed index 7.6 to 8.7 g for *G. hirsutum* L. Seed index of Purnima and SRT-1 from *G. hirsutum* L. had values of 7 g and 6.8 g respectively, while cotton cultivars Rohini and Eknath from *G. arboreum* L.showed 5 g and 5.5 g seed index respectively (Singh and Deshpande, 1989).

Heterosis for seed index was generally negative. SP-37 had highest seed index value (93.9 mg/seed). Application of nitrogen increased the weight of seed up to 120 kg per ha after which the weight decreased, indicating that increase was not statistically significant. Similar effect of nitrogen was observed in combination with P and K separately. Phosphorus application alone had very little influence, while the potash application increased the seed weight significantly, which may be attributed to the higher production of dry matter. The percentage increase in seed weight as a result of P x K interaction was highest but not significant (Singh *et al.*,1969).

The increase in the added N-level resulted in increasing of seed index significantly, which can be due to the enhancement in photosynthetic activity in leaves, where N is essential for photosynthesis, as a component of chlorophyll, enzymes and cell membranes (Nevins and Loomis, 1970). Chakravorty and Singh (1979) and Gomaa *et al.*, (1981) came to same conclusion.

Application of K_2O increased seed weight significantly in comparison to P_2O_5 + K_2O in combination but this improvement was at par with P_0K_0 statistically. Higher seed weight in response to K_2O has been reported by Singh *et al.* 1969.

Growth regulators application significantly increased seed index, with no particular difference in response between them. This might be attributed to that, applied of these substances at the right concentration and proper time during development it seems, can increase the mobilization of photosynthates of chloroplasts. The sum of the per cent protein and per cent oil was correlated significantly (r= 0.56)with the seed index (El-Nockrashy *et al.*, 1969).

2.1.2 Hull to kernel ratio

Murthi and Achaya (1975) reported the hull to kernel ratio for *G. hirsutum* L. 0.51 to 1.02, *G. barbadense* L. 0.65 to 0.77, *G. herbaceum* L. 0.74 to 1.01 and *G. arboreum* L. 0.77 to 1.14. The hull to kernel ratio for *G. hirsutum* L. was 0.56 (Chakravorty and Singh, 1979).

2.1.3 Moisture

Cotton seed kernels of *G. hirsutum* L. contained about 7 per cent moisture (Zhuge *et al.*, 1988). The moisture content of cotton-seeds obtained from different parts of the world viz., USA 9.0-12.8 per cent, India - A (Indian varieties) 6.8 - 10.6 per cent, India-B (American varieties) 9.0 to 12.1 per cent, Egypt 7.8 - 9.5 per cent; Brazil 6-7 per cent and Sudan 5-6 per cent. The cottonseed revealed 5-12.8 per cent moisture for *G. hirsutum* L. (Altschul *et al.*, 1958). The cultivars of

cotton from *G. hirsutum* L. showed 5.70 to 6.84 per cent moisture (Singh *et al.*, 1969). Murthi and Achaya (1975) reported the moisture content of cotton seed for American cotton cultivars (*G. hirsutum* L.) 5.6 to 14.1 per cent and for *Desi* cotton cultivars (*G. herbaceum* L. and *G. arboreum* L.) 5.8 to 11.3 per cent.

Application of nitrogen did not influence the moisture percentage of cotton seed significantly, singly or in combination. However, application of 180 kg N per ha increased moisture percentage slightly, which was probably related to an increase in the protein content of the seed. Similarly, P slightly decreased moisture percentage. But application of K at 60 kg per ha decreased the moisture percentage significantly, which might be owing to the higher production of dry matter at the cost of moisture as a result of luxurious consumption of potash. The combination of P x K indicated that K decreases the moisture level while P increased it. None of the other interactions influenced the moisture content in the seed (Singh *et al.*, 1969).

2.1.4 Oil and oil quality

Traditionally, cotton seed has been used as cattle feed in our country. The use of cotton seed for extraction of oil on commercial scale is comparatively of recent origin. The growing shortage of other edible oils in the country during the sixties and seventies gave greater impetus to increased usage of cotton seed oil.

Cotton seed oil did not find acceptance as an edible oil till a few decades back due to the presence of small quantity of gossypol, a toxic phenolic compound which also contributes to the colour of the oil in unrefined state. Methods are now available for easy removal of gossypol both from the oil and from the meal remaining after extraction of oil. The refined cotton seed oil which contains practically no gossypol is pale yellow in colour and can be used directly as a cooking medium (Sitaram *et al.*, 1988). In fact, in Egypt, cotton seed oil is the main oil used for cooking purposes. Extensive trials at Cotton Technological Research Laboratory have shown that keeping quality of cotton seed oil is comparable to that of safflower oil but inferior to that of sesame and groundnut oil. However, it may be noted that the cotton seed oil is rarely used as a direct cooking medium in India and its use as an edible oil is mainly as a component oil for the manufacture of Vanaspati or hydrogenated fat.

Cotton seed oil has a light golden colour and a mild pleasant nutty/buttery flavour. The fatty acid profile of cottonseed oil generally consists of 22 per cent saturated fatty acid, primarily palmitic and oleic acid, mono unsaturated fatty acid and approximately 54 per cent linoleic acid, which is an essential fatty acid. The composition gives cotton seed oil its ability to resist oxidation (King and Camire, 1989). The triacylglyceride composition of cotton seed oil also is unique. About 22 per cent of the molecules contain two linoleic and one saturated fatty acid, the next most

common type consists of two saturated fatty acids, usually palmitic, at the one and three acyl position and either oleic or linoleic at two position. Cottonseed oil also is rich in tocopherols, which are natural antioxidants with varying degree of vitamin E activity.

Crude cotton seed oil contains 0.7 to 2.7 per cent phosphatides but the colour of the lecithin is too dark to make it attractive for human consumption. Other non fat components are phenolic pigments gossypol, sterols and cyclopropenoid fatty acids (CPFA) of which sterculic acid is one of the best known example. As much as 1 per cent cyclopropenoid material has been reported in some cotton seed oils (Hamilton, 1987). A major part of cotton seed oil (about 72 % of the cotton seed oil produced in the USA) is used in the manufacture of lard substitutes, about 11 per cent as a cooking oil and 7 per cent for margarine, the remaining is used for soap.

In the breeding material so far analysed oil contents of up to 42.5 per cent and protein content upto 51.4 per cent were found in kernels from individual plants. The maximum linoleic acid content in the oil was as high as 64.8 per cent (Hussein *et al.*, 1986). The oil obtained from cotton seed, which reaches an order of magnitude of 3-4 million tonnes (Fochem, 1985), is used in its refined state principally in human nutrition (Baltes, 1975). The residues from oil production are used as protein source in animal feed in the form of coarse meal or cotton seed cake (Lennerts, 1984).

The percentage of oil in kernels is around 34 per cent irrespective of varieties from any species, however kernel oil content of *G. hirsutum* L. was 32 to 36 per cent, *G. barbadense* L. 35 per cent, *G. herbaceum* L. 34 to 35 per cent and *G. arboreum* L. 32 to 33 per cent (Bhatawadekar *et al.*, 1999). The tetraploid cottons (*G. hirsutum* L. and *G. barbadense* L.) have normally higher oil content than diploids (*G. herbaceum* L. and *G. arboreum* L.) ones (18-24 %) and their contribution to total edible oil supplies is also considerably high (Singh *et al.*, 1995). Cotton seed output in India is 4 million tonnes per year, constituting 9-10 per cent of the total edible oil production and its oil cake is a valuable animal feed with high protein content. The oil content of upland cotton (*G. hirsutum* L.) varied from 20.45 per cent (GHH-1668) to 24.85 per cent (GHH-1691) and the gossypol content in oil from 1.21 (optical density/g oil) in GHH-1668 to 5.87 (optical density/g oil) in GHH-1702. The desirable quality of the seed is high oil content with low level of gossypol (Varghese *et al.*, 1995).

Oil content in cotton seed varied from 14.6 to 25.6 per cent. The seeds of *Desi* cotton (*G. herbaceum* L. and *G. arboreum* L.) cultivar showed an average oil content of 19 per cent whereas *G. hirsutum* L. and *G. barbadense* L. yielded higher average oil content of 21 per cent (Sitaram *et al.*, 1988). Selection from AC-134 contained 28 per cent oil. Russian breeders had succeeded in developing lines (*G. hersutum* L.) having 28.7 per cent oil in the seed (Bredihina, 1970). These figures almost approach the oil content values of safflower.

Seed oil content of 595 cotton cultivars averaged 39.03, 34.76 and 35.01 per cent for *G. hirsutum* L., *G. arboreum* L. and *G. africanum* L. respectively (Sun *et al.*, 1987).

Cotton seed oil content, estimated by nuclear magnetic resonance, was 14 - 25.8 per cent in 162 germplasm lines of *G. arboreum* L. var. *nadam* (Syn. *G. barbadense* L.) with EC-97635 and Egyptian-1 having the highest oil content and 14.5 - 24.5 per cent in 765 lines of *G. arboreum* L. with H-474 highest oil content (Singh, 1988). Ash-25 (*G. hirsutum* L.) had a high seed kernel percentage (57.65 %), seed oil content (24.65 %) and kernel oil content (42.76 %) (Pulatov and Gubanova, 1986). Seed oil content, estimated by nuclear magnetic resonance ranged from 15.4 to 27.3 per cent in 650 *G. hirsutum* L. germplasm lines from the collection of 3000 at Nagpur (Singh and Singh, 1985). Average weight of kernel was 64 per cent in intact seed but its contribution towards oil composition was 98.4 per cent of the total. The seed kernel oil content of *G. hirsutum* L. cultivars was 36 per cent. The low oil content in big seeds was due to less oil synthesis in the kernel (Chakravorty and Singh, 1979). El-Nockrashy *et al.* (1969) reported crude oil content of cotton seed kernel of wild species of cotton like *G. triphyllum* L. is 24.5 per cent. Crude oil content in cottonseed cultivars from *G. hirsutum* L., Purnima was 14.6 per cent, SRT-1, 14.4 per cent and from *G. arboreum* L., Rohini 15.1 per cent and Eknath 16.8 per cent (Singh and Deshpande, 1989).

The iodine value of cottonseed oil extracted from *G. hirsutum* L. was 110.8 which was greater than the groundnut oil (90.9) and mustard oil (101.5). The acid value of cottonseed oil was 0.50 mg KOH / g oil which was less than the groundnut oil (6.96), sesame oil (4.79), mustard oil (3.0) and safflower oil (3.12). The saponification value, specific gravity and refractive index of cottonseed oil was 195.6, 0.9040 and 1.478, respectively (Kamla *et al.*, 1984). The acid value of crude oil extracted from cottonseed kernel of *G. barbadense* L. ranged 0.12 to 0.13 mg KOH / g oil (Sawan *et al.*, 1988). The cottonseed oil was characterised by an iodine value of 90-113 and a saponification value of 180-198. The malvalic acid content determined by HBr titration varied from a low of 0.56 per cent to high of 1.17 per cent. Iodine values of the oil extracted from *G. hirsutum* L. and *G. barbadense* L. ranged from 96.8 to 111.6 (Bailey *et al.*, 1966). Definite correlation could not be established between iodine value and malvalic acid content. Murthi and Achaya (1975) reported the iodine value of crude oil extracted from cotton seed kernel of *Desi* cotton cultivars (*G. herbaceum* L. and *G. arboreum* L.) 91-111 and of American cultivars (*G. hirsutum* L.) 94 to 113. The iodine value of crude oil extracted from *G. hirsutum* L. ranged 92.9 to 114 (Hamilton, 1987) and from *G. barbadense* L. ranged 105.89 to 106.01 (Sawan *et al.*, 1988). The high degree or correlation suggested that for commercial oil the fatty acid composition could be estimated from the iodine value. Stausbury and Hoffpanir (1952) made a systematic study of the relationship between

iodine value and fatty acid composition of oils from a number of native varieties and types. It is well known that the fatty acid composition and iodine value of the oil depend upon environmental factors during development of the seed.

Positive correlation was obtained between refractive index and iodine value ($r = 0.92$). For breeding purpose, varieties with a high iodine value or refractive index with medium seed weight are the most useful (Eissa and EL-Nakhlawy, 1988). The seed oil properties, i.e. acidity, iodine values and saponification values, tended to decrease slightly by increasing N-rate and the application of growth substances, while a reverse trend was noticed by raising P-level (Sawan *et al.*, 1988). Nachaev *et al.* (1983) studied biochemical changes in the oil content, acid number, fatty acid composition of total lipids and the content of free gossypol during the storage of cotton seed under controlled atmosphere.

The fatty acids of the oil contents of palmitic, oleic and linoleic acid, which usually make up more than 90 per cent of total fatty acids (Raie *et al.*, 1983). The mean values for the genotypes between 52.3 - 56.2 per cent and were thus within a range also found by other authors (Yazicioglu and Wetherilt, 1985; Yazicioglu and Karaali, 1983) in plants of Turkish provenance. The level of heritability ($h^2 = 0.28$) was low compared with palmitic and oleic acid and showed that in cottonseed oil also the degree of saturation was affected by environmental factors, a situation often encountered in

other oil plant (Marquard, 1980). The negative correlations between palmitic and oleic acid on the one hand and between oleic and linoleic acid on the other are the results of the substrate/product relationship in the biosynthetic fatty acid pathway.

The cotton seed oil contains 40 to 55 per cent linoleic acid, 20 to 25 per cent palmitic acid, 2 to 7 per cent stearic acid, 18 to 30 per cent oleic acid, and a small proportion of myristic and arachidic acid with about 0.5 to 2 per cent cyclopropenoid acid. Cotton seed contains cyclopropenoid fatty acids (CPFA), malvalic and sterculic acids, in the form of glycerides that are extractable with hexane. CPFA occurring in cotton seed oil affect several species. The laying hens deposit these fatty acids in the egg yolk, on storage the yolks become rubbery and the white turn pink (Phelps *et al.*, 1965). These act synergistically with aflatoxins as liver carcinogens (Hendricks *et al.*, 1980). Adverse effects have not been noted in humans, it is presumed that humans are not affected at normal levels of ingestion (Mattson, 1973). Processing lowers the content of CPFA in crude cottonseed oil especially during deodorization and hydrogenation (Harris *et al.*, 1964; Phelps *et al.*, 1965). Jones (1981) has reviewed the possible effects of CPFA ingested by poultry and animals.

The fatty acid composition of seed oils was determined by G.L.C. Technique from *G. hirsutum* L. grown on dry land and contained 26 per cent palmitic fatty acid which was more than mustard oil (3.5 %), groundnut oil (7.2 %), safflower oil (8.5 %) and

sesame oil (9.9 %). Palmitic, oleic and linoleic acids accounted for more than 95 per cent of the total dry seed fatty acids. Saturated fatty acids consisted of myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic acid ($C_{18:0}$), while unsaturated fatty acids were palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acid, which is usually associated with phosphotidyl ethanolamine and phosphatidyl inositol, was detected in E-2, Pima-S-3, P-23, P-21, Pima-S-4 samples. The correlation coefficients of unsaturated to saturated fatty acid ratio with palmitic, oleic and linoleic acids were 0.99, 0.10 and 0.95, respectively (Bartkowski *et al.*, 1977).

A significantly positive correlation (r = 0.98) existed between unsaturated to saturated fatty acid ratios of total seed lipid and polar lipid. The unsaturated to saturated fatty acid ratios of the neutral lipid fraction, comprised mainly of triacylglyceride skeletons, was not significantly correlated with those ratios obtained from total seed lipid (r = 0.59) (Bartkowski *et al.*, 1977). The average value of the coefficients for correlation of fatty acids with age of seed were for myristic -0.1, for palmitic 0.2, for palmitoleic -0.4, for stearic - 0.3 for oleic -0.4 and linoleic 0.3.

Fatty acid profiles were generally consistent with the fatty acid pattern found in *G. barbadense* L. seed oil. The main fatty acids were palmitic acid (15.57 - 34.33 %), oleic acid (21.86 - 38.27 %) and linoleic acid (23.63 - 49.75 %). Differences were observed in oleic acid : linoleic acid ratios. Crude seed oil of *G. barbadense L.* was more

resistant to oxidation deterioration than the other oils (Awatif and Mohamod, 1997). The phosphatidylinositol with an unusual distribution of fatty acids was detected in cottonseed after 19 hr of germination, 58 per cent of saturated fatty acids (55.4 per cent palmitic acid) was detected in the Sn-2 position and 53 per cent unsaturated acid (34.7 % oleic acid and 17.4 linoleic acid) in the Sn-1 position (Gazizov and Glushenkova, 1996). Stausburg *et al.* (1954) have reported that variety and environment had highly significant influence on oil content. Bairamova (1976) found that raising P level resulted in seed oil percentage increment. Chakravorty and Singh (1979) mentioned that seed weight and seed protein content increased, while seed oil content decreased by raising N-rate. Sawan *et al.* (1982) applied IBA to cotton plant and found that, cottonseed yield, seed index and seed oil content increased without any response on seed protein. El-Halawany (1979) observed that, oil refractive index decreased with N-level increment. The oil acidity, iodine value and saponification values exhibited a very slight reduction as a result of increased N rate.

Light seeds contained low oil percentage and heavy seed also varied in size and oil composition. The percentage weight of kernel in heavy big seed was more but synthesis of oil was less. The synthesis of oil was little affected by agronomical factors, seasonal variations and location (Malik and Khan, 1964; Ali and Ahmed, 1965). Reduction in the oil content of cottonseeds as a result of nitrogen

fertilization has been shown (Khan and Karim, 1968). Similar decrease at higher nutrient level was also observed by Vanden Driessche, (1963). On the other hand Bhatt *et al.* (1961) did not find any marked effect of N application on oil synthesis while protein content went up with graded dose of N (Singh *et al.*, 1969) and average percentage composition of both were of same order (Lopes, 1970). The infection by the pathogen caused a more pronounced alternation in the fatty acid composition of the lipid strongly bound to proteins in the resistant than in the susceptible cultivar, while the reverse was true for the lipids weakly bound to proteins (Gusakova *et al.*, 1995).

2.1.5 Protein and protein quality

Cottonseed meal contains as high as 60-62 per cent protein but it is rendered unfit for use in human diet on account of the inevitable presence of gossypol. The new gossypol free seed from the glandless varieties offers a valuable source of vegetable protein for human and animal nutrition. Edible grade glandless cottonseed and the liquid cyclone processed flour from glandless cottonseed are expected to play an important role in coping with the world protein shortage (Harder and Yang, 1975). Cottonseed meal is finding several applications in various fields in addition to its use as cattle feed. In fact, trials have been conducted on the use of gossypol free cotton-seed meal as a protein suppliment to children in Central American Countries under the auspices of UN agencies. Gossypol free cotton-

seed meal is also being used as nutrient medium in certain fermentation processes, such as for production of penicillin. However, great care has to be taken to ensure that the cottonseed meal is free from contamination by other organisms and also has the required protein content.

The cottonseed kernel protein content varied from 46 to 59 per cent. The protein content in defatted kernel meal of cotton cultivars of *G. hirsutum* L. ranged from 52 - 57.4 per cent, *G. barbadense* L. 47 - 51.2 per cent, *G. herbaceum* L. 46.2 - 52.8 per cent and *G. arboreum* L. 52.4 - 53.9 per cent (Bhatawadekar *et al.*, 1999). The 15 days old seeds of *G. arboreum* L. were having protein content in the range of 12.09 - 13.06 %, which reached a range of 20.95 to 21.05 per cent for fully matured seeds of 60 days old. It could be seen that protein content of seed increased throughout the development (Taneja *et al.*, 1991b). The *G. hirsutum* L. strain SRT-1 had significantly higher protein content (21.1 %) than the *G. arboreum* L. strain Rohini (19.6 %), Eknath (19.8 %) and *G. hirsutum* L. strain Purnima (18 %) (Singh and Deshpande, 1989). The highest protein content in cotton seed was 42.8 per cent found in a *G. hirsutum* L. variety. In general, *G. hirsutum* L. varieties had higher average protein content of 35.5 per cent. The protein content in cotton seed of *G. barbadense* L. 36.6 per cent, *G. herbaceum* L. 34.6 per cent and *G. arboreum* L. 33.8 per cent. The cotton-seed kernel is most important as it contains high percentage of protein (Sitaram *et al.*, 1988).

Sawan *et al.* (1988) reported the protein content in defatted seed meal of *G. barbadense* L. 20.92 to 21.97 per cent. Seed protein content of 595 cotton cultivars averaged 34.64, 38.78 and 38.21 per cent for *G. hirsutum* L., *G. arboreum* L. and *G. africanum* L. respectively (Sun *et al.*, 1987). Protein content in defatted kernel meal of *G. hirsutum* L. cotton is 20.7 to 50.9 per cent (Hussein *et al.*, 1986). The distribution of seed protein percentage in the Texas and Stoneville collections (*G. hirsutum* L.) were similar to those for seed oil percentage (Kohel, 1978). The Texas collection contained a wider range of values (12 to 32 %) than the Stoneville collection (16 to 32 %) and the mean seed protein percentage was lower in the Texas collection (22 %) than that of the Stoneville collection (24 %). The Texas collection has consistently more oil and less protein than the Stoneville collection. The relation between percentage of protein and oil were significantly negative except for a non-significant correlation between embryo oil and embryo protein percentages (Kohel, 1985). Taneja *et al.* (1993) have estimated the protein content in the seeds from developing cotton bolls of *G. hirsutum* L. genotype.

Protein content in Kernel meal of *G. hirsutum* L. cotton ranged 35.7 to 43.5 per cent (Pandey and Thejappa, 1976). Protein content in whole seed of *G. hirsutum* L. cotton ranged 15.83 to 23.10 per cent (Parmar *et al.*, 1973; Pathak and Sood, 1972 and Singh *et al.*, 1969). Protein content in seed of wild cotton species like *G. triphyllum* L. was 61.8 per cent (El-Nockrashy *et al.*, 1969).

Seed protein per cent significantly increased by the added nitrogen fertilizers. This result suggested that the high N-rates might enhance the protein synthesis in the cotton leaves (Rzaev *et al.*, 1973). Over the low rate and stimulate the accumulation of protein in seed more than oil (Elmore *et al.*, 1979). The sugar and protein contents in the cottonseed are reported to be negatively associated (Pathak and Sood, 1972). In the case of protein contents the low level of heritability shows that this characteristics is influenced by other environmental factors e.g. nitrogen supply to the plants (Leffler *et al.*, 1977).

The principal proteins of cottonseed are globulins (Alpha and beta), phosphoproteins, glutelin and glycoproteins. Among these, globulins are major storage proteins of the kernels. The globulins are grouped as 12 S and 7 S and 2 S proteins on the basis of their sedimentation coefficients (Martinez *et al.*, 1970). The 12 S and 7 S are the storage proteins and constitute the major fraction of cottonseed proteins. Reddy and Narasinga Rao (1988a) have named these proteins as gossypin and congossypin. These proteins have a globular shape, an oligomeric structure with subunits. The proteins are rich in glutamic acid, arginine and aspartic acid, while relatively low in sulfur containing amino acids (Reddy and Narasinga Rao, 1988b, 1988c). The soluble protein content in whole seed of *G. hirsutum L.* cotton was 0.2 to 9 per cent (Elmore and Leffl

A toxic polyphenolic pigment :sent in cotton seed, is known to interact with protei ocessing

of cotton seed kernels. Damaty and Hudson (1975) indicated that the major form of binding is the formation of Schiffs base by condensation of formyl group of gossypol with amino group of lysine. They further established that application of heat to flours or isolate containing free gossypol resulted in the formation of insoluble unhydrolyzable products due to irreversible copolymerization between gossypol and cotton seed proteins. Reddy and Narasinga Rao (1988c) reported that interaction between gossypol and storage proteins of cottonseed was involved. The low association constants suggested that the binding was of weak type and involved non covalent interactions.

The 12 S and 7 S proteins dissociated to very low molecular weight monomers under acidic conditions. These monomers reassociate and dissolve in alkaline solution but may not necessarily assume their original configuration. The 12 S and 7 S proteins also undergo association phenomena with changes in ionic strength of alkaline buffers. Protein complement analysis of isolated protein storage vacuoles of dry cotton seed with one dimensional SDS-PAGE gels revealed similar major storage proteins, viz., 53 and 48 KDa, with difference in lower molecular mass proteins. Radicle protein storage vacuoles have apparently more 35-KDa and less 22 KDa storage protein than cotyledon protein storage vacuoles. The major storage protein for cotyledon protein storage vacuoles is similar to that for the insoluble fraction from dry cotton seed (Dure *et al.*, 1983). The SDS-PAGE data provide evidence that the major storage

proteins of cottonseeds are present in the protein PSVs cotyledons and radicles (Vigil *et al.*, 1996). The electrophoretic analysis of seed proteins of cotton extracted using 2.5 per cent NaCl and 50 per cent isopropanol indicated that most of them are water soluble with few being salt or alcohol soluble (Zhang *et al.*, 1998).

The metabolic conversion of supply amino acids into demand amino acids probably occurs in all developing seeds. Clearly, significant shifts occurred in the composition of the free amino acid pools of both cottonseed and field pea (Flinn and Pate, 1968). Two non-protein amino acids, GABA (gamma amino butyric acid) and ethanolamine, were always found in the NPN (Non-protein nitrogen) fraction. These may be two of the unknown amino acids described by Carter *et al.* (1966) in a alcoholic extract of mature cottonseed. The free amino acid content in seed of *G. hirsutum L.* cultivar was 0.027 mg/100 g (Balasubramanian and Gopalan, 1981). Raju and Reddy (1989) reported the free amino acid content in seed of cotton cultivars from *G. hirsutum* L. to be 0.01 to 0.032 mg/100 g, *G. barbadense* L. 0.019 to 0.023 mg/100 g, *G. herbaceum* L.0.021 to 0.023 mg/100 g and *G. arboreum* L.0.020 to 0.024 mg/100 g. In *G. hirsutum* L.the superior genotype for total amino acids are Acala-II X Tamcot SP 21, T X Maroon- 2-78, EL-500 and G-9030-8-5 and in *G. barbadense* L. Sea Island-339, No-6002 and Giza-12 (Singh *et al.*, 1995).

Accompanying the increase in seed protein were changes in the concentration of amino acids (Leffler *et al.*, 1977). These arised

from the N fertilization effects on seed protein types. Seed N was basically composed of three types of amino N plus a fraction of non-amino N such as nucleic acid N. These three amino N fractions were the storage proteins, the metabolic protein (enzymes) and the free amino acids (some times called non-protein nitrogen) (Altschul *et al.*, 1966; Elmore and Leffler, 1976). The free amino acid fraction was quite small in cotton (much less than 1 % of the total). Even if it were influenced by fertilizer application its overall effect would be small (Elmore and Leffler, 1976 and Leffler *et al.*, 1977).

The free amino acid content was high in the susceptible than the tolerant varieties. Free amino acids were more in PRS-72 than in GS-23 and HB-69. Free amino acid or other amino acids such as aspartic acid, glutamic acid, proline and threonine were not found in PRS-72, glycine and tryptophan in GS-23 and alanine, glycine and histidine in HB-69, but Krishnanada (1973) reported that susceptible cotton varieties contained high levels of aspartic acid, glutamic acid, alanine, tyrosine, glycine and threonine. The leaf hopper infestation increased the amino acid content in the order GS-23 > PRS-73 > HB-69 (Balasubramainan and Gopalan, 1981). The positive correlations with harvest dates were noted only for total amino acids, lysine and methinone (Kohel and Cherry, 1983).

The histidine requirement in children is high whereas that of arginine is high in adults, both are high in cottonseed protein (Pandey, 1984). The glutamic acid content in seed proteins of the

diploid species was higher than in seed proteins of other species. *G. barbadense* L.seed can be identified in relation to some amino acid of albumins (histidine and leucine), globuline (histidine, alanine, valine and total essential amino acids) and glutelins (valine and total essential amino acids). These indices of amino acid differed from those of the seed proteins of *G. hirsutum* L. and triploid species. The cultivar specificity of amino acid composition of seed glutelins was of interest from the view point of exploring the possibility of using these indices in selection of seed in directed breeding (Koryakina *et al.*, 1988). Carter *et al.* (1966) reported that the methionine content in seed of cotton cultivars from *G. hirsutum* L. was 1.35 g/16 g N, *G. barbadense* L. 1.65 g/16 g N, *G. herbaceum* L. 1.20 g/16 g N and *G. arboreum* L. 1.28 g/16 g N. The methionine content in seed of cotton cultivars from various species 1.32 g/16 g N, 1.34 g/16 g N, 1.43 g/16 g N and 1.74 g/16 g N of *G. herbaceum* L. , *G. arboreum* L., *G. hirsutum* L. and *G. barbadense* L.,respectively also reported by El-Nockrashy *et al.*(1969). *G. hirsutum* L. of cotton cultivars Stoneville contains 1.3 to 1.7 g/16 g N and Starkville contains 1.4 g/16 g N of methionine (Leffler *et al.*, 1977).

According to the FAO (1970), isoleucine is the first limiting amino acid and sulfur containing amino acids are secondary limiting. Although there is appreciable lysine present, it is generally regarded as low as even limiting in cotton seed meal (Bressani and Elias, 1974). This may be because gossypol complexes with lysine

during processing (Damaty and Hudson, 1975). Berardi and Cherry (1980) reported that tryptophan content in seed of cotton cultivars of *G. hirsutum* L. was 1.4 per cent . 55-225 μg/g of fresh weight tryptophan content in *G. hirsutum* L. species (Balasubramanian and Gopalan, 1981).

The higher levels of glutamic acid (4.09 mg/100 mg) leucine (2.60 mg/100 mg), and phenylalanine (2.24 mg/100 mg) were observed in *G. barbadense* L. and of aspartic acid (3.28 mg/100 mg), glutamic acid (6.09 mg/100 mg), phenylalanine (2.68 mg/100 mg) and arginine (2.55 mg/100 mg) in *G. hirsutum* L. The glutamic acid content was maximum in all samples. AC-3377 and K-9 from *G. arboreum* L. showed maximum percentage 7.2 and 6.8 respectively. Presently, there is a lot of interest in supplementing the microbial culture medium with protein through seed meals having high glutamic acid content in producing biopesticides. Cotton seed meal has been found to contain higher glutamic acid content compared to soybean and groundnut. Proline is an important amino acid imparting resistance to drought, pest and diseases (Bhatawadekar *et al.*, 1994).

The protein and their amino acid composition in the case of different species and varieties of the genus Gossypium showed that a great variability exists in this aspect (Carter *et al.*, 1966; El-Nockrashy, 1969). Four cotton cultivars were given 0 or 50 kg N and 0, 25 or 50 kg P_2O_5/ha. N application increased seed protein content, while the application of 50 kg P_2O_5 decreased it (Patil *et al.*, 1997).

Cotton seed protein concentrates obtained by wet operations, ninety percent ethanol gives an optimum extraction of residual lipid and sugars with minimum removal of nitrogen (Berardi and Cherry, 1980). A light yellow bland concentrate with a high nitrogen solubility, contains < 400 ppm free gossypol and < 3000 ppm total gossypol (Zhuge *et al.*, 1988). Cotton seed protein isolates (90 % protein or higher) are conventionally prepared by extracting protein from cotton seed flour (Berardi *et al.*, 1969). Osman *et al.* (1987) and Hanumantha Rao *et al.* (1987) prepared a low gossypol cottonseed protein isolate using different solvents and studied its functional properties. The isolate exhibited good whipping capacities. The foaming capacities allowed its use in toppings, chiffon mixes and confectionery products.

2.1.6 Ash

Cotton seed kernel ash content of *G. arboreum* L. species varied according to their cultivar such as AK-235 6.4 per cent, Sanjaj 5.7 per cent, *G. herbaceum* L. of Digvijay 8.9 per cent, G.cot-13, 8 per cent, Gujarat-11, 8.9 per cent, Jayadhar, 8.7 per cent and V-797, 8 per cent. *G. hirsutum* L. of Deviraj, 7 per cent, Gujarat-12, 7 per cent, LH-900, 7.6 per cent and SRT-1, 7.7 per cent, while *G. barbadense* L. of Surin, 8.7 per cent. The ash content of interspecific hybrid DCH-32 and hybird-6 was 9.7 per cent and 7.5 per cent respectively (Bhatawadekar *et al.*, 1999). Ash content of defatted kernel meal of cotton cultivar from *G. hirsutum* L. ranged from 6.2 to 20.3 per cent (Lawhon *et al.*, 1974). Martinez *et al.* (1970) reported the ash content of

defatted kernel meal of cotton cultivar of *G. hirsutum* L. 7.9 to 11.4 per cent.

2.1.7 Minerals and vitamins

Generally minerals from plant sources are less available than from animal sources. In recent years, interest has been focused on the bioavailability of minerals which is governed by factors like digestibility, chemical forms of minerals, dietary levels of other nutrients, presence of mineral chelates, particle size of the food and food processing conditions (Reddy *et al.*, 1989). The dietary importance of minerals is very well recognized. Phosphorus is an essential constituent of every known tissue and cell in the body. Zinc is found to act as a cofactor in a variety of enzyme systems. Zinc is also concerned with the fundamental process of RNA and protein synthesis, while iron containing compounds, haemoglobin (heam protein) and myoglobin play a vital role in oxygen transportation (Anonymous, 1993).

Phosphorus content in whole seed of cotton cultivars from *G. hirsutum* L. ranged from 2.1 to 5.35 per cent (Martinez *et al.*, 1970 ,Singh *et al.*, 1972 and Lawhon *et al.*, 1974). Potassium content in seed of cotton cultivars from *G. hirsutum* L. ranged from 0.94 to 1.97 per cent (Balasubramanian and Gopalan, 1978 and Rao *et al.*, 1975). Shanmugham and Bhatt (1990) reported the potassium content in cottonseed of *G. barbadense* L. ranged from 0.95 to 1.21 per cent.

Calcium and magnesium content of defatted cottonseed meal of *G. hirsutum* L. showed 180 mg/100 g and 330 mg/100 g respectively (Altschul *et al.*, 1958). Martinez *et al.* (1970) reported the calcium content in seed of cotton of *G. hirsutum* L. ranged from 100 to 500 mg/100 g. Magnesium content in defatted seed meal of cotton cultivars of *G. barbadense* L. ranged from 140 to 190 mg/100 g (Shanmugham and Bhatt, 1990).

The cotton cultivars of *G. arboreum* L. showed Fe 15 to 35.25 mg/100 g, Mn 3.5 to 5.25 mg/100 g, Zn 1.57 mg/100 g and Cu 1.92 to 3.75 mg/100 g (Taneja *et al.*, 1991c).

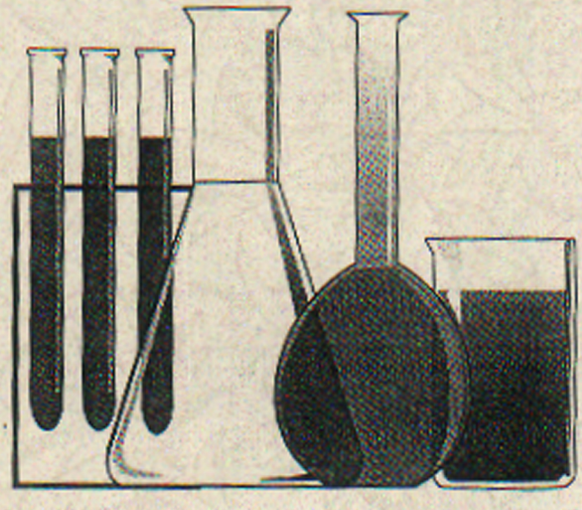
X-ray maps of protein storage vaculoes (PSVs) of dry cotton seed *in situ* indicated that P, K and Mg were mainly localized in protein storage vacuoles while `S' randomly distributed throughout the cross fracture area of cells (Vigil *et al.*, 1996).

Cottonseed are a good source of minerals. The seeds contain low sodium and high potassium and are good source of phosphorus. However, the major portion of phosphorus is present as phytic acid. Kernels are also a good source of thiamin, riboflavin, niacin, pantothenic acid and inositol. The concentration of N and P varied more in burs (Carpel walls) than in seed and P concentration was low in *G. arboreum* L. and high in *G. barbadense* L. Variation in K concentration was small, but the concentration was higher in burs (Carpel walls) of *G. arboreum* L. and *G. herbaceum* L. Phosphorus is involved in the enzymatic reaction in carbohydrate metabolism

including inter-conversion of carbohydrates and improving respiratory energy for the chemical reduction of nitrates. The concentration of Mg was higher in burs than in seeds (Krishna *et al.*, 1990).

Three sprays particularly with a basal dose of 25 kg K/ha increased the N, P, K and Mg contents of cottonseed by 0.18 to 0.43 per cent, 0.04 to 0.06 per cent, 0.04 to 0.06 per cent and 0.02 to 0.06 per cent, respectively and on soil application of 37 kg K/ha by 0.90 to 1.15 per cent, 0.11 to 0.13 per cent, 0.14 to 0.19 per cent and 0.08 to 0.09 per cent, respectively compared with the control. Foliar sprays of K not only increased the K content in cottonseed but also increased the Mg content (Shanmugham and Bhatt, 1990).

Ca and Mg were more in the susceptible PRS-72 and the tolerant GS-23 than in the resistant HB-69, the percentage of increase being 22.72 per cent and 9.09 per cent for Ca and 49.51 per cent and 11.65 per cent for Mg. Singh *et al.* (1972) observed that high amounts of Ca and Mg helped in increasing the osmotic concentration of the cell sap. PRS-72 and GS-23 had 45.45 per cent and 10.91 per cent more Ca content as a result of jassid infestation. The infested RS-72 and GS-23 had 18.51 per cent and 1.66 per cent more Ca than the healthy plants. Jassid infestation reduced the Mg content by 6.49 per cent and 13.91 per cent in PRS-72 and GS-23, respectively, but it increased the Mg content by 58.25 per cent in the resistant HB-69 (Balasubramanian and Gopalan, 1978).



MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Seeds

The seeds of eighteen cotton cultivars of *Gossypium hirsutum* L. were obtained from Cotton Breeder, Cotton Improvement Project , Mahatma Phule Krishi Vidyapeeth, Rahuri (Table 1). The seeds were cleaned manually and stored at 4^0C until used for experiments. Kernels and hulls were manually separated. Kernels were ground into fine powder (60 mesh) using coffee grinder. Fine powder of cottonseed kernels was defatted using petroleum ether and defatted powder was used for their chemical analysis.

3.1.2 Chemicals

Most of the chemicals used in this study were of analytical grade (Merck, S.d. fines, Qualigens, SRL or Himedia).

3.2 Methods

3.2.1 Seed index

The seed index was calculated by weighing the 100 g of seed of cotton cultivars.

3.2.2 Hull to Kernel ratio

The hull to kernel ratio was determined by dividing weight of hulls by weight of kernels of seed of cotton cultivars.

Table 1. Pedigree and salient features promising cotton cultivars

Sr. No.	Cultivars	Pedigree	Salient features
1.	PKV-081	G-47/4 x LL	3-4 g boll wt, 38 % ginning, white colour fibre, 10-12 q/ha kapas yield
2.	LRA-5166	Laxmi x Reba-B-50 x Acala-122	20 bolls/plant, 4.3 g average boll wt, 35.6 % ginning, 546 kg/ha kapas yield, cultivated in M.S., A.P., and T.N.
3.	JLH-168	Reba-B-50 x IAN-579/188	3-3.5 g boll wt., 27.2 mm mean fibre length, white and smooth fibre, 10-12 q/ha kapas yield
4.	DS-28	-	Boll large, roundish, 4-5 loculed, seed medium size, dull seed, white fuzzy
5.	G.cot.100	-	Released from Cotton Research Station, Surat, Boll elongated and medium size, seed medium size
6.	GB-20	Reba TK-1 x G.cot.10	GB-20 is popular name of G.cot.16, released to replace G.cot.10, 27 mm fibre length, 34 % ginning, 16 q/ha kapas yield
7.	SRT-1	Selection from KW-66-2096	SRT-1 is popular name of G.cot 10, 3-3.5 g boll wt., 35 % ginning, white and coarse fibre, 25 mm fibre length, 12-15 q/ha kapas yield
8.	NHH-44	BN-1 x AC-738	Released from Cotton Research Station, Nanded in 1983 recommended for Maharashtra, Karnataka and A.P., roundish and medium size boll
9.	BN	-	Released from Bikaner Research Station roundish and medium size boll
10.	ERB	Introduced	Original nomenclature is ERB-4492, 33.6 % ginning, good spinning potential, 4.2 q/ha kapas yield
11.	RHC-0191	-	31 bolls/plant, 4.0 g. average boll weight, 34.3 % ginning, 1350 kg/ha seed cotton yield
12.	RHC-0688	-	23 bolls/plant, 3.9 g average boll wt., 37.2 % ginning, 1701 kg/ha seed cotton yield

Table 1 contd.....

Sr. No.	Cultivars	Pedigree	Salient features
13.	RHC-994	-	1816 kg/ha seed cotton yield
14.	RHC-1088	-	1218 kg/ha seed cotton yield
15.	RHC-1189	-	29 bolls/plant, 4.1 g average boll wt., 36.7 % ginning , 1408 kg/ha seed cotton yield
16.	RHC-1794	-	598 kg/ha seed cotton yield
17.	RHC-3194	-	24 bolls/plant, 4 g average boll wt., 36.6 % ginning, 1101 kg/ha seed cotton yield
18.	RHC-9740	-	35 bolls/plant, 4.3 g average boll wt., 34.4 % ginning, 1545 kg/ha seed cotton yield

3.2.3 Moisture

The cottonseed kernel moisture was estimated by A.O.A.C., (1990).

One grams of the cottonseed kernel defatted sample was accurately weighed and dried at 105^{0}C for 5 hrs. After cooling in the desicator, it was weighed again. Drying was continued for one more hour and the sample was again weighed. The drying and weighing were repeated until constant weight was obtained. The loss in weight was recorded as the moisture content.

$$\% \text{ Moisture} = \frac{\text{Weight of sample before drying (g)} - \text{Weight of sample after drying (g)}}{\text{Weight of sample taken (g)}} \times 100$$

3.2.4 Oil and oil quality

3.2.4.1 Crude oil

The crude oil content was determined by the ether extraction using soxhlet apparatus (A.O.A.C.,1990).

Reagent

Petroleum ether having a boiling point of 40-60^{0}C.

Procedure :

Five grams of powdered sample (60 mesh) was accurately weighed and transferred to a thimble. The thimble was plugged with cotton and connected the thimble to the pre-weighed oil extraction flask. The petroleum ether was poured into the thimble till one siphoning was over (approximately 250 ml). Then again refilled the

petroleum ether with sufficient quantity (approximately 150 ml) into the thimble. Connected the thimble along with the oil extraction flask to the condenser and started running tap water and heating at 60^{0}C. Continued the extraction till 8-10 siphonings were completed. Disconnected the assembly and evaporated excess ether from extraction flask over water bath until no odour of ether remains in the oil flask. Cooled the oil extraction flask in desicator and weighed the flask. Repeated heating again and weighed till the constant weight was obtained and recorded the weight. Calculated the per cent crude oil content in the sample using following formula.

$$\% \text{ crude oil} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample taken (g)}} \times 100$$

3.2.4.2 Identification of oil colour

Oil colour of different cultivars of cotton seed kernels was identified by visual observation and comparison with Methuen Colour Chart (Kornerup and Wanscher, 1978).

3.2.4.3 Iodine value

The iodine value of crude oil was estimated by the method of A.O.A.C. ,(1990).

Reagents

1. Lipid solvent [Chloroform ($CHCl_3$)].
2. Hanus reagent : 13.2 g pure iodine were dissolved in acetic acid containing 3 ml of bromine solution and final volume made to 1 litre with acetic acid.

3.	Potassium iodide	:	15 per cent
4.	Sodium thiosulphate	:	0.1 N
5.	Starch indicator	:	1 %

Procedure

The 0.5 g oil was weighed accurately in 250 ml iodine flask and dissolved in 10 ml of lipid solvent. Hanus reagent (25 ml) was added to it and allowed to stand for 30 min in dark. The content of the flask was shaken occasionally. Subsequently, 10 ml of 15 per cent potassium iodide solution was added, mixed thoroughly and titrated with 0.1 N sodium thiosulphate until yellow colour turned almost colourless. Then, few drops of starch indicator were added and the titration was continued until blue colour disappeared. The quantity of sodium thiosulphate required for titration was used for calculation of iodine value. A blank containing all reagents except oil was run simultaneously. The titre value was determined by subtracting samples reading from the blank.

$$\text{Iodine value} = \frac{(B - S) \times N \times 12.69}{\text{Weight of sample (g)}}$$

Where,

B = ml of $Na_2S_2O_3$ (0.1 N) required for titration of blank

S = ml of $Na_2S_2O_3$ (0.1 N) required for titration of sample

N = Normality of $Na_2S_2O_3$ (0.1 N)

3.2.4.4 Acid value

The acid value of crude oil was determined by A.O.A.C., (1990) method.

Reagents

1. Lipid solvent [ethanol + petroleum ether, 50 : 50 (v/v)]
2. Potassium hydroxide : 0.01 N
3. Phenolphthalein indicator

Procedure

The crude oil was accurately weighed (0.5 g) and dissolved in 25 ml lipid solvent in a conical flask in triplicate. Phenolphthalein indicator (3 to 4 drops) was added to flask and mixed thoroughly. The contents were titrated with 0.01 N potassium hydroxide until pink colour persisted for 20 to 30 seconds. The amount of alkali required for neutralisation was used for calculation of the acid value. A blank titration without oil was run simultaneously. The sample titre value was calculated by subtracting the blank reading from sample reading.

$$\text{Acid value} = \frac{\text{Titre value x N of KOH x 56.1}}{\text{Weight of sample (g)}}$$

3.2.5 Protein

3.2.5.1 Crude protein

The protein content was determined by microkjeldhal method (A.O.A.C., 1990).

Reagents

1. Concentrated sulphuric acid (Sp. gr. 1.84, purity 98.08 per cent and nitrogen free)
2. Catalyst mixture : Potassium sulphate, mercuric oxide and copper sulphate were weighed, 99, 4.1 and 0.8 g respectively. Mixed and ground into fine powder.
3. Sodium hydroxide (50 %) : 50 g of sodium hydroxide and 5 g of sodium thiosulphate were dissolved in distilled water separately then mixed and the volume was made upto 100 ml with distilled water.
4. Boric acid (4 %) : 4 g of boric acid was dissolved in distilled water and the volume was made upto 100 ml with distilled water.
5. Hydrochloric acid (0.02 N) : 0.17 ml of hydrochloric acid (Sp. gr. 1.18, purity 35.4 %) was dissolved in distilled water and the volume was made upto 100 ml with distilled water.
6. Hydrogen peroxide : 30 % (v/v) commercially available in the market.
7. Mixed indicator : Mixed indicator was prepared by dissolving 0.1 g of bromocresol green and 0.1 g of methyl red in 100 ml of 95 per cent alcohol separately. Ten parts of bromocresol green and 2 parts of methyl red indicator were mixed together and transferred to a bottle provided with stopper.

Procedure :

Powdered and defatted sample (200 mg) was accurately weighed and transferred to digestion flask. One gram of catalyst mixture was added and mixed thoroughly with the sample. Five ml of concentrated sulphuric acid and 5 ml of hydrogen peroxide were carefully added and sample was digested in digestion chamber. Initially the flasks were heated slowly for 10 to 15 min and then the temperature was raised gradually so that the contents boiled briskly. The digestion was continued until the sample became clear and colourless. Then flasks were cooled to room temperature and after cooling the contents were transferred to volumetric flasks. The digestion flasks were washed 3 to 4 times with distilled water. All the washings were transferred to volumetric flasks and volume was made to 50 ml.

Ten ml of boric acid solution was pipetted into 100 ml beakers and 6 to 8 drops of mixed indicator solution were added. The beaker was placed under condenser of the distillation unit. At the end of distillation, the tip of condenser was washed to collect all ammonia. The distillate was then titrated with standard hydrochloric acid (0.02 N) solution. Before distillation the colour of boric acid plus indicator was pink which changed to blue green during distillation and finally to pink red at the end of the titration. Blank titration without sample was also carried out. The percentage of nitrogen content was calculated from the quantity of standard hydrochloric acid required

for titration of sample. The protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

$$\% \text{ Nitrogen} = \frac{(S - B) \times N \times 14.007}{\text{Weight of sample (g)}} \times \frac{\text{Volume made}}{\text{Volume taken}} \times 100$$

Where,

S = ml of hydrochloric acid required for sample titration

B = ml of hydrochloric acid required for blank titration

N = Normality of HCl (0.02 N)

% Protein = % Nitrogen x 6.25

3.2.5.2 Soluble protein

The soluble proteins present in the defatted meal of cotton seed kernels were determined by the colorimetric method described by Lowry *et al.,* (1951) using bovine serum albumin as standard protein.

Reagents

1. Reagent `A' (Alkaline sodium carbonate) : Two grams of sodium carbonate were dissolved in 0.1 M sodium hydroxide and volume was made to 100 ml with 0.1 M NaOH.
2. Reagent `B' (Copper sulphate - Sodium potassium tartarate solution) : Five hundred milligrams of $CuSO_4.5H_2O$ were dissolved in 1 % (w/v) sodium potassium tartarate solution

and volume was made to 100 ml with 1 % sodium potassium tartarate solution.

3. Alkaline copper tartarate reagent : Fresh alkaline copper tartarate reagent was prepared just before use by mixing 50 ml of reagent `A' with 1 ml of reagent `B'.
4. Folin-Ciocalteau (Phenol) reagent : It was prepared by diluting one part of commercial grade reagent with one part of distilled water on the day of use. This was a solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid.
5. Stock solution of Bovine serum albumin (BSA) : The BSA was weighed 100 mg accurately and dissolved in distilled water and volume was made 100 ml with distilled water.
6. Working standard solution of BSA : Ten ml of the stock solution was pipetted in the 100 ml volumetric flask and volume was made with distilled water. The concentration of this solution was 100 µg per ml.

Procedure :

A. Extraction of soluble protein

0.5 g of finely powdered sample was weighed and extracted in 20 ml distilled water on mechanical shaker for 60 min. It was then centrifuged at 10,000 rpm for 30 min and supernatant was collected in 50 ml volumetric flask. This process was repeated again two times and supernatants were combined to make final volume

upto 50 ml with distilled water and clean supernatant was used for the estimation of soluble proteins.

B. Colour development

0.1 ml supernatant was pipetted in the test tube in triplicate, mixed with 5 ml of alkaline copper reagent and kept for 10 minutes at room temperature. To this, 0.5 ml of diluted Folin - ciocalteau reagent was rapidly added with immediate mixing. The intensity of blue colour was measured after 30 min at 660 nm on Spectronic-20.

C. Calibration of standard curve :

The 0, 0.1, 0.2, 0.3, ... , 1, ml (i.e. 10, 20, 30, ..., 100 μg) of the working standard solution of BSA was pipetted in a series of the test tubes in triplicate. The volume was made 1 ml with distilled water and colour was developed as above (section B). The absorbance was measured after 30 min. at 660 nm and standard curve was prepared by plotting absorbance against concentration of standard protein (Fig 1)

The soluble protein was calculated from the standard curve and results were expressed as g of soluble protein per 100 gram dry weight of sample.

3.2.6 Amino acids

3.2.6.1 Free amino acids

The free amino acids were determined by ninhydrine method as described by Rosen (1957).

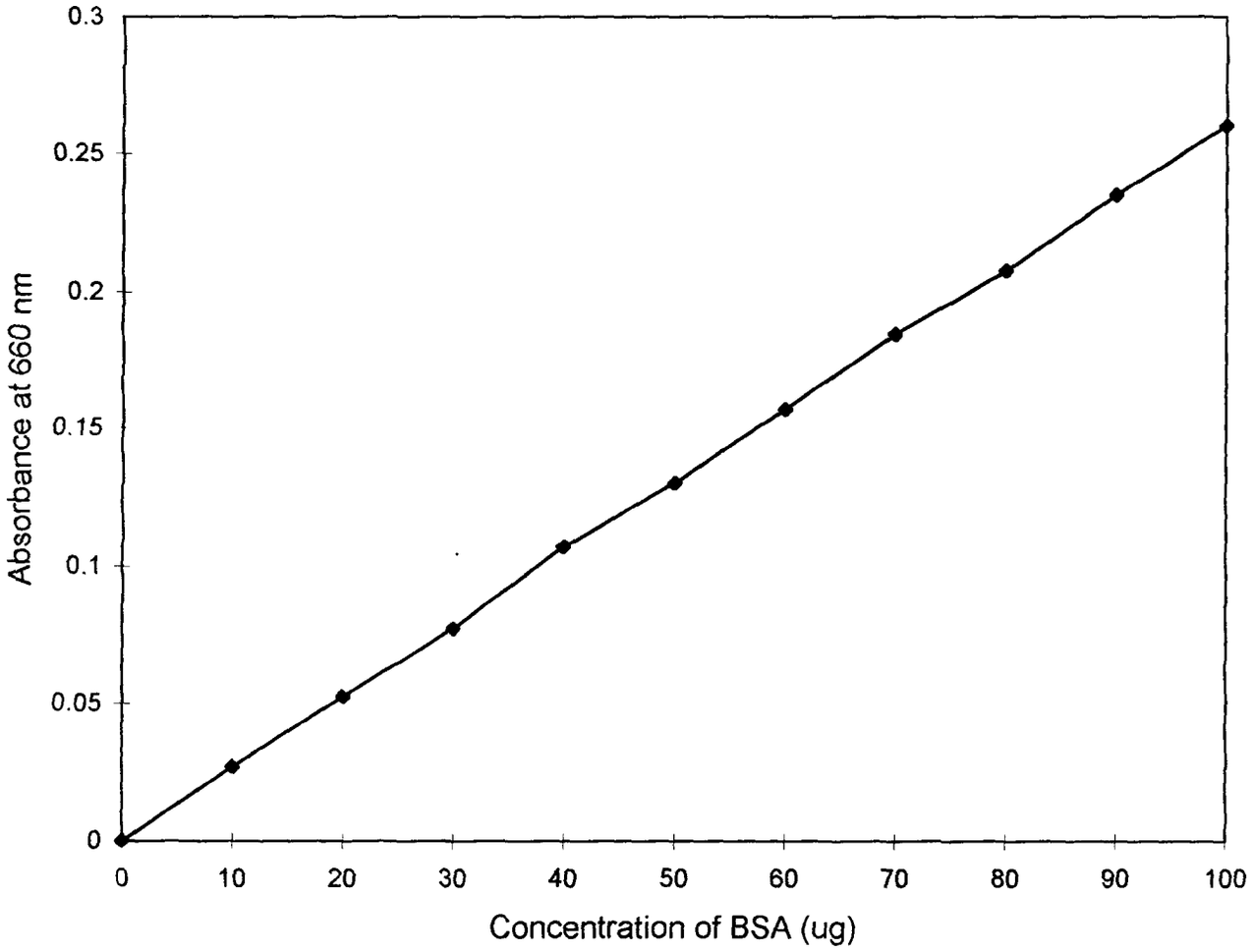


Fig. 1. Standard curve for the estimation of soluble protein

Reagents

1. Citrate Buffer (0.05 M, pH 5.0) :

A. 0.1 M citric acid : Dissolved 2 g of citric acid in 100 ml distilled water.

B. 0.1 M sodium citrate : Dissolved 2.94 g sodium citrate in 100 ml distilled water.

Mixed 20.5 ml of `A' and 29.5 ml of B and diluted to 100 ml with distilled water.

2. Diluent (isopropanol : water) : Mixed equal volumes of isopropanol and water (1:1) to make required volume.
3. Ninhydrin reagent (3 %) : Dissolved 1.5 g of ninhydrine in 2-methoxy ethanol and volume made to 50 ml.
4. Standard amino acid solution (100 μg/ml) : Dissolved 10 mg L-leucine in distilled water and volume made to 100 ml. The concentration of L-leucine was 100 μg/ml.
5. Ethanol (70 %) : (70 ml ethanol plus 30 ml distilled water)

Procedure :

A. Extraction of amino acid from sample

Weighed 0.5 g of finely powdered defatted sample and transferred it to a capped centrifuse tube. Added 4 ml of ethanol, shaked on mechanical shaker for 3 hrs and centrifused at 10,000 rpm for 15 minutes. Repeated the extraction two more times and collected all supernatants in beaker. Allowed to evaporate excess ethanol on

hot water bath till 10 ml solution remained in the beaker. The volume of extract was made to 100 ml with distilled water. Used this extract for estimation of free amino acids.

B. Colour development

One ml of above extract was taken in test tube in triplicate, then added 1 ml of water, 0.5 ml of citrate buffer and 0.5 ml of ninhydrin reagent. Heated all test tubes in hot water bath for 15 minutes (bluish purple colour formed). Allowed them to cool and added 5 ml diluent, mixed thoroughly on cyclo mixer. The intensity of colour was measured at 570 nm on Spectronic-20.

C. Preparation of standard curve

Zero to 2 ml of L-leucine was taken in duplicate in test tubes. Volume made in each test tube to 2 ml with distilled water. Colour was developed as described for sample. Standard graph was drawn (Fig 2) and the amount of free amino acids present in given sample was calculated by using the standard graph

3.2.6.2 Methionine

This amino acid was estimated by a method described by McCarthy and Paille (1959).

Reagents

1. 2 N Hydrochloric acid : Diluted 86.2 ml of 11.6 N HCl (reagent grade) to 500 ml with distilled water.
2. 10 per cent sodium hydroxide : Dissolved 10 g of NaOH in distilled water and final volume made to 100 ml.

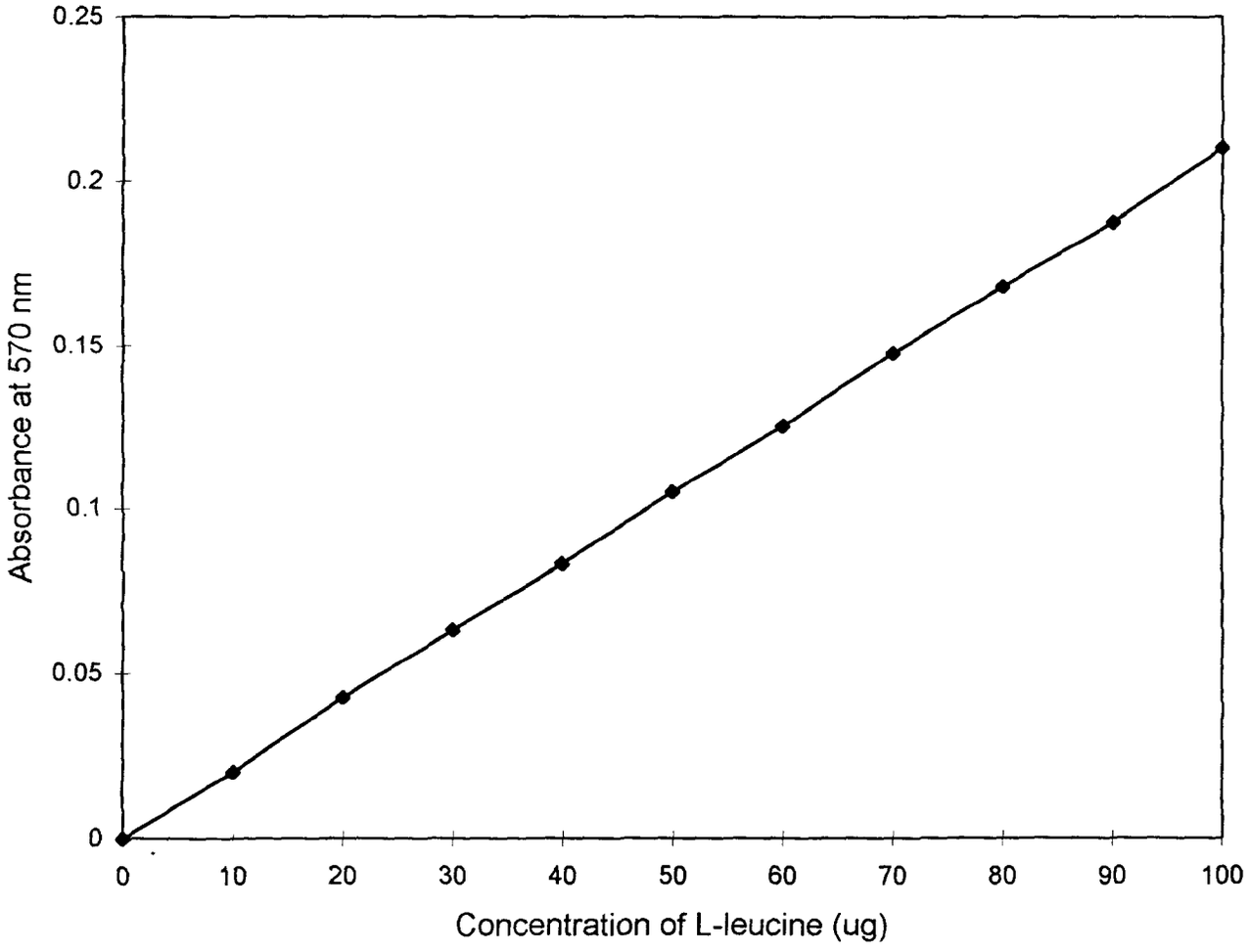


Fig. 2. Standard curve for the estimation of free amino acids

3. 10 per cent sodium nitroprusside : Dissolved 10 g of sodium nitroprusside in distilled water and final volume made to 100 ml.
4. 10 N sodium hydroxide : Ten grams of NaOH dissolved in distilled water and final volume made to 100 ml with distilled water.
5. 3 per cent glycine : Dissolved 3 g of glycine in distilled water and final volume made upto 100 ml with distilled water.
6. Concentrated phosphoric acid commercially available.
7. Standard methionine solution : Hundred mg of DL-methionine was dissolved in 0.5 ml of 20 per cent hydrochloric acid and further diluted to 100 ml with distilled water. This solution contained 1 mg DL-methionine per ml.

A. Extraction of methionine :

Methionine was extracted according to the method described by Gupta and Das (1955). One gram of defatted sample was autoclaved with 25 ml of 2 N HCl at 15 lbs pressure for one hour. The hydrolysate was treated with a pinch of activated charcoal to get rid of colour, heated to boiling and filtered. The charcoal was washed 3 to 4 times with hot water quickly and the washings were collected. The colour free extract was neutralized with 10 N NaOH to bring the pH to about 6.5. The volume was made to 100 ml.

B. Colour development

The colour was developed according to the method described by McCarttry and Paille (1959). The extract (50 ml) in duplicate were taken in 250 ml conical flask and 6 ml of 10 per cent NaOH were added. This was followed by addition of 0.3 ml of sodium nitroprusside and contents were kept for 10 min with occassional shaking. After 10 min, 2 ml of glycine were added, shaken well and allowed to stand for 10 min. Then 4 ml of concentrated orthophosphoric acid were added. Shaken vigorously and colour intensities were measured after 10 min on Spectronic-20 at 540 nm.

C. Standard curve

For preparation of a standard curve, different concentrations of methionine (2, 4, 6 and 8 mg) were taken in duplicate, water was added to make up the volume to 50 ml. The colour was developed in the same way as described for samples. The methionine content in sample was calculated from standard curve (Fig. 3) and expressed as per cent as well as gram per 16 g N.

3.2.6.3 Tryptophan

Tryptophan present in the samples was determined by the colorimetric method as described by Spice and Chambers (1949).

Reagents

1. 19 N sulphuric acid. Diluted 52.7 ml concentrated 36 N H_2SO_4 to 100 ml with distilled water.

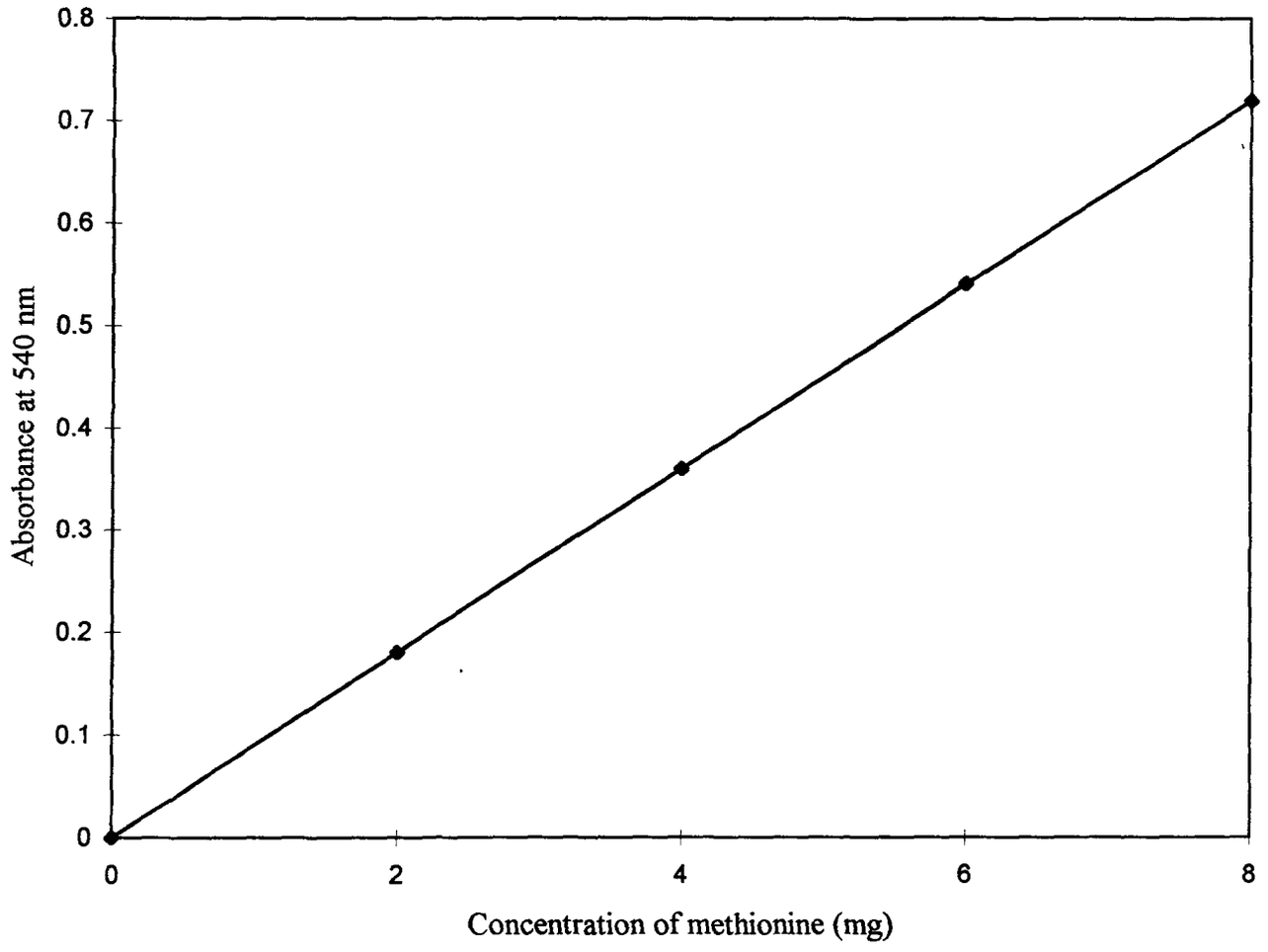


Fig. 3. Standard curve for estimation of methionine

2. 20.4 N sulphuric acid : Thirty six normal H_2SO_4 56.7 ml was diluted to 100 ml with distilled water.
3. 0.045 per cent sodium nitrite : Dissolved 0.045 g sodium nitrite in water and final volume was made upto 100 ml.
4. *P*-dimethyl amino benzaldehyde.
5. Standard tryptophan solution : L-tryptophan (20 mg) was dissolved in 100 ml distilled water (2 drops of 10 per cent NaOH were added to facilitate solubilization of tryptophan). This solution contained 200 µg L-tryptophan/ml.

Procedure :

A. Tryptophan in sample

The ground defatted sample (40 mg) was taken in 50 ml conical flask in triplicate and 30 mg of *P*-dimethyl amino benzaldehyde were added to each flask. The third flask in each sample was kept for blank. Then, 10 ml of 19 N H_2SO_4 were added to each flask. After keeping the flasks in dark for 20 hrs at 30^0C, 0.1 ml of $NaNO_2$ was added to each flask except in blanks, shaken gently and kept in dark for 30 min for colour development. The contents were filtered through glass wool and the colour intensities were measured at 600 nm on Spectronic-20.

B. Standard curve

The standard tryptophan solution (0 to 120 µg) was taken in 50 ml concial flask in triplicate. The water was added to make up volume to 0.6 ml in each flask. Then 9.4 ml of 20.4 N H_2SO_4 were

added to each flask in order to make 19 N as final strength of the acid. The *P*-diamethyl amino benzaldehyde (30 mg) was then added to each flask and flask were kept in dark for 20 hrs at 30^{0}C. Rest of the procedure was same as described for samples. The tryptophan content in samples was determined from the standard curve (Fig.4) after making the necessary corrections for the blank values for each sample. Tryptophan was expressed as per cent in meal and g per 16 g N.

3.2.7 Ash

The ash content of sample was estimated by the method of A.O.A.C., (1990).

One gram of the powdered sample was accurately weighed into a pre-weighed silica crucible. It was then carbonised in silica crucible on burner followed by heating at about 600 ^{0}C for 6 hrs in the muffle furnace to get complete white coloured ash. Allowed them to cool in the furnace. Then transferred the crucible to a desicator and weighed as quickly as possible to prevent moisture absorption. The ash was calculated using following formula.

$$\% \text{ Ash} = \frac{\text{Wt. of crucible with ash} - \text{Wt. of crucible}}{\text{Wt. of sample in g}} \times 100$$

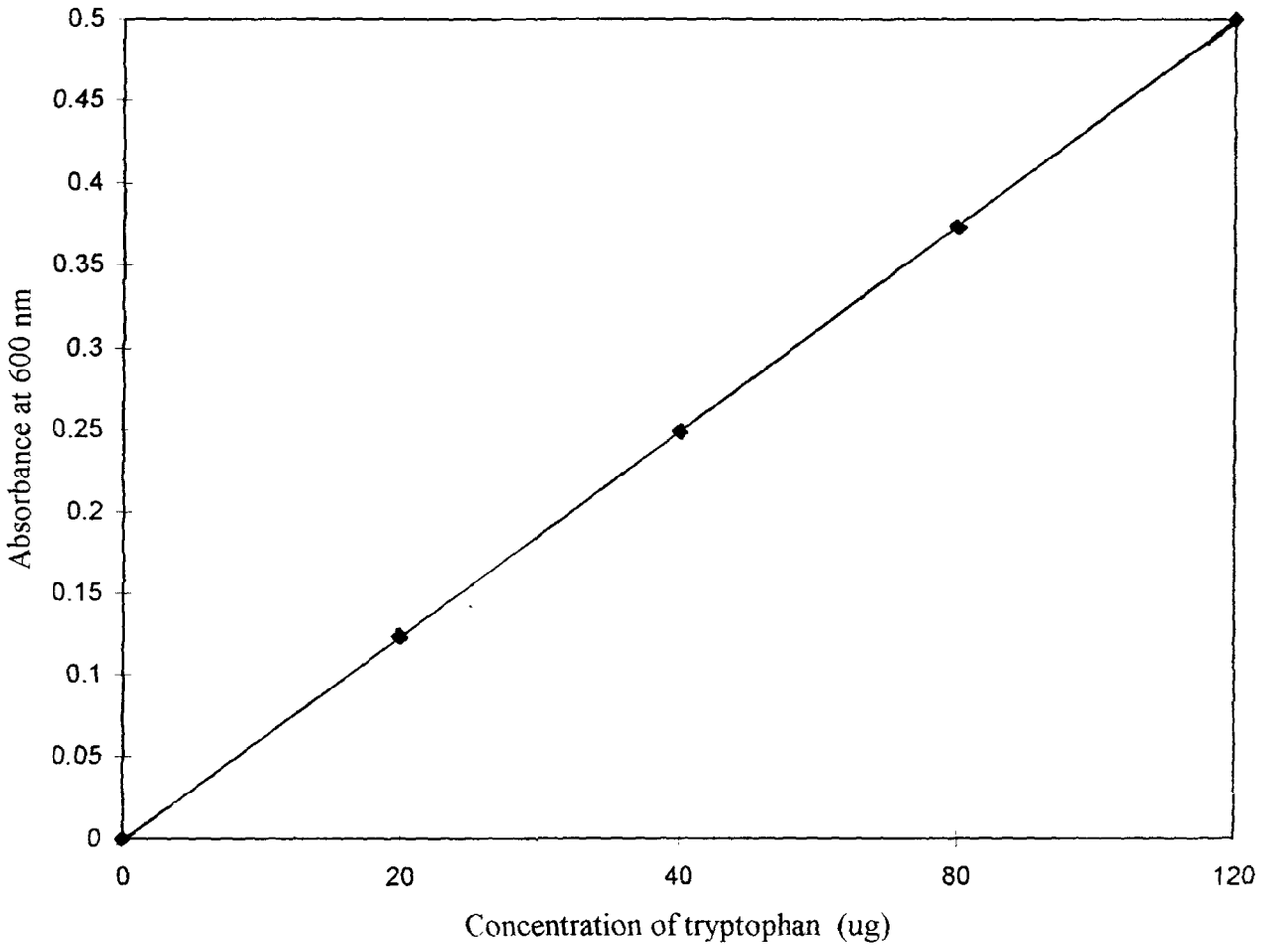


Fig. 4. Standard curve for estimation of tryptophan

3.2.8 Minerals

3.2.8.1 Phosphorus

The phosphorus in the meal was determined by colorimetric method as described by Chapman and Pratt (1961).

Reagents

1. Ammonium molybdo-vanadate reagent : Ammonium molybdate (22.5 g in 400 ml distilled water), ammonium vanadate (1.25 g in 300 ml boiling distilled water) and 250 ml concentrated nitric acid were mixed together and volume was made to 1000 ml with distilled water.
2. Standard orthophosphate solution : Potassium dihydrogen phosphate (0.2194 g) was added in distilled water and diluted to 100 ml. This solution contained 50 µg phosphorus per ml.

Procedure

A. Digestion of sample

Ignited the powdered sample (1 g) to ash as described under section 3.2.7. Digested the ash sample with 20 ml H_2O, 5 ml H_2SO_4 and 25 ml HNO_3 mixture. The digestion was carried out at low temperature initially and continue heating until all nitrate fumes are removed. The content was cooled and filtered through Whatman No.1 filter paper and volume made to 50 ml.

B. Phosphorus in the sample

The digested sample (1 ml) was taken in 50 ml volumetric flask and 10 ml ammonium molybdo-vanadate reagent was added.

The contents were diluted to 50 ml and mixed well. The absorbance was measured after 30 min at 470 nm on a Spectronic-20. The total phosphorus in sample was calculated from the standard curve.

C. Standard curve

Standard orthophosphate solution 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml was taken into 50 ml volumetric flasks and 10 ml of the ammonium molybdate-vanadate reagent was added. The contents were diluted to 50 ml with distilled water and mixed well. The absorbance was measured after 30 min at 470 nm on Spectronic-20 and graph was prepared (Fig. 5).

3.2.8.2 Potassium

The potassium content in defatted cottonseed kernel meal was estimated by flame photometer method as described by Chapman and Pratt (1961).

Reagents

1. Stock solution of potassium chloride : Potassium chloride, 1.907 g was accurately weighed and dissolved in 1000 ml distilled water.
2. Working standard solution of potassium chloride : Ten ml of stock solution was diluted to 100 ml with distilled water. This solution contains 10 µg of potassium per ml.

Procedure

A. Potassium in sample

Exactly 0.1 ml of digested extract (digestion described as under 3.2.8.1) was diluted to 50 ml with distilled water in a

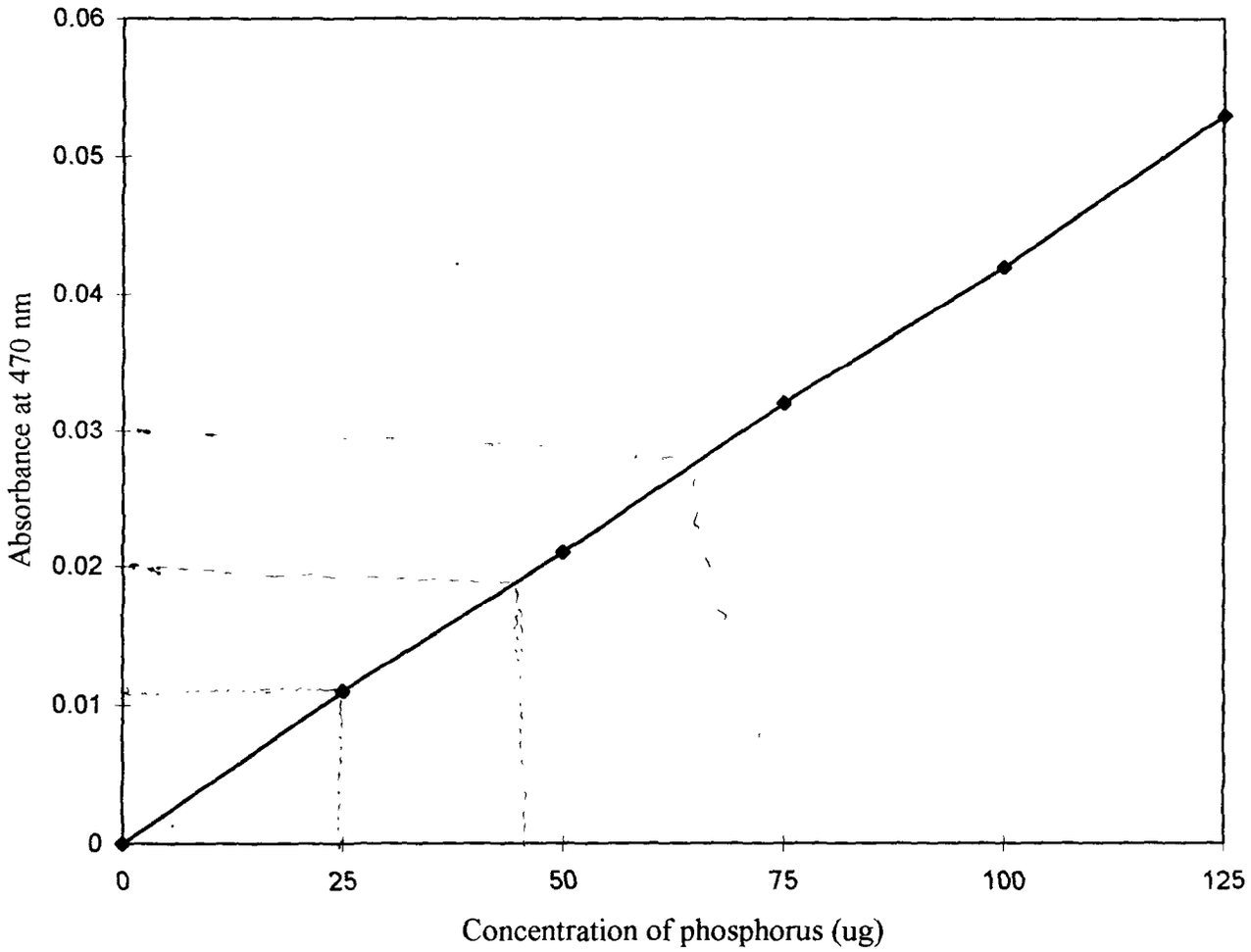


Fig. 5. Standard curve for estimation of phosphorus

volumetric flask. The readings of diluted samples were taken on flame photometer (Aimil Sales and Agencies Pvt. Ltd., Bangalore).

B. Calibration of standard curve

The 0, 0.5, 1, 1.5 ...., 5 ml of working standard solution of KCl was pipetted in the 50 ml volumetric flask in duplicate and the volume was made with distilled water. The readings were recorded on flame photometer and standard graph was prepared by plotting flame photometer reading against concentration of potassium (Fig. 6).

The potassium content in the sample was determined using standard graph and results were expressed in percentage on dry weight basis.

3.2.8.3 Calcium

The calcium content in the defatted meal of cotton kernels was determined by the method as described by Black *et al.* (1965).

Reagents

1. Standard calcium solution (0.01 N) : Pure dried calcium carbonate 0.5 g was weighed and dissolved in 10 ml of 0.2 N HCl. The solution was boiled till the CO_2 was completely driven off. Then it was cooled and volume was made to 1 litre accurately.
2. Standard ethylenediamine tetra acetic acid (EDTA) solution : Two grams of EDTA and 0.03 g of $MgCl_2$. $6H_2O$ were dissolved separately in distilled water, these solutions were mixed and volume was made to 1000 ml with distilled water.

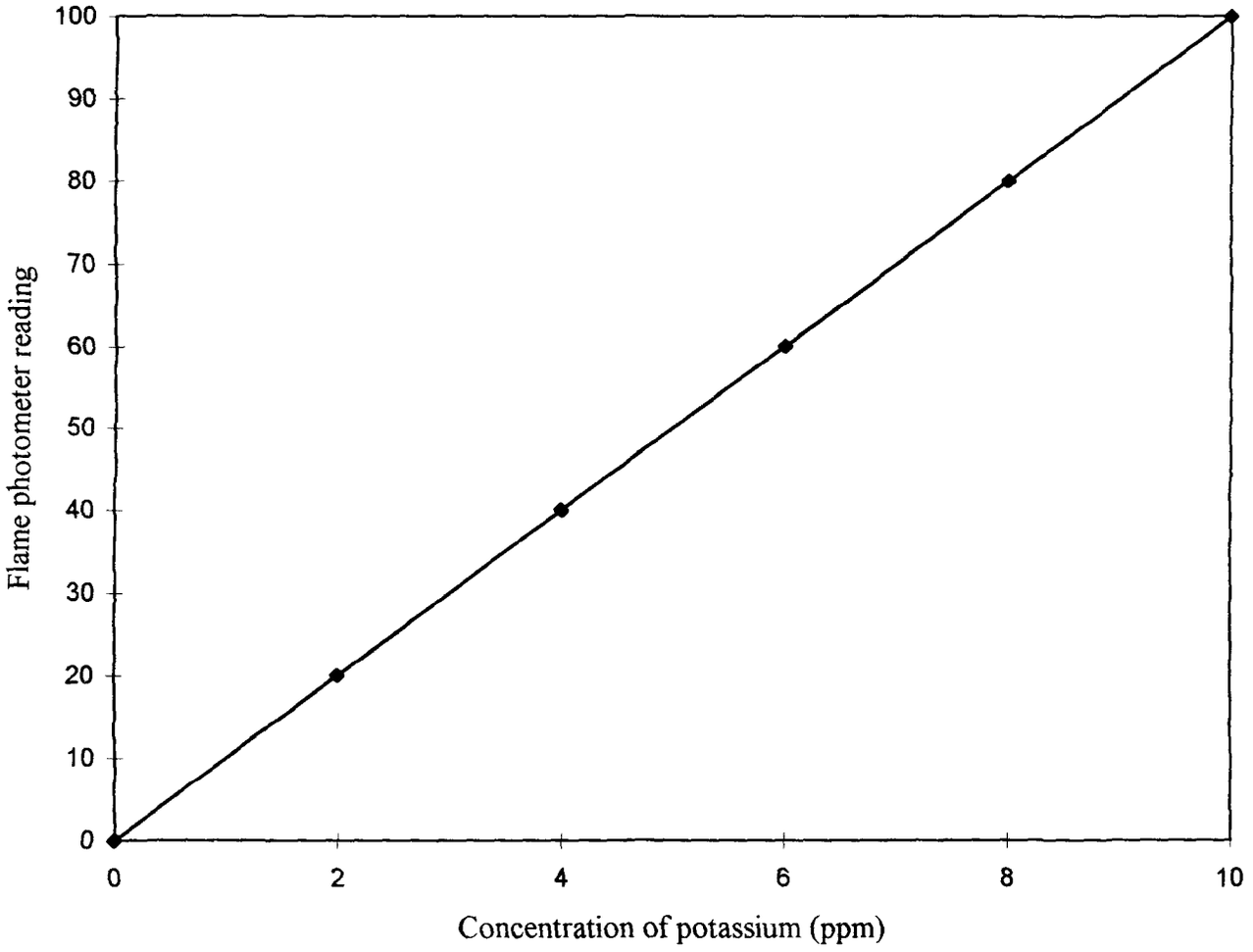


Fig. 6. Standard curve for estimation of potassium

3. Sodium hydroxide (10 %, w/v) : Sodium hydroxide, 10 g was dissolved in distilled water and final volume was made to 100 ml.
4. Potassium ferrocyanide solution (4 %, w/v) : Four grams of K_4FeCN_6 were dissolved in distilled water and volume was made to 100 ml.
5. Erichrome Black T indicator (EBT) : EBT, 0.4 g was dissolved in 100 ml methanol.
6. Calcon indicator : Calcon powder, 40 mg was dissolved in 100 ml of methanol.
7. Triethanolamine (TEA)

Standardization of EDTA

Standard calcium solution (0.01 N) of 25 ml was pipetted into a conical flask. Few drops of EBT indicator were added and it was titrated with EDTA solution and titre reading was recorded. The normality of EDTA was standardized by using the formula $N_1V_1 = N_2V_2$.

Procedure

Determination of calcium :

To the 5 ml of digested extract (digestion described as under 3.2.8.1), 10 ml of distilled water was added, followed by 5 ml of 10 per cent NaOH and 5 to 6 drops each of potassium ferrocyanide solution and triethanolamine (TEA). The flasks were allowed to stand for 10 minutes. After 10 min ten drops of calcon indicator were added

and it was titrated with standard EDTA solution, until colour changes from wine-red to sky blue. From the volume of standard EDTA solution required for titration, calcium was estimated in mg/100g.

3.2.8.4 Magnesium

The magnesium content was calculated as the difference between Ca + Mg and Ca (Black *et al.*, 1965).

Reagents

1. Buffer solution : Ammonium chloride 67.5 g was dissolved in 200 ml distilled water. To this, 570 ml of ammonium hydroxide (ammonia) solution was added and final volume was made to one litre with distilled water.

Remaining reagents were same as described for calcium.

Procedure :

Determination of Ca + Mg

Five ml of digested extracted (digestion described as under 3.2.8.1) was taken into a conical flask, to which 10 ml of distilled water, 5 ml of buffer solution and 5 to 6 drops each of potassium ferrocyanide solution and triethanolamine (TEA) were added. After 5 minutes, few drops of EBT indicator were added and it was titrated with standard EDTA solution until colour changes from wine red to blue. Volume of EDTA solution required to neutralise the content in the flask was recorded and Ca + Mg was calculated.

3.2.8.5 Iron

The iron content in cotton seed meal was estimated by the standard method of A.O.A.C., (1990).

Reagents :

1. Hydroxylamine hydrochloride (10 %, w/v) : Exactly 10 g of hydroxylamine hydrochloride was dissolved in distilled water and volume was made to 100 ml.
2. Orthophenanthroline (0.1 %, w/v) : 0.1 g of orthophenanthroline was dissolved in 25 ml of alcohol and final volume was made 100 ml with distilled water.
3. Sodium acetate solution (2 M) : Dissolved 272.18 g of sodium acetate in distilled water and final volume was made upto volume 1000 ml.
4. Stock solution of iron : Ferric chloride ($FeCl_3$) was weighed 1.45 g and dissolved in distilled water and volume was made to 100 ml with distilled water.
5. Working standard solution of iron : From the above stock solution, 1 ml standard iron solution was diluted to 100 ml which contained 50 µg iron per ml.

Procedure :

A. Preparation of standard graph

The standard working solution of 0, 0.1, 0.2, 0.3, 0.4 ..., 0.9 and 1 ml was taken in 50 ml volumetric flasks and 1 ml hydroxylamine hydrochloride was added in each flask, then flasks

were rotated and kept for few minutes. Then 9.5 ml 2 M sodium acetate solution and 1 ml orthophenanthroline solution were added in the flask. The contents were diluted to 50 ml with distilled water and mixed well. The flasks were allowed to stand for few minutes and absorbance was read on Spectronic-20 at 510 nm and graph was prepared (Fig. 7).

Iron in the sample

Four ml of digested extract of sample (digestion described as under 3.2.8.1) was taken in 50 ml volumetric flask and 1 ml hydroxylamine hydrochloride in each flask, then flasks were rotated and kept for few minutes. Then 9.5 ml 2 M sodium acetate solution and 1 ml orthophenanthroline solutions were added in each flask. The contents were diluted to 50 ml with distilled water and mixed well. The flasks were allowed to stand for few minutes for colour development and absorbance was read on Spectronic-20 at 510 nm.

3.2.8.6 Manganese, Zinc and copper

The manganese, zinc and copper from the digested extract (see section 3.2.8.1) of the defatted kernel meal of cotton seed cultivars were estimated with the help of Atomic Absorption Spectrophotometer (A.A.S., Perkin Elmer Model 2380).The digested extract after necessary dilution (5 ml to 50 ml) was used for taking readings on Atomic Absorption Spectrophotometer (AAS) using appropriate cathode.

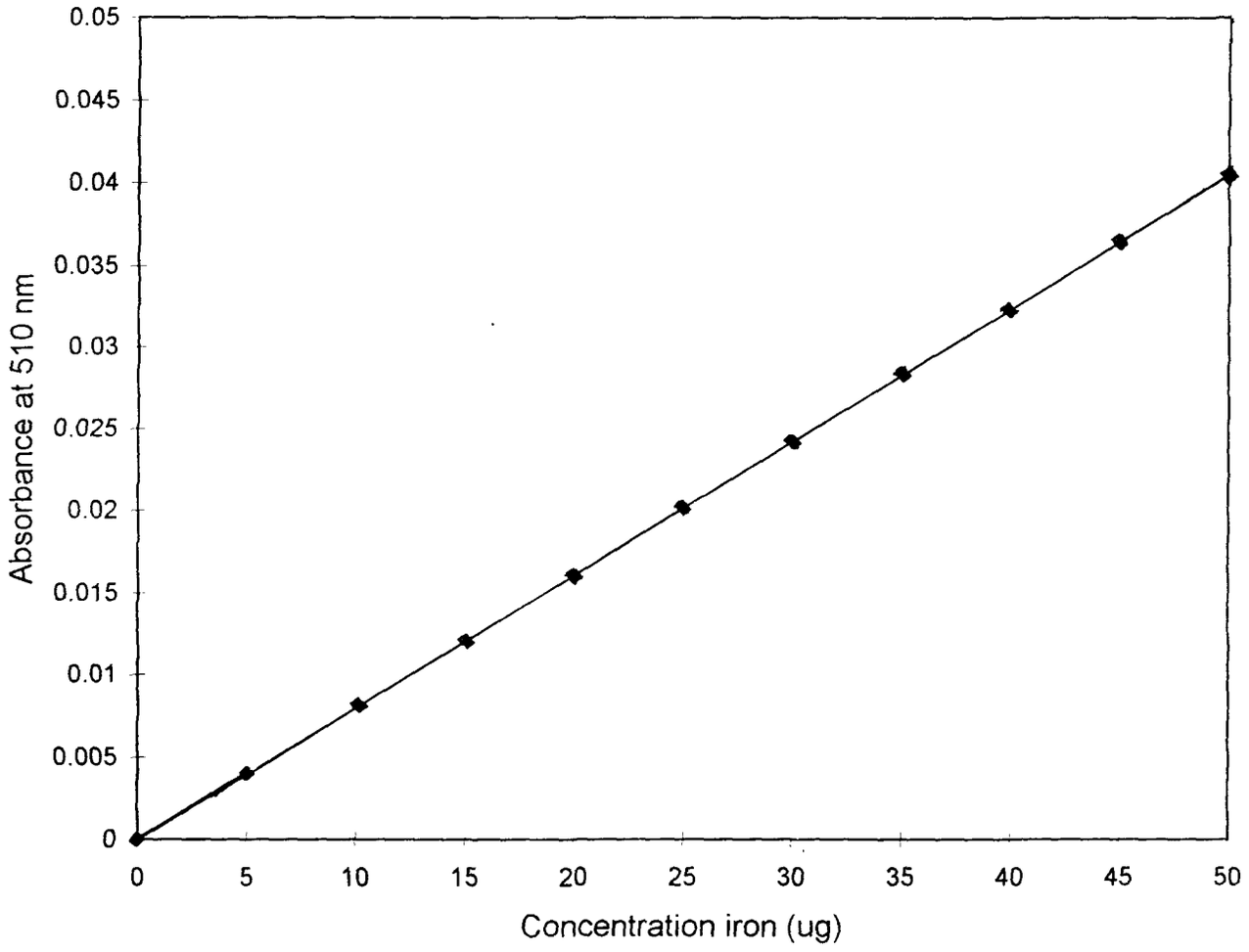


Fig. 7. Standard curve for the estimation of iron

3.3 Statistical analysis

All the experiments were planned using completely randomised block design (CRD) with three replications each and mean, range of chemical composition have been reported.

The data obtained in the present investigation were analysed for the statistical significance according to the procedure given by Panse and Sukhatme (1967). The appropriate standard error (S.E.) and critical difference (C.D.) at 0.05 probability were worked out and are given at the appropriate places.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

In the present investigation the seeds of eighteen cotton cultivars of *Gossyipum hirsutum* L. were obtained from the Cotton Breeder, Cotton Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. The hulls and kernels were manually separated and kernels were used for analysis of biochemical and mineral constituents. The results of these investigation are presented and discussed in this section.

4.1 Seed index

Seed index is the weight of 100 seeds in gram. The seed index of eighteen cultivars of cotton were calculated and results are summarized in Table 2. The seed index was found to range from 5.60 to 8.82 gram. The cultivar JLH-168 had the lowest seed index (5.60 g), while cultivar RHC-1189 had the highest seed index (8.82 g). The mean of the seed index of different cultivars of cotton was 7.67 g.

Several investigators have studied the seed index of cotton and it revealed different ranges such as 1.0 to 8.5 g for *Gossypium hirsutum* L. (El-Nockrashy *et al.*, 1969), 5.48 to 6.39 g for *G. hirsutum* L. (Singh, 1969), 2 to 8.15 g for *G. hirsutum* L. of Laxmi cultivar (Pandey, 1972), 9.5 to 11.7 for *Gossypium barbadense* L., 6.9 to 11.1 for *G. hirsutum* L., 5.4 to 7.1 g for *Gossypium herbaceum* L., 4.3 to 6.9 g for *Gossypium arboreum* L. (Murthi and Achaya, 1975), 7.6 to 13.6 g for *G. hirsutum* L. (Pandey and Thejappa, 1976), 7.6 to 8.7 g for *G.*

Table 2. Seed index, hull to kernel ratio of cotton seed and moisture content of defatted kernel meal

Sr. No.	Cultivar	Seed index [wt. of 100 seeds (g)]	Hull to Kernel ratio	Moisture (%)
1.	PKV-081	7.34	0.65	7.40
2.	LRA-5166	8.20	0.68	8.30
3.	JLH-168	5.60	1.28	10.60
4.	DS-28	6.83	0.72	9.20
5.	G.Cot.100	8.56	0.65	9.40
6.	GB-20	7.42	0.80	8.70
7.	SRT-1	8.60	0.78	10.50
8.	NHH-44	8.13	0.71	9.10
9.	BN	6.87	1.04	10.30
10.	ERB	7.16	0.85	12.10
11.	RHC-0191	8.40	0.83	10.40
12.	RHC-0688	7.20	1.17	12.50
13.	RHC-994	8.43	0.73	13.10
14.	RHC-1088	8.20	0.97	16.10
15.	RHC-1189	8.82	0.73	10.70
16.	RHC-1794	7.30	1.64	11.20
17.	RHC-3194	7.52	0.86	11.88
18.	RHC-9740	7.50	0.82	13.86
	Range	5.60-8.82	0.65-1.64	7.4-16.10
	Mean	7.67	0.89	10.85
	S.E.±	0.29	0.12	0.09
	CD at 5 %	0.85	0.34	3.12

hirsutum L. (Chakravorty and Singh, 1979), 7 to 6.8 g for *G. hirsutum* L., 5 to 5.5 g for *G. arboreum* L. (Singh and Deshpande, 1989). The present results of seed index of eighteen cotton cultivars developed at Cotton Improvement Project, M.P.K.V., Rahuri are in the range of previous reported values.

4.2 Hull to kernel ratio

The results for hull to kernel ratio of eighteen cotton cultivars are presented in Table 2. The hull to kernel ratio of cotton cultivars ranged from 0.65 to 1.64. The highest hull to kernel ratio (1.64) was obtained in RHC-1794, while the lowest (0.65) hull to kernel ratio was found in PKV-081 and G.Cot. 100. The mean of hull to kernel ratio of different cultivars of cotton was 0.89.

The reported values of hull to kernel ratio in cotton are 0.65 to 0.77 for *G. barbadense,* 0.51 to 1.02 for *G. hirsutum,* 0.74 to 1.01 for *G. herbaceum,* 0.77 to 1.14 for *G. arboreum* (Murthi and Achaya, 1975) and 0.56 for *G. hirsutum* (Chakravorty and Singh, 1979). The hulls to kernel ratio mostly depend upon the thickness of hull and size of the kernel. The variation in thickness and size may be dependent upon varietal variation, climatic condition as well as agronomic practices.

4.3 Moisture

The results for moisture content in defatted cotton seed kernels of different cultivars are presented in Table 2. The moisture content in the defatted cotton seed kernels of eighteen cultivars

ranged from 7.4 to 16.10 per cent. The maximum moisture content was present in RHC-1088 (16.10 %) and the minimum (7.4 %) in PKV-081 cultivar. The mean of the moisture content in the defatted kernel powder of different cultivars of cotton was found to be 10.85 per cent.

The reported values of moisture content (%) in cotton seed kernel are 5 to 12.8 per cent for *G. hirsutum* (Altschul *et al.*, 1958; Singh *et al.*, 1969; Zhuge *et al.*, 1988), 5.8 to 11.3 per cent for *G. herbaceum* and *G. arboreum*, 5.6 to 14.1 for *G. hirsutum* (Murthi and Achaya, 1975). In general the present results are in agreement with those reported by earlier research workers.

4.4 Oil and oil quality

4.4.1 Crude oil

The values for crude oil content in the cotton seed kernels of eighteen cultivars are presented in Table 3. The crude oil content (%) in the cotton seed kernels ranged from 30.20 to 37.70 per cent. The highest oil content of 37.70 per cent was found in ERB followed by LRA-5166 (34.70 %), BN (34.40 %), GB-20 (34.10 %) and RHC-0191 (34.10 %), while the lowest of 30.20 per cent was found in DS-28 cultivar. The mean value for crude oil content was 33.07 per cent.

The literature values for crude oil content in the cotton seed ranged from 15.4 to 36 per cent for *G. hirsutum* (Chakravorty and Singh, 1979 and Singh and Singh, 1985), seed oil 24.65 per cent and kernel oil 42.76 per cent for *G. hirsutum* (Pulatov and Gubanova,

Table 3. Crude oil content, oil colour, iodine value and acid value of different cotton cultivars kernel oil

Sr. No.	Cultivar	Crude oil (%)	Colour of crude oil	Iodine value	Acid value (mg KOH/g oil)
1.	PKV-081	33.30	Carrot red	106	0.39
2.	LRA-5166	34.70	English red	101	0.42
3.	JLH-168	32.60	Brownish orange	105	0.69
4.	DS-28	30.20	English red	100	0.49
5.	G.Cot.100	33.60	Carrot red	102	0.43
6.	GB-20	34.10	Carrot red	104	0.42
7.	SRT-1	33.30	Brownish orange	100	0.40
8.	NHH-44	32.30	English red	103	0.67
9.	BN	34.40	English red	102	0.38
10.	ERB	37.70	English red	104	0.36
11.	RHC-0191	34.10	Brownish orange	103	0.65
12.	RHC-0688	33.50	English red	98	0.48
13.	RHC-994	31.30	English red	103	0.52
14.	RHC-1088	30.80	Carrot red	95	0.56
15.	RHC-1189	31.50	English red	96	0.49
16.	RHC-1794	33.50	English red	102	0.55
17.	RHC-3194	33.20	Carrot red	99	0.51
18.	RHC-9740	31.20	Carrot red	104	0.53
	Range	30.20-37.70	-	95-106	0.36-0.69
	Mean	33.07	-	101.50	0.50
	S.E.±	1.62	-	1.02	0.05
	CD at 5 %	N.S.	-	2.93	0.14

N.S. = Non-significant

1986), 39.03, 34.76 and 35.01 per cent for *G. hirsutum*, *G. arboreum* and *G. africanum* respectively (Sun *et al.*, 1987) 14.5 to 25.6 per cent for *G. arboreum* (Singh, 1988). *G. herbaceum* and *G. arboreum* showed an average oil content of 19 per cent whereas *G. hirsutum* and *G. barbadense* have higher average oil content of 21 per cent (Sitaram *et al.*, 1988), 14.6 to 16.8 for *G. hirsutum* and *G. arboreum* (Singh and Deshpande, 1989), 18 to 24 per cent for *G. arboreum* and *G. herbaceum* (Singh *et al.*, 1995). Kernel oil content was of *G. aboreum* 32 to 33 per cent, *G. herbaceum* 34 to 35 per cent, *G. hirsutum* 32 to 36 and *G. barbadense* 35 per cent (Bhatawadekar *et al.*, 1999).

The present results are in the accordance with the previous reported values. The varietal difference in the oil content were statistically non-significant.

4.4.2 Colour of crude oil

The colour of crude oil extracted from cotton seed kernels of eighteen cultivars are presented in Table 3. The carrot red colour of crude oil was found in the cultivars PKV-081, G.Cot. 100, GB-20, RHC-1088, RHC-3194 and RHC-9740. The English red colour of crude oil was found in LRA-5166, DS-28, NHH-44, BN, RHC-0688, RHC-994, RHC-1189 and RHC-1794. The brownish orange colour of crude oil was found in the cultivars JLH-168, SRT-1 and RHC-0191.

The literature information regarding oil colour is pale yellow colour (Sitaram *et al.*, 1988) and light golden colour (King and Camire, 1989). The colour of crude oil mostly depends upon the

pigmented material present in the cotton kernels. This pigmented material or constituents may be extracted with oil and that are mostly responsible to give the colour to the crude oil. The present results are different from the previous findings and this may be due to the higher concentration of pigmented material in the newly developed cotton cultivars.

4.4.3 Iodine value

The iodine values of the oils extracted from eighteen cultivars of cotton are summerized in Table 3. The iodine value of oil extracted from different cotton seed kernel meals of cotton cultivars ranged from 95 to 106. The highest iodine value of 106 was found in PKV-081 while the lowest was 95 in RHC-1088. The mean of the iodine value of oil extracted from different cultivars of cotton was 101.5. The mean differences of iodine values between the varieties were statistically significant.

The literature values for iodine value of oil extracted from cotton seed kernels were 96.8 to 111.6 for *G. hirsutum* and *G. barbadense* (Bailey *et al.,* 1966), 91 to 111 for *Desi* cultivars (*G. herbaceum* and *G. arboreum*) and 94 to 113 for American cultivars (*G. hirsutum*) (Murthi and Achaya, 1975), 110.8 for *G. hirsutum* (Kamla *et al.,* 1984), 92.9 to 114 for *G. hirsutum* (Hamilton, 1987) and 105.89 to 106.01 for *G. barbadense* L. (Sawan *et al.,* 1988). The iodine value indicates the level of unsaturation and probability of contents of essential fatty acids. From nutritional point of view the highest iodine

value is desirable. However too much unsaturation as seen in safflower oil is undesirable owing to its susceptibility to auto-oxidation and decreases the shelf-life for edible oils. An iodine value of 80 to 100 is often considered as optimum. Based on this, the cotton seed oil extracted from new developed cultivars at Cotton Improvement Project, M.P.K.V., Rahuri were seen to be nutritionally quite balanced.

4.4.4 Acid value

The results on acid value of oil extracted from kernels of various cotton cultivars are presented in Table 3. The acid values of oil ranged from 0.36 to 0.69 mg KOH/g oil. Among the cultivars studied JLH-168 had (0.69 mg KOH/g oil) highest acid value, while ERB the lowest (0.36 mg KOH/g oil) acid value in their oil but the oil content was highest (37.70 %). The mean of the acid value of oil extracted from cottonseed kernel of different cultivars was 0.50 mg KOH/g oil.

The reported values of acid value for oil of cotton seed were as follows : 0.50 mg KOH/g oil for *G. hirsutum* (Kamla *et al.*, 1984) and 0.12 to 0.13 mg KOH/g oil for *G. barbadense* (Sawan *et al.*, 1988). The acid value indicates the extent of free fatty acids in the oil. The acid value needs to be low for the edible oils. As per the Indian Standard Institute (ISI) the acid value should be less than 2 mg KOH/g oil. In this context, the crude oils of all the cotton seed cultivars studied showed a safe range.

4.5 Proteins

4.5.1 Crude protein

The results of crude protein content of various cotton seed kernels of different cultivars are presented in Table 4. The data revealed that the crude protein content of defatted kernels of cottonseed cultivars ranged from 22.30 to 47.6 per cent. The mean of the crude protein content of defatted kernels of cotton seed cultivars was found to be 38.18 per cent. The crude protein content was higher in the cultivars DS-28 (47.60 %), RHC-9740 (47.60 %) followed by LRA-5166 (45.40 %), RHC-1189 (44.60 %), G.Cot.100 (42.40 %) and SRT-1 (42.40 %). The cultivar DS-28 with lower oil content (30.20 %) contained maximum of 47.60 per cent protein, while cultivar ERB with maximum oil content (37.70 %), had the lowest protein value (22.30 %) (Fig. 8). The crude protein and crude oil contents were negatively correlated (r = -0.552). These results clearly indicated that a defatted cotton seed kernel meal is a good potential source of dietary proteins. The variation in the protein content of different cultivars may be due to the genetic variation in the biosynthesis of protein in the particular variety as well as application of fertilizers and agronomic practices.

The literature values of protein content in the defatted meal of cotton seed kernel ranged from 35.7 to 43.5 per cent for *G. hirustum* (Pandey and Thejappa, 1976) and 20.7 to 50.9 per cent for *G. hirsutum* (Hussein *et al.*, 1986). Protein content was in whole seed of

Table 4. Crude protein, soluble protein and free amino acids content of defatted kernel meal of different cotton cultivars

Sr. No.	Cultivar	Crude protein (%)	Soluble protein (%)	Free amino acids mg/100 g
1.	PKV-081	35.10	10.40	0.35
2.	LRA-5166	45.40	13.80	0.06
3.	JLH-168	33.40	4.11	0.19
4.	DS-28	47.60	4.40	0.05
5.	G.Cot.100	42.40	4.12	0.08
6.	GB-20	28.30	13.70	0.15
7.	SRT-1	42.40	11.01	0.07
8.	NHH-44	38.70	9.80	0.11
9.	BN	38.70	3.80	0.15
10.	ERB	22.30	9.30	0.88
11.	RHC-0191	29.70	5.60	0.15
12.	RHC-0688	37.20	12.90	0.06
13.	RHC-994	39.40	4.81	0.05
14.	RHC-1088	35.70	9.20	0.11
15.	RHC-1189	44.60	2.69	0.06
16.	RHC-1794	37.90	11.30	0.08
17.	RHC-3194	40.90	11.71	0.12
18.	RHC-9740	47.60	4.91	0.33
	Range	22.30-47.60	2.69-13.80	0.05-0.88
	Mean	38.18	8.20	0.17
	S.E.±	1.54	0.91	0.05
	CD at 5 %	4.43	2.60	0.14

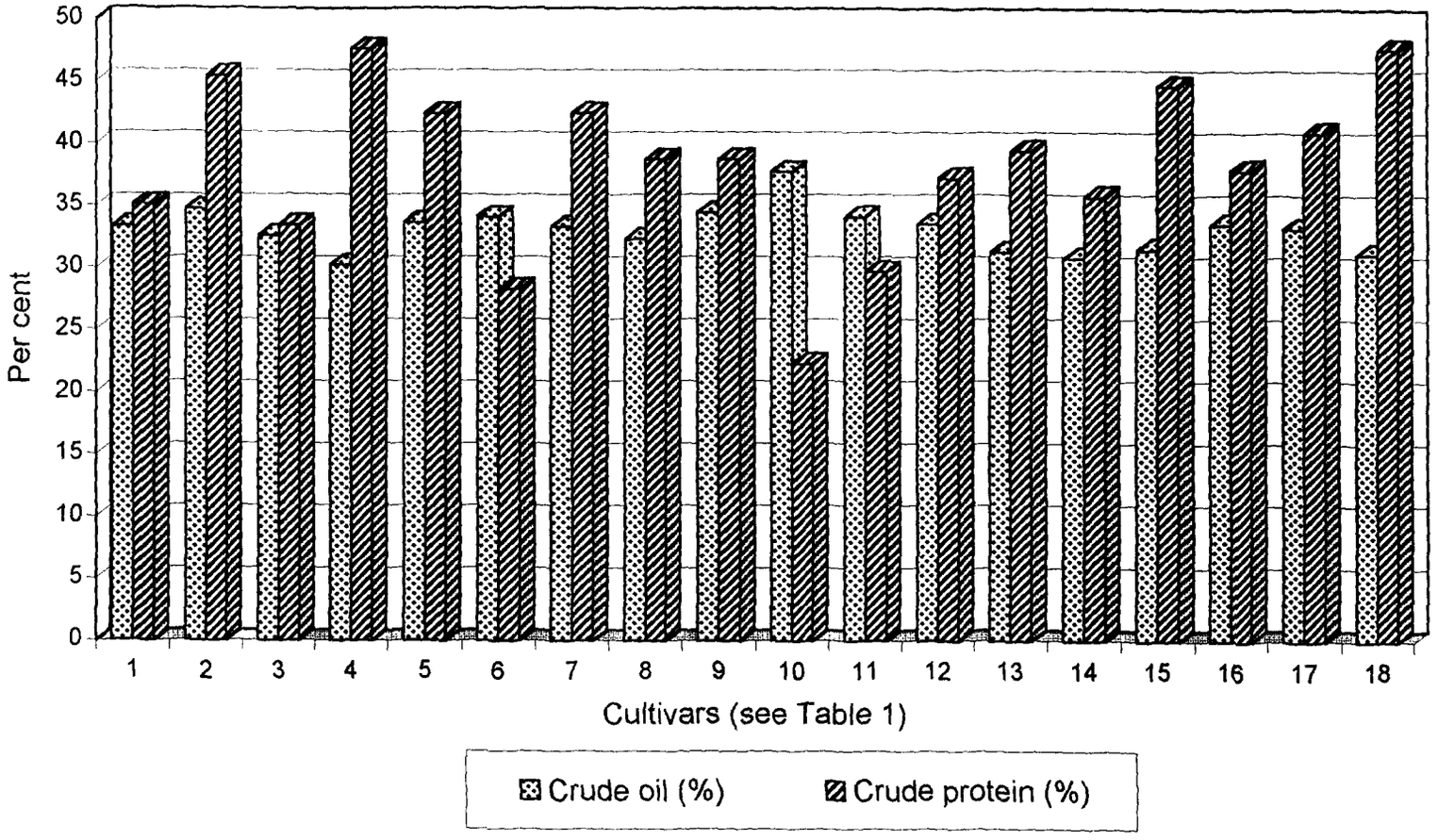


Fig.8. A graphical view of crude oil and crude protein content in kernel meal of cotton cultivars

G. hirsutum 35.5 per cent, *G. herbaceum* 34.6 per cent, *G. barbadense* 36.6 per cent and *G. arboreum* 33.8 per cent (Sitaram *et al.*, 1988). The protein content was ranged from 20.92 to 21.97 per cent for *G. barbadense* (Sawan *et al.*, 1988), 18 to 21.1 per cent for *G.hirsutum* and 20.95 to 21.05 per cent for *G. arboreum* (Singh and Deshpande, 1989 and Taneja *et al.*, 1991b). The protein content in defatted kernel meals of cotton cultivars was 52.4 to 53.9 per cent, 46.2 to 52.8 per cent, 52 to 57.4 per cent and 51.2 per cent was found for *G. arboreum, G. herbaceum, G. hirsutum* and *G. barbadense* respectively (Bhatawadekar *et al.*, 1999). The results of protein content in newly developed cultivars are similar to those results reported earlier by several investigators.

4.5.2 Soluble protein

The defatted kernel meal of cotton cultivars were analysed for soluble protein and the result are presented in Table 4. The soluble protein content in defatted kernel meal of cotton cultivars ranged from 2.69 to 13.80 per cent. The highest soluble protein content of 13.8 per cent was obtained in LRA-5166, while the lowest (2.69 %) soluble protein was found in RHC-1189. The mean of the soluble protein content in defatted kernel meals of cotton was 8.20 per cent.

The previous reported values for soluble protein content in defatted seed meal of cotton cultivars as observed by earlier researchers ranged from 0.2 to 9 per cent for *G. hirsutum* (Elmore and

Leffler, 1976). The globuline were major storage protein of the kernels. The globulines were grouped as 12 S and 7 S and 2 S proteins on the basis of their sedimentation coefficients (Martinez *et al.*, 1970). Reddy and Narsinga Rao (1988a) have named 12 S and 7 S protein as gossypin and congossypin. The protein complement analysis of isolated protein storage vacuoles (PSVS) of dry cotton seed with one dimensional SDS-PAGE gels revealed similar major storage viz., 53 KDa and 48 KDa, with difference in lower molecular mass proteins (Vigil *et al.*, 1996).

The higher soluble proteins content in the defatted flour of cotton is beneficial to improve the quality of feed or food in which they are going to be added. It also helps in cohesiveness of the product and water holding capacity of the product.

4.6 Amino acids

4.6.1 Free amino acids

The defatted kernel meals of cotton cultivars were analysed for free amino acid content and the results are presented in Table 4. The data in Table 4 revealed that free amino acid content of defatted kernel meal of cotton cultivars ranged from 0.05 to 0.88 mg/100 g. Among the cultivars studied, ERB contained (0.88 mg/100 g) higher free amino acid, while that of DS-28 and RHC-994 exhibited the lowest free amino acid content (0.05 mg/100 g). The mean of free amino acid content of defatted kernel meal of different cultivars of cotton was 0.17 mg/100 g.

The reported values of free amino acid content in cotton seed were 0.027 mg/100 g for *G. hirsutum* (Balasubramanian and Gopalan, 1981) and 0.01 to 0.032 mg/100 g, 0.021 to 0.023 mg/100 g, 0.020 to 0.024 mg/100 g and 0.019 to 0.023 mg/100 g for *G. hirsutum, G. herbaceum, G. arboreum* and *G. barbadense* respectively (Raju and Reddy, 1989).

The present results for free amino acids of some of the cultivars showed higher values than the literature values and this may be due to improper utilization of primary source (free amino acids) to synthesize storage protein in their metabolism. Another reason may be harvesting of cotton seed before full maturity.

4.6.2 Limiting amino acids

The limiting amino acids were determined from defatted kernel meal of different cultivars of cotton and the results are presented in Table 5, 6 and composition is shown in Fig. 9.

4.6.2.1 Methionine

The methionine content (g/16 g N) in defatted kernel meal of cotton ranged from 1.12 to 2.73 g/16 g N with a mean value of 1.87 g/16 g N. Among the eighteen cultivars studied, JLH-168 had the highest methionine content of 2.73 g/16 g N, followed by ERB with 2.72 g/16 g N. The lowest value for methionine content was found in RHC-1088 (1.12 g/16 g N). The methionine content as g/100 g meal in defatted kernel meal of cotton cultivars ranged from 0.41 to 0.91 (Table 6).

Table 5. Limiting amino acids (methionine and tryptophan) content in defatted kernel meal of different cultivars of cotton (g/16 g N)

Sr. No.	Cultivar	Methionine (g/16 g N)	Tryptophan (g/16 g N)
1.	PKV-081	2.28	1.28
2.	LRA-5166	1.37	1.43
3.	JLH-168	2.73	1.71
4.	DS-28	1.53	1.01
5.	G.Cot.100	1.94	1.35
6.	GB-20	1.62	1.34
7.	SRT-1	1.91	0.97
8.	NHH-44	2.30	1.34
9.	BN	1.73	1.19
10.	ERB	2.72	2.06
11.	RHC-0191	2.49	1.89
12.	RHC-0688	1.72	1.32
13.	RHC-994	2.21	1.14
14.	RHC-1088	1.12	1.63
15.	RHC-1189	1.43	1.05
16.	RHC-1794	1.48	1.27
17.	RHC-3194	1.61	1.17
18.	RHC-9740	1.55	1.06
	Range	1.12-2.73	0.97 - 2.06
	Mean	1.87	1.35
	S.E.±	0.28	0.18
	CD at 5 %	0.81	0.52

Table 6. Limiting amino acids (methionine and tryptophan) content in defatted kernel meal of different cultivars of cotton (g/100 g meal)

Sr. No.	Cultivar	Methionine (g/100 g meal)	Tryptophan (g/100 g meal)
1.	PKV-081	0.80	0.45
2.	LRA-5166	0.62	0.65
3.	JLH-168	0.91	0.57
4.	DS-28	0.73	0.48
5.	G.Cot.100	0.82	0.57
6.	GB-20	0.46	0.38
7.	SRT-1	0.81	0.41
8.	NHH-44	0.89	0.52
9.	BN	0.67	0.46
10.	ERB	0.61	0.46
11.	RHC-0191	0.73	0.56
12.	RHC-0688	0.64	0.49
13.	RHC-994	0.87	0.45
14.	RHC-1088	0.41	0.58
15.	RHC-1189	0.64	0.47
16.	RHC-1794	0.56	0.48
17.	RHC-3194	0.66	0.48
18.	RHC-9740	0.74	0.52
	Range	0.41-0.91	0.41-0.65
	Mean	0.70	0.50
	S.E.±	0.118	0.075
	CD at 5 %	0.34	0.22

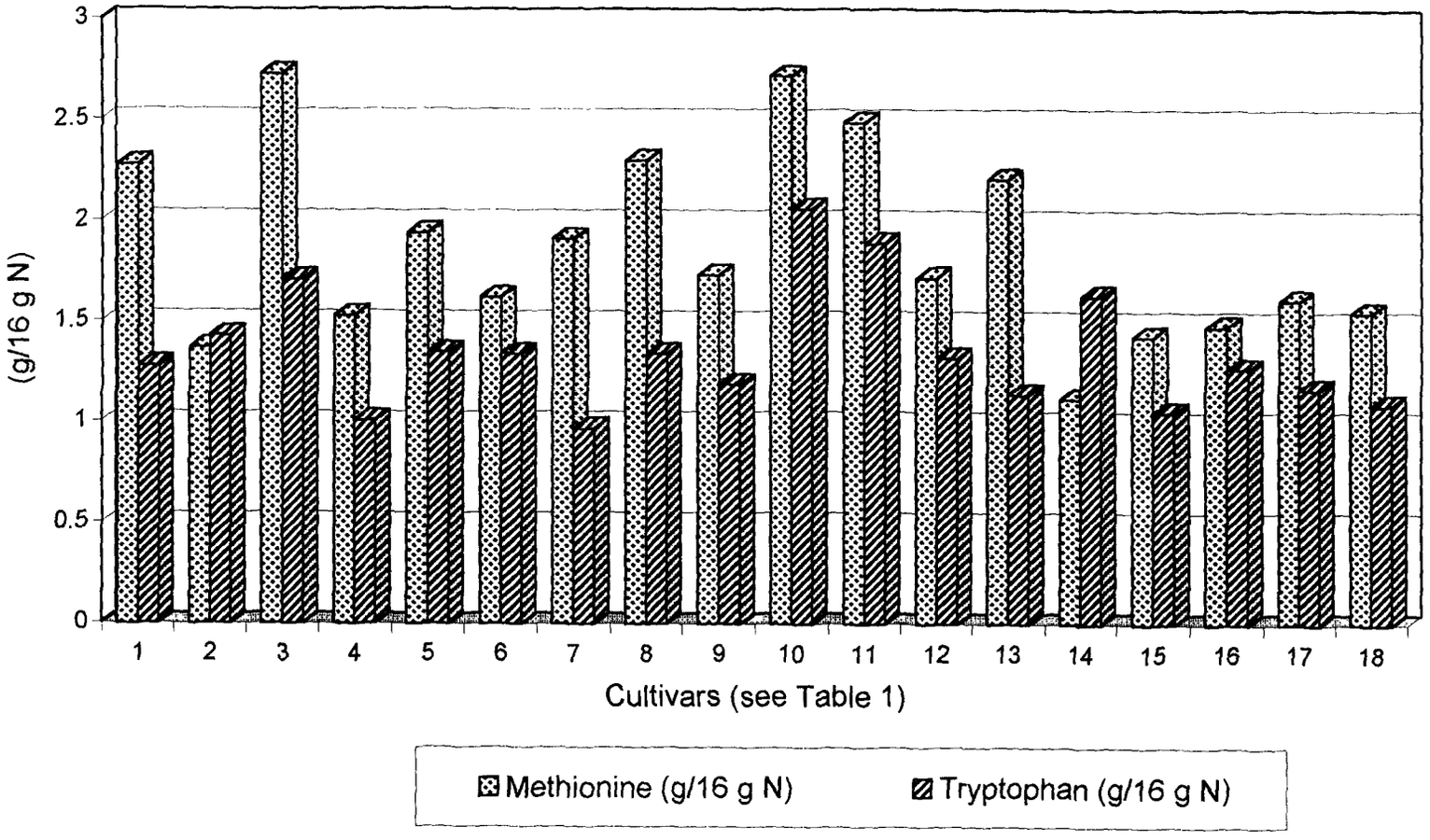


Fig.9. A graphical view of limiting amino acids (methionine and tryptophan) content in defatted kernel meal of different cotton cultivars

The earlier reported values of methionine content in cotton seed kernel meal were 1.20 g/16 g N, 1.28 g/16 g N, 1.35 g/16 g N and 1.65 g/16 g N for *G. herbaceum, G. arboreum, G. hirsutum* and *G. barbadense* respectively (Carter *et al.*, 1966) and 1.32 g/16 g N, 1.34 g/16 g N, 1.43 g/16 g N and 1.74 g/16 g N for *G. herbaceum, G. arboreum, G. hirsutum* and *G. barbadense* respective (El-Nockrashy *et al.*, 1969). *G. hirsutum* of cotton cultivars Stoneville contained 1.3 to 1.7 g/16 g N and Starkville contained 1.4 g/16 g N of methionine (Leffler *et al.*, 1977). The present results of methionine content in newly developed cultivars are in agreement to the earlier results.

4.6.2.2 Tryptophan

The tryptophan content in defatted kernel meal of different cultivars of cotton ranged from 0.97 to 2.06 g/16 g N, with a mean value of 1.35 g/16 g N. The highest content of tryptophan was observed in ERB (2.06 g/16 g N). The cultivar SRT-1 had the lowest tryptophan content (0.97 g/16 g N). The tryptophan content as g/100 g meal in defatted kernel meal of cotton cultivars ranged from 0.41 - 0.65 (Table 6).

Berardi and Cherry (1980) and Balasubramanian and Gopalan (1981) reported 130 µg/g of fresh weight and 1.4 per cent dry weight respectively tryptophan content in defatted seed meal of cotton cultivars from *G. hirsutum* species.

The values for tryptophan content in different cultivars of cotton except SRT-1 are higher than the standard value of tryptophan

given by FAO (1970). These results indicated that the cotton seed meal is a good source of limiting amino acids.

4.7 Ash

The results on ash content in defatted kernel meal of cotton cultivars are presented in Table 7. The data revealed that the total ash content of defatted kernel meal of different cotton cultivars ranged from 6.60 to 9.80 per cent. The cultivar GB-20 had the highest ash content of 9.80 per cent, followed by PKV-081, JLH-168, BN and RHC-1794 with 8.90 per cent. The cultivar RHC-1189 had the lowest ash content of 6.60 per cent. The mean of the ash content in defatted kernel meal of cotton cultivars was 8.29 per cent.

Martinez *et al.* (1970) and Lawhon *et al.*, (1974) reported 7.9 to 11.4 per cent and 6.2 to 20.3 per cent respectively, ash content in defatted seed meal of cotton cultivars from *G. hirsutum.* Bhatawadekar *et al.* (1999) reported the ash content in defatted kernel meal of cotton cultivars ranged from 5.7 to 6.4 per cent, 8 to 8.9 per cent, 7 to 7.7 per cent and 7.2 to 8.7 per cent for *G. arboreum, G. herbaceum, G. hirsutum* and *G. barbadense* respectively. The present results are similar to previous reported results of ash content in cotton cultivars.

4.8 Minerals

4.8.1 Phosphorus

The phosphorus content of defatted kernel meal of cotton cultivars was analysed and results are represented in Table 7. The

Table 7. Ash, phosphorus and potassium content in defatted kernel meal of different cultivars of cotton

Sr. No.	Cultivar	Ash (%)	Phosphorus (%)	Potassium (%)
1.	PKV-081	8.90	1.57	1.72
2.	LRA-5166	8.70	1.32	2.12
3.	JLH-168	8.90	1.47	2.33
4.	DS-28	7.70	1.76	1.97
5.	G.Cot.100	8.80	1.81	2.08
6.	GB-20	9.80	2.03	2.39
7.	SRT-1	7.80	1.36	1.66
8.	NHH-44	8.80	1.54	1.97
9.	BN	8.90	1.39	2.16
10.	ERB	7.90	1.62	1.93
11.	RHC-0191	7.80	1.38	1.88
12.	RHC-0688	7.70	1.84	1.86
13.	RHC-994	8.00	1.49	1.61
14.	RHC-1088	8.30	1.55	2.02
15.	RHC-1189	6.60	1.32	1.81
16.	RHC-1794	8.90	1.53	1.96
17.	RHC-3194	7.80	1.52	2.13
18.	RHC-9740	8.00	1.61	2.06
	Range	6.60-9.80	1.32-2.03	1.61-2.39
	Mean	8.29	1.56	1.98
	S.E.±	0.74	0.26	0.28
	CD at 5 %	N.S.	N.S.	N.S.

N.S. = Non-significant

phosphorus content of defatted kernel meal of different cotton cultivars ranged from 1.32 to 2.03 per cent. The highest phosphorus content of 2.03 per cent was observed in GB-20 followed by RHC-0688 (1.84 %), G.Cot 100 (1.81 %), DS-28 (1.76 %) and ERB (1.62 %), while the lowest was in LRA-5166 and RHC-1189 (1.32 %).

Many research workers presented the phosphorus content in cotton seed ranged from 2.1 to 5.35 per cent for *G. hirsutum* (Martinez *et al.*, 1970; Singh *et al.*, 1972 and Lawhon *et al.* 1974). Phosphorus content of newly developed cotton cultivars is in the range of literature values.

4.8.2 Potassium

The defatted kernel meal of different cotton cultivars were analysed for potassium content and the results are represented in Table 7. The potassium content in defatted kernel meal of cotton cultivars ranged from 1.61 to 2.39 per cent. The highest potassium content of 2.39 per cent was obtained in GB-20, followed by JLH-168 (2.33 %), BN (2.16 %), RHC-3194 (2.13 %) and LRA-5166 (2.12 %), while the lowest (1.61 %) was found in RHC-994. The mean potassium content of eighteen cotton cultivars is 1.98 per cent.

Rao (1975) and Balasubramanian and Gopalan (1978) reported 0.94 to 1.97 per cent potassium content in defatted seed meal of cotton cultivars. Shanmugham and Bhatt (1990) also analysed several cultivars of cotton from *G. barbadense* for their potassium content and it was reported in the range of 0.95 to 1.21 per cent on a

dry weight basis. The present results of newly developed eighteen cultivars of cotton for potassium also in the same line of previous results reported by earlier research workers.

4.8.3 Calcium

The results on calcium content of various cotton cultivars are presented in Table 8. The calcium content in defatted kernel meal of cotton cultivars ranged from 109 to 215 mg/100 g. The highest calcium content of 215 mg/100 g was obtained in RHC-994, followed by GB-20 (196 mg/100 g), RHC-0191 (196 mg/100 g), RHC-1088 (177 mg/100 g) and RHC-1794 (173 mg/100 g), while the lowest 109 mg/100 g calcium was found in BN. The Mean calcium content of eighteen cotton cultivars was 151.33 mg/100 g.

Altschul *et al.* (1958) and Martinez *et al.* (1970) analysed several cultivars of cotton from *G. hirsutum* for calcium content in defatted seed meal and reported that the calcium content ranged from 100 to 500 mg/100 g sample on dry weight basis. Currently analysed cotton cultivars for calcium content are also in same range.

4.8.4 Magnesium

The magnesium content of the eighteen cotton cultivars was calculated by subtracting the calcium from the calcium plus magnesium and is presented in Table 8. The results indicated that the magnesium content in defatted kernel meal of cotton cultivars ranged from 80 to 177 mg/100 g. The highest magnesium content of 177 mg/100 g was observed in BN, while the lowest (80 mg/100 g)

Table 8. *Calcium, magnesium, iron, manganese, zinc and copper* content in defatted kernel meal of different cultivars of cotton

Sr. No.	Cultivar	Calcium mg/100 g	Magnesium mg/100g	Iron mg/100g	Manganese mg/100g	Zinc mg/100g	Copper mg/100 g
1.	PKV-081	127	156	18	1.8	1.5	5.8
2.	LRA-5166	143	149	29	2.6	1.7	7.2
3.	JLH-168	151	137	22	2.0	1.4	10.7
4.	DS-28	121	121	22	2.5	1.6	4.6
5.	G.Cot.100	140	155	38	2.3	2.2	6.0
6.	GB-20	196	120	28	2.5	1.5	7.2
7.	SRT-1	127	175	36	2.5	2.3	6.4
8.	NHH-44	145	147	21	2.1	1.7	5.8
9.	BN	109	177	16	2.1	1.3	9.3
10.	ERB	163	129	42	3.2	1.6	17.0
11.	RHC-0191	196	80	18	1.9	1.6	11.9
12.	RHC-0688	127	159	30	2.0	1.2	5.1
13.	RHC-994	215	91	24	2.0	1.3	13.6
14.	RHC-1088	177	110	21	2.2	1.5	6.0
15.	RHC-1189	145	113	23	2.1	1.3	4.6
16.	RHC-1794	173	150	27	2.5	1.3	5.8
17.	RHC-3194	136	162	29	2.1	1.3	6.4
18.	RHC-9740	133	170	24	2.4	2.0	6.0
	Range	109-215	80-177	16-42	1.8-3.2	1.2-2.3	4.6-17.0
	Mean	151.33	138.94	26.00	2.27	1.57	7.75
	S.E.±	5.46	5.32	4.15	0.34	0.25	0.47
	CD at 5 %	15.68	15.30	11.92	N.S.	N.S.	N.S.

N.S. = Non significant

magnesium was found in RHC-0191. The mean value of magnesium content of the eighteen cotton cultivars was 138.94 mg/100 g.

The literature values for magnesium content in the seed meal of cotton cultivars ranged from 330 mg/100 g for *G. hirsutum* (Altschul *et al.* 1958) and 140 to 190 mg/100 g for *G. barbadese* (Shanmugham and Bhatt, 1990). The current results are in agreement with earlier research workers.

4.8.5 Iron

The results for iron content in defatted kernel meal of different cotton cultivars are presented in Table 8. The iron content in defatted kernel meal of cotton cultivars ranged from 16 to 42 mg/100 g, the maximum iron content (42 mg/100 g) was observed in ERB followed by G.Cot 100 (38 mg/100 g), SRT-1 (36 mg/100 g), RHC-0688 (30 mg/100 g), LRA-5166 (29 mg/100 g) and RHC-3194 (29 mg/100 g) and the minimum (16 mg/100 g) in BN cultivars. The mean of iron content in defatted kernel meal of different cultivars of cotton was 26 mg/100 g.

The earlier reported values of iron content in cotton cultivars ranged from 15 to 35.25 mg/100 g for *G. arboreum* (Taneja *et al.* 1991c). In general the present results are in agreement with those reported by earlier research worker.

4.8.6 Manganese

The results on manganese content of various cotton cultivars are presented in the Table 8. The results given in Table 8

revealed that the manganese in cotton cultivars ranged from 1.8 to 3.2 mg/100 g. The highest manganese content of 3.20 mg/100 g was found in ERB, while the lowest (1.80 mg/100 g) manganese was found in PKV-081. The mean value of manganese content of cotton cultivars was 2.27 mg/100 g.

The literature values of manganese content in cotton ranged from 3.5 to 5.25 mg/100 g for *G. arboreum* (Taneja *et al.*,1991c). Manganese content in newly developed cultivars at Cotton Improvement Project, M.P.K.V., Rahuri is slightly lower than the literature values. This may be due to the varietal variation in the biosynthesis of mineral content in the seed.

4.8.7 Zinc

The zinc content in the defatted kernel meal ranged from 1.2 to 2.3 mg/100 g (Table 8). The highest zinc content of 2.30 mg/100 g was found in SRT-1, while the lowest (1.20 mg/100 g) zinc was found in RHC-0688. The mean value of zinc content of cotton cultivars was 1.57 mg/100 g. Similar results were reported by Taneja *et al.* (1991c) for cotton cultivars from *G. arboreum*.

4.8.8 Copper

The results for copper content in the defatted kernel meal of eighteen cotton cultivars are presented in Table 8. Copper content in kernel meal of cotton ranged from 4.6 to 17 mg/100 g. The mean copper content in defatted kernel meal of different cultivars of cotton was 7.75 mg/100 g.

Taneja *et al.* (1991) reported that cotton seed kernel contained 1.92 to 3.75 mg/100 g copper for *G. arboreum*. Copper content in newly developed cotton cultivars are slightly higher than the values reported by Taneja *et al.* (1991c). This may be due to soil condition or varietal variation.

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Cotton (*Gossypium spp.*) is one of the most important cash crop grown in several countries such as India, USA, USSR, China, Egypt and Africa, mainly for the fibre, which is mostly used for the production of luxury fabrics. India is unique in cultivating all the four species of cotton and is the first country to commercialise hybrid cottons. The total production of seed cotton is over 80 lakh tonnes which yields 52 lakh tonnes of cottonseed annually. Cottonseed is widely distributed as oilseed in tropical and subtropical areas and it plays an important role in the economics of Agricultural Cum Industrial Development. India is the fourth largest country in oil economy in the world. Cotton seed oil has high stability against oxidation. Cotton seed oil is also rich in tocopherols, which are natural antioxidents with varying degrees of vitamin E activity. This too contributes to its stability. The histidine requirement in children is high whereas that of arginine is high in adult, both are high in cotton seed protein. Cottonseed protein has been successfully used to rehabilitate malnourished infants. The role of superior cultivars for oil and protein profile in crop improvement for cotton seed is brought out in the present study, the investigations of proximate composition, crude oil, colour of crude oil, iodine value, acid value, protein content, soluble protein, free amino acids, limiting amino acids like methionine, tryptophan and mineral composition of

eighteen cotton cultivars are also carried out. The results obtained are briefly summarised in this section.

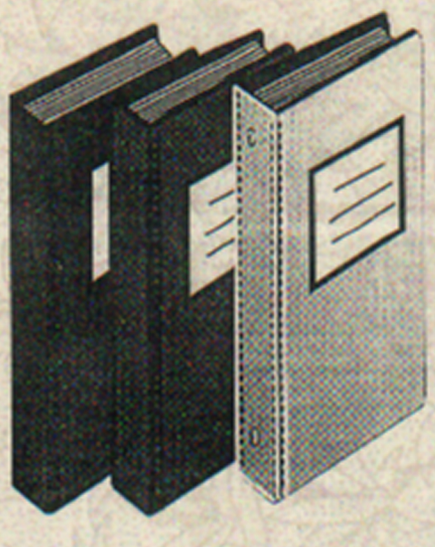
1. Seed index (g per 100 seeds) ranged from 5.60 to 8.82 g in different cultivars. The higher seed weight is generally associated with higher oil and protein content. The hull to kernel ratio ranged from 0.65 to 1.64. The quality of lint depends upon the quality of cotton seed kernel. The defatted kernel meal of eighteen cotton cultivars showed significant variation in moisture content that ranged from 7.4 to 16.10 per cent. The maximum moisture content was found in RHC-1088 (16.10 %) and the minimum (7.4 %) in PKV-081 cultivars.
2. The crude oil content ranged from 30.20 to 37.70 per cent in different cultivars. Among the cultivars studied, ERB exhibited maximum oil content of 37.70 per cent followed by LRA-5166 (34.70 %) and BN (34.40 %). For colour of crude oil studied, English red colour was found in most of the cultivars followed by carrot red and brownish orange colour. The iodine value of the extracted oil varied from 95 to 106. The highest iodine value of 106 was found in PKV-081, while the lowest of 95 was found in RHC-1088. The acid value of the extracted oil ranged from 0.36 to 0.69 mg KOH/g oil, while the levels of free fatty acids and oxidation in fresh oil were found minimum and below the safe level. Iodine value data indicated that cotton seed oil has a fair proportion of unsaturated fatty acids.

3. The defatted meal of different cultivars contained 22.30 to 47.6 per cent crude proteins. The cultivar DS-28 with least crude oil content (30.20 %) showed a maximum of 47.60 per cent crude protein. While a cultivar ERB with maximum of 37.70 per cent crude oil contained a lowest of 22.30 per cent crude protein. The correlation studies showed a significant negative correlation between crude oil and crude protein content. The soluble protein content in defatted kernel meal of cotton cultivars ranged from 2.69 to 13.80 per cent. The higher soluble protein content in the defatted flour of cotton is beneficial to improve the quality of feed or food and also helps in cohesiveness of the product and water holding capacity of the product.
4. Free amino acids ranged from 0.05 to 0.88 mg/100 g. Methionine content ranged from 1.12 to 2.73 g/16 g N, while the tryptophan content varied from 0.97 to 2.06 g/16 g N.
5. The defatted kernel meal of eighteen cotton cultivars showed significant variation in ash content that ranged from 6.60 to 9.80 per cent. The phosphorus content in the defatted kernel meal of cotton cultivars varied from 1.32 to 2.03 per cent. The potassium content in the eighteen cotton cultivars ranged from 1.61 to 2.39 per cent. The calcium content also varied from 109 to 215 mg/100 g. The magnesium content ranged from 80-177 mg/100 g. The variation in iron content was from 16 to 42

mg/100 g. A wide variation was also observed in manganese content ranging from 1.8 to 3.2 mg/100 g. The zinc content varied within the range of 1.2 to 2.3 mg/100 g. Similarly, a considerable variation was observed in the copper content among the cotton cultivars ranging between 4.6 to 17 mg/100 g.

Among eighteen cotton cultivars studied ERB, LRA-5166, BN, GB-20, RHC-0191 were higher in oil content, while DS-28, RHC-9740, LRA-5166, RHC-1189, G.Cot. 100 and SRT-1 were higher in protein content. Phosphorus content was higher in GB-20, RHC-0688, G.Cot.100, DS-28 and ERB. Potassium was higher in the cultivars GB-20, JLH-168, BN, RHC-3194 and LRA-5166. Calcium content was higher in the cultivars RHC-994, GB-20, RHC-0191, RHC-1088 and RHC-1794, while iron content was higher in the cultivars ERB, G.Cot. 100, SRT-1, RHC-0688, LRA-5166 and RHC-3194.

The data on the chemical constituents of cotton seed presented through this research will help breeders and the seed meal and oil users in making proper selections of cotton cultivars. The study has clearly brought out that varieties from *Gossypium hirsutum* L., have maximum variation in kernel to hull ratio, oil, protein and mineral content and breeders can select proper variety for improvement in oil and protein contents.



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VITA

7. VITA

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of

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