INTRODUCTION

Protein deficiency is very common amongst Indians of low and average income groups. Children and villagers, particularly of Madhya Pradesh and Orissa states of India, are the worst hit by protein malnutrition. Therefore, the chemical investigation of edible grade protein rich plants, both of cultivated and wild varieties including forest products of India, is a very important field. Proteins, forming the major portion of the present investigation, have been briefly reviewed below.

Proteins

Proteins are important constituents of all plants, being the seat of and directing the course of their manifold activities, as well as serving as reserve materials for their various functions. The main functions of proteins in single and multicelled plants are (1) as nucleo proteins in the genes, controlling cell division and heridity; and (2) as enzymes in the cell cytoplasm, catalyzing various chemical reactions essential for the maintenance of cell life. In higher plants, proteins serve as important reserve of amino acids in the seeds. While plants are able to utilize source of fixed nitrogen like ammonia, nitrite and nitrate, the man and other animals for the most part are dependent on a source
of amino acids to build their body proteins. They obtain their nitrogen requirements directly or indirectly from plants. Thus proteins are the most essential organic nutrients of human and animal diet.

Proteins may be defined as complex organic compounds of high molecular weight which invariably contains carbon, hydrogen, nitrogen, oxygen and sulphur. Besides these elements, many of them also contain some phosphorus, iron, iodine and cobalt. Proteins are made up of amino acids. 18 to 20 being of common occurrence in proteins of all the major classes of living plants and other organisms. The known amino acids are those (cystine, cysteine, glycine, alanine, serine, threonine, valine, methionine, leucine, isoleucine, aspartic acid, glutamic acid, lysine, arginine, phenylalanine, tyrosine, tryptophane, histidine, proline and hydroxyproline.) and are described in standard works.¹,²

With the exception of proline and hydroxyproline, all amino acids contain \(-\text{CH}-\text{COOH}\) group attached to the \(\text{NH}_2\) rest of the amino acid molecule (ie, characteristic group R). In case of proline and hydroxyproline, amino group is a part of the cyclic ring; that is, it is present in the form of an imino \(-\text{NH-CH}\) group and these two acids in reality are imino acids. Since the acids are the
integral part of the protein molecules and also their reactions are similar to those of other amino acids, these are usually referred to as amino acids. Besides these amino acids, many more amino acids of unusual structure exist in proteins of some plants, animals or other micro organism in small amounts. Examples of these are - citrulline, hydroxylysine, and lathionine etc.

**FORMATION OF AMINO ACIDS IN PLANTS**

Plants synthesize all the amino acids needed for their growth from carbohydrates and simple nitrogenuous compounds such as ammonium salts, various nitrates, nitrites and urea etc. An enzyme nitrate reductase reduces nitrate to ammonia. This enzyme contains molybdenum. In the plants of Leguminosae family, the symbiotic bacteria of their root nodules or other micro-organisms (several species of Azotobacter) take up nitrogen directly from atmosphere and convert it into hydroxylamine or ammonia. In plants probably aspartic acid is first formed by the action of hydroxylamine or ammonia with oxalacetic acid in accordance with the following scheme.

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Oxal acetic acid, on reductive amination with ammonia in presence of an enzyme NADP

Oxidation

Reduction

Aspartic Acid.

In a similar manner, glutamic acid is formed by the action of hydroxyl amine or ammonia on alpha keto glutaric acid in a manner represented below.

Alpha ketoglutaric acid, on direct-amination with ammonia in presence of an enzyme NADP

Aspartic acid and glutamic acid react with more of ammonia to form amides, asparagine \[\text{CONH}_2\text{CH}_2\text{CONH}_2\] and glutamine \[\text{CONH}_2\text{CH}_2\text{CH}_2\text{CONH}_2\] respectively.
Plant tissues contain enzyme called "transaminases" which are capable of catalysing the reactions between aspartic acid or glutamic acid with other alpha keto carboxylic acids (formed as intermediates of Krebs cycle or from fatty acids especially in germinating seeds or by deamination of amino acids) to form other amino acids. This biochemical reaction which involves an exchange of functional group of the reacting molecules, is called transamination. This reaction provides a link between carbohydrate and protein metabolism through the transference of amino groups from amino acid to alpha keto carboxylic acid. Pyridoxal (Vitamin B6) is the co-enzyme for the transaminase enzyme. Probably pyridoxal acts as the real active catalyst and it functions through metal chelate formation. The metal which acts as activator for pyridoxal are copper(II), iron (II & III), aluminium(III), nickel (II) and cobalt(II). The transaminase reaction was found to occur rapidly in aqueous solution even in the absence of the enzyme. The mechanism of non enzymatic transamination between an amino acid and a keto acid by metal activated pyridoxal can be represented as follows:—

\[
\text{R-C=O} + \text{M}^{+} + \text{H}_{2}\text{N-CH}_{2} + \text{CH}_{2}\text{OH} \rightarrow \text{R-C-CH}_{2}\text{OH}
\]
Pyridoxamine formed as above, may then transfer the amino group, it has taken up from an amino acid, to a keto acid, proceeding in a reverse way, with pyridoxal acting as a catalyst. The overall change involving the formation of alanine by reaction between aspartic acid or glutamic acid with pyruvico acid is represented as follows:

\[
\text{HOOC-CH} = \text{CH} - \text{CH} - \text{COOH} + \text{CH}_3 - \text{C} = \text{COOH} \rightarrow \text{CH}_3 - \text{CH} = \text{COOH} \\
\text{NH}_2 \quad \text{O} \quad \text{NH}_2
\]

Aspartic acid + Pyruvic acid \rightarrow Alanine + Oxal acetic acid.

\[
\text{HOOC-CH} = \text{CH} - \text{CH} - \text{CH} - \text{COOH} + \text{CH}_3 - \text{C} = \text{COOH} \rightarrow \text{CH}_3 - \text{CH} = \text{COOH} \\
\text{NH}_2 \quad \text{O} \quad \text{NH}_2
\]

Glutamic acid + Pyruvic acid \rightarrow Alanine + Alpha keto glutaric acid.

The formation of Schiff's base between the amino acid and pyridoxal, stabilised by complex formation with metal ion, provides a clue to this type of reaction. A schematic representation of the formation of amino acids in plants is given below.
A SCHEMATIC REPRESENTATION OF AMINO ACID FORMATION
IN PLANTS

Sources of fixed nitrogen

Nitrogen of the atmosphere is directly assimilated by plants of the Leguminosae family in presence of symbiotic bacteria and other micro-organisms at their root nodules.

Ammonium Nitrites & Nitrate Salts on reduction under the influence of enzymes nitrite/nitrate reductase.

\[
+ \text{NH}_3 \rightarrow \text{NH}_2\text{OH} \rightarrow \text{NH}_3 \leftarrow \text{Ammonia.}
\]

Reductive amination of oxalacetic acid under the catalytic influence of an enzyme NADPH.

\[
\text{Asparagine} \rightarrow \text{Aspartic acid} \rightarrow \text{keto acids} \rightarrow \text{Glutamic acid} \rightarrow +\text{NH}_3
\]

(on transamination in presence of enzymes)

* Transaminases */ direct amination of keto acids

\[
\text{Glutamine.}
\]

\[
\text{Other amino acids.}
\]
Many amino acids are, however, formed by the directamination of keto acids.

Micro-organisms during their growth in nutrient media, consisting of mineral salts, nitrogenuous compounds like ammonium salts, nitrates and nitrites and urea etc; and glucose or other simple sugars, convert fixed nitrogen into amino acids probably by reactions similar to those which take place in various plants.

CLASSIFICATION OF AMINO ACID

Nutritive Classification:

This type of classification involved division of amino acids into three groups called indispensable, semi-indispensable and dispensable amino acids.

Indispensable amino acids are those which can not be synthesised by the animal at a rate sufficient for optimum growth from materials ordinarily available in the diet.

Dispensable amino acids are those which can be synthesised by the animal at a rate sufficient for optimum growth from materials ordinarily available in their diet.

Semi-indispensable amino acids are those which can replace partly some indispensable amino acids, e.g., tyrosine.
can spare but not completely replace phenylalanine; cystine possesses sparing effect for methionine; and nicotinic acid partly replaces tryptophane.

Following tables No. 1, represents the nutritive classification according to Block.17

<table>
<thead>
<tr>
<th>Indispensable/Essential</th>
<th>Semi-Indispensable</th>
<th>Dispensable/Non-essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>Glycine</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>Serine</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Methionine</td>
<td>Cystine</td>
<td>Alanine</td>
</tr>
<tr>
<td>Threonine</td>
<td>Tyrosine</td>
<td>Proline</td>
</tr>
<tr>
<td>Valine</td>
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<td>Hydroxyproline</td>
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<td>Leucine</td>
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<tr>
<td>Isoleucine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine**</td>
<td></td>
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</tbody>
</table>

+ Nicotinic acid is a vitamin of B group.
++ Arginine and glycine are essential for chicks and turkeys.
Serine possess sparing effect for lysine.
Recent investigations have shown that arginine and histidine are non-essential for human beings.
Formation of Proteins in Plants:

Amino acids formed in plants react together to form proteins needed for their growth. The condensation of amino acids which leads to protein formation takes place in the presence of guanine triphosphate.

Nitrogen requirements of animals:

Proteins are synthesised by the living organism from 19 or 20 amino acids which are usually supplied in the form of dietary proteins. The proteins after ingestion are first broken down into their constituent amino acids by the enzymes of the gastrointestinal tract (abbreviated as GI tract). The amino acids thus liberated are absorbed into the body. However, the hydrolytic enzymes and other tissue constituents which are involved in the digestion of food, are also proteins and they too undergo hydrolysis in the GI tract to their constituent amino acids. In fact, it may well be that the largest part of the amino acids absorbed from the intestine originates from the digestion of proteins secreted by the organism into the gut (Fig 1).

Fig 1. Diagrammatic representation of sources of amino acids supplied to the portal circulation.

Food Proteins

Peptic, pancreatic, intestinal enzymes and other proteins of the gut.

Gastrointestinal tract.
Hydrolysis of dietary proteins

Amino acids for absorption
Thus the pattern of amino acids presented to the organism during digestion is almost, though obviously not entirely, constant.

The absorbed amino acids are then recombined into the myriads of specific proteins of which the organism is composed. It should always be remembered that no protein can be made unless every amino acid of which it is composed, is available at the site of synthesis. Furthermore, the quantity of protein—which can be made—is limited by the amino acid which is available in relatively the smallest amount. This fact generally accepted intuitively by nutritionists, has been demonstrated experimentally using amino acids labelled with isotopes. These experiments show that all the amino acids needed for synthesis of a protein molecule must be lined up on the "template" and are then linked together by almost simultaneous dehydration to form the finished product or at least, that peptides are synthesized, which do not accumulate to any appreciable extent and these in turn are synthesized to the tissue proteins. In either event protein synthesis is governed by the law of minimum.

Folin suggested that there are two different types of proteins metabolism, i.e., endogenous and exogenous metabolism. Folin and Rubner believed that the tissues of an adult body slowly wastes away due to the "wear and tear" of living. For this purpose amino acids have to be supplied in the food to enable the organism to replace
the lost protein. When an excess quantity of amino acids is ingested by the adult, these surplus amino acids are broken down to yield CO₂, H₂O, energy and urea. When new tissue is being formed the demands for proteins are obviously greater than in the metabolically quiescent adult. This type of protein metabolism is represented diagrammatically in Fig. 2.

Later on Borsok and especially Scheonheimer modified the ideas of Folin. According to Scheonheimer the living tissues are not metabolically inert, but that the tissue proteins are in a continual state of degradation and resynthesis. The concept of a dynamic state of body constituents is represented diagrammatically in Fig. 3.
Tissue Proteins

Hydrolysis

Food Proteins

Hydrolysis in the G.I. tract.

Synthesis

Amino acid pool of the body

Catabolism

Urea, carbon dioxide, water and heat.

Fig. 3: Diagrammatic scheme of Schoenheimer's hypothesis of dynamic equilibrium in proteins metabolism.

Many investigators on the basis of labelled amino acid feeding experiments have established that amino acid turnover is not uniformly rapid in all tissues. It is very fast in some, like liver, intestinal walls and some glands; and very slow in others like muscle, brain and collagen etc. The greatest amount of amino acids reaching the body amino acids pool are derived from the G.I. tract. The quantity of certain amino acids supplied to the pool will vary with amount and type of gastrointestinal secretion which in turn is influenced by composition of the dietary proteins.

CLASSIFICATION OF PROTEINS

Till recently little was known about the exact chemical nature of any protein and it was difficult to
classify them on the basis of their precise structure.
A number of operational classification systems which distinguish among protein classes on different basis are as under.

(A) Classification on the basis of their composition:

1. Simple Proteins: They contain only amino acids as their structural components.

2. Conjugated Proteins: They contain one or more compound in addition to amino acids as their structural components, like (a) Chromoproteins contain amino acids and some pigments, eg; hemoglobin; (b) Glycoproteins and Mucoproteins, contain amino acids and carbohydrates, eg, Mucin of saliva. (c) Phosphoproteins, contain amino acids and phosphoric acid, eg, Casein of milk; (d) Lipoproteins contain amino acids and lipids, eg, Lipovitellins of the egg. (e) Nucleoproteins contain amino acids and nucleic acid, eg, virus proteins are of this type.

(B) Classification based on shape of the molecule:

1. Globular Proteins: are those which are relatively spheric or ovoid shaped. These as a rule are relatively soluble.

2. Fibrous Proteins, are those which resemble long ribbons or fibres in nature. These are insoluble and are usually
found as components of the tougher type of the tissues, e.g., Keratins of the skin, hair and feathers; elastins of the ligaments; and silk fibroin of the silk fibres.

(C) **Classification based on solubility**:

1. **Albumins** are soluble in distilled water, dilute salt solutions, dilute acids and bases. They are precipitated without denaturation by saturating them with ammonium sulphate.

2. **Globulins** are those proteins which are insoluble in distilled water but soluble in dilute salt solutions (e.g., 5% NaCl solution). These are precipitated by half saturation with ammonium sulphate.

3. **Protamines and Histones** are highly soluble, small and stable proteins. They are not coagulated by heat. They contain very high percentage of basic amino acids and form salts with mineral acids, nucleic acid and acidic proteins. The major difference between the two proteins is that histones are somewhat weaker bases and are insoluble in ammonium hydroxide solution whereas protamines are soluble.

4. **Glutelins** are insoluble in distilled water and alcohol but soluble in dilute acid or base solutions. They are commonly found in various plant seeds. Stickiness of dough in wheat flour is because of the presence of glutelins.
5. **Prolamines** are insoluble in distilled water, but soluble in dilute acids or bases and also in 70 to 80% alcohol solutions.

6. **Scleroproteins** are those which are insoluble in water and other common solvents. These are mainly the fibrous proteins described above.

**FIXED OILS**

Fats and oils are another important type of nutrient of the diet of man and animals. They also play an important role in many industries. The demand of fats and oils is increasing day by day.

The fats are present to different extent in every living material in addition to proteins and carbohydrates. As a source of energy, fats come at the top, as the calories supplied (9 calories per gram) is double that of proteins (4 calories per gram) and carbohydrates (4 calories per gram). They are composed of an alcohol (glycerol) esterified with organic acids, which belong to the aliphatic straight chain type with few exceptions. The fatty acids occur in nature in various combinations, the most important being glycerides (A) as in fats; phosphatides (B) as in phospholipids like lecithin, and esters of monohydric alcohols (C) as in waxes like spermaceti consisting of esters of cetyl alcohol (C\(_{16}\)H\(_{33}\)OH) with lauric acid, myristic acid and palmitic acid.
where $R, R'$ and $R''$ stand for alkyl groups alike or different, and $B$ stands for nitrogen base like choline.

The oils and fats are widely distributed in both plant and animal kingdoms. In plants, they are mainly present in spores, seeds and fruit but they are also present in leaves, roots and other vegetative organs. Their function in the leaves is not yet clear, but those in the spores, seeds and some tubers constitute a food reserve to be drawn during germination and the early life of the plant. The fats, with a few exceptions from tropical plants are characterised by containing a notable percentages of saturated acids, whereas those from plants growing under colder regions contain large proportions of unsaturated acids. Consequently, drying and semidrying oils are of more frequent occurrence in the plants of temperate climate than in those found in tropics. On the other hand those of the non drying class predominate in tropical regions. With the applications of modern methods, particularly GLC technique and methods developed by Hilditch and coworkers, it has become possible to determine the component glycerides of a fat and a more
intimate insight has been obtained not only as to their character and quality but also the way in which these are combined to form glycerides.

**UNSAPOONIFIABLE MATTER**

The term unsaponifiable matter includes all those substances which are not saponified by alkali and are soluble in both light petroleum ether and also in solvent ether. The unsaponifiable matter consists of sterol, higher alcohols like tocopherols and hydrocarbons such as squalene \( \text{C}_{30}\text{H}_{50} \); tricontane \( \text{C}_{30}\text{H}_{62} \); and pristane \( \text{C}_{18}\text{H}_{30} \) etc. These may be separated from sterols and other alcohols by standard methods\(^{19}\).

**STEROLS**

The sterols (from sterces—solid) are crystalline polycyclic hydroaromatic, secondary alcohols containing an aliphatic side chain. They are widely distributed in nature occurring both in the free state and as ester of higher fatty acids and may be classified on the basis of occurrence as zoosterols (in animals), phytosterols (in plants) and mycosterols (in kryptogams particularly in fungi). They play an important role in animal metabolism, and have been reviewed by Bergmann\(^{20}\), Fieser and Fieser\(^{21}\).
CARBOHYDRATES

The term "Carbohydrates" is applied to polyhydroxy aldehydes and ketones and their derivatives, including deoxy sugars, amino sugars and even sugar alcohols and acids. Pigman²²; Pigman and Goepp²³ have reviewed carbohydrates.

MODERN METHODS OF ANALYSIS OF PLANT MATERIALS

The vast development in the Chemistry of Plant products in the last two or three decades or so, is the outcome of the applications of the recent physical and biochemical methods of their studies in addition to the conventional methods of investigating the nature of hydrolytic, reductive, and oxidative type of degradation products and specific colour reactions of various types of organic compounds of plant origin. This has led to the discovery of a large number of organic compounds, elucidation of their structures and their synthesis as well.

The following techniques are of great help in phytochemical investigations:

(1) Chromatography, (2) Colorimetry, (3) Polarography, (4) Ultra violet, infra red, NMR and Mass spectroscopy etc.

Some of these techniques are briefly reviewed below.--

Chromatography--

Identification of different types of organic compounds particularly amino acids may be done by various
techniques of chromatography like paper chromatography, paper electrophoresis, combination of paper electrophoresis and paper chromatography, paper chromatography on special ion exchange papers and thin layer chromatography. Separations of organic compounds including amino acids may also be achieved by adsorption, partition, or ion exchange resin column chromatography.

In 1930 the technique of chromatography was successfully applied to the carotenoid field. It was quickly recognised as a powerful analytical tool, in the natural product field generally, facilitating the separations of minor components not necessarily coloured, for there are many ways to locate them—from highly complex mixtures of largely unknown compositions. Chromatography has now become the most common and universally adopted as an efficient method of isolation, purification and characterisation of the constituents of plant, animal or synthetic origin. A number of reviews\textsuperscript{24-28} are now available which describe chromatography both from theory and practical application point of view.

\textbf{Paper Chromatography}:

Paper chromatography of sugars has been described by Shellard\textsuperscript{29}, Glegg\textsuperscript{30}, Partridge\textsuperscript{31}, Bogge\textsuperscript{32}, Williams\textsuperscript{33}, Radhakrishnamurthy\textsuperscript{34}, Bersin\textsuperscript{35}, Kawerau\textsuperscript{36}, Giri\textsuperscript{37}, Parihar\textsuperscript{38}, etc; and the separations of their osazones by Barry and Mitchell on circular paper disc.
Fatty acids are separated by reversed phase chromatography on filter paper by Kaufmann, Puchmann, Michael, Birayama, Inouye, Pink and Pink, etc. Other derivatives of fatty acids have been separated by Michael, Birayama, Inouye, Pink and Pink, etc.

Resolution of amino acids on filter paper has been widely studied by Consden, Matthiaia, William and Kirby, Rutter, Marchal and Wittwar, Giri, Giri, Levy and Chung, Dent, Redfield, and Hausman, etc. Knight, Peterson and Smit have described chromatographic separations of amino acids using special ion exchange resin paper.

Quantitative Estimations of resolved substances on paper chromatograms, can be performed either by examination of the spot on the filter paper itself, or by elution of the substances and subsequent determinations with the eluates.

Fisher, Parsons and Morrison showed that the area of the round or oval shaped spots increase as the logarithm of the spot content (concentration). This relationship holds well for a wide range of concentrations. To obtain more accurate and reproducible results, the spot area of unknown substance must be compared with spots obtained from the same volume of a known concentration of the same substance, spotted and
developed under identical conditions on the same filter paper sheet.

This procedure has been used successfully by Block, Fromageot, Ohtsu and others for the estimation of amino acids.

Reid and Lederer applied area measurement and comparison method to the estimation of volatile organic acids.

Quantitative Estimations using Photoelectric instruments for measurement of spot intensity:

Photodensitometric Studies: This method involves direct photometric determinations of the spots after imparting some transparency to the fully developed chromatogram by immersing it for 5 minutes in anisol, benzyl alcohol, or in a mixture of o-c bromonaphthalene and liquid paraffin (24:76, parts by volume). The spots can then be estimated with the help of a photoelectric densitometer. The instrument consists of a photocell arrangement with direct reading and a device for moving paper strips past a lightbeam at regular intervals with or without direct recording device.

This method was adopted by Block, McFarren, Patton, Salander, Gassener, Polson, Kosikovsky.
Roland, Farmon and W. Sequenzburg, and Keil etc. for the quantitative estimation of amino acids separated on unidimensional paper chromatograms.

Fisher et al. and Gustafsson took photographs of chromatograms and measured the densities of the spots on the negative, photodensitometrically.

Block, Bolling, Sochon, Rockland, Levy, Nehring, Gerok, Bode, Ackerfeldt, Marcoek, Schwerdtfeger, Chinnall, Woitwood, Pope and Stevens, Rauen et al., Kofranyi, Wellington, Giri et al., Naftalin etc. have reviewed the quantitative estimations of amino acids by measuring colour density after elution of the colour from the spot on the paper chromatogram in some solvent like water, alcohol solution or aqueous butanol etc. and then measuring the intensity of this solution with some photoelectric colorimeter or a spectrophotometer.

Thin layer chromatography -

It is a valuable supplement to column and paper chromatography and has become of great importance in achieving the separations of natural products. It not only combines the advantages of paper and column chromatography but in certain respects, is superior to them. Microscopic TLC technique using microscopic slides instead of glass plates has also recently been used.
Kiehner et al., Reitsma, Stahl, Demole, Wollish et al., Von Aul and Neher, etc., have published a number of papers dealing with the separations of various types of organic compounds by thin layer chromatography.

Several books by Renderth, Truter, Bobbit, Stahl, Marini and Calvin have reviewed TLC both in theory and in practice.

Recently a number of workers separated amino acids and amines as their dansyl derivatives on thin layer plates. Dansyl derivatives of amino acids or amines, suitable as qualitative standards are conveniently prepared by mixing 5 mg of Dansyl reagent (1-dimethyl amino naphthalene-5-sulphonyl chloride) in acetone with an amino acid or amine (about 1 mg) dissolved in 1 ml of 0.1 molar sodium bicarbonate solution. In most cases one hour is sufficient for complete conversion, but it is often convenient to leave overnight at room temperature. The addition of 8 ml of acetone, precipitates sodium bicarbonate. Dansyl derivatives thus prepared were separated by thin layer chromatography technique.

Adsorption, partition and ion exchange chromatography are reviewed by Partridge, Elsden and Synge, Synge, Moore and Stein, Hire et al., Martin et al., and Gordon et al., etc.
Ion exchange chromatography has been used by Cheronis\textsuperscript{137} who has given a general method of fractionation of plant constituents on ion exchange resin column. This method is applicable to any aqueous plant tissue extract and is of immense value.

**GAS LIQUID CHROMATOGRAPHY**

The technique of gas liquid chromatography (abbreviated as GLC) was introduced for the first time by James and Martin\textsuperscript{128} in 1952. It has been used to separate complex mixtures of compounds like terpenes, fractions of essential oils, and their oxygenated derivatives, even when they are present in trace quantities and with boiling ranges \(-20^\circ - 400^\circ\) C. These substances are analysed both qualitatively and quantitatively with great accuracy and in short time. James\textsuperscript{139}, Purnell\textsuperscript{140}, Hardy and Pollard\textsuperscript{141}, Rose\textsuperscript{142}, DalNogare\textsuperscript{143}, Peason\textsuperscript{144}, Keulemans\textsuperscript{145} and Goloy\textsuperscript{146} have published comprehensive reviews on this technique.

The technique has also been extended to the investigations of unsaponifiable matter, aliphatic alcohols, tocopherols, triterpenoid alcohols, fatty acids as their methyl or ethyl esters derivatives and amino acids as their trifluoroacetyl n-butyl esters.
In plant chemistry, many factors such as climate, soil, fertilizers, cultural operations, as well as growth, variety and maturity stages etc are known to influence the composition of the plant of a particular species to a great extent. Besides this, the results of phytochemical investigations also depend on the methods used for extraction and other treatment given to the extracts or isolates and subsequent determinations both qualitative and quantitative with various extracts or isolates.

Phytochemistry is a dynamic science and much of the fascination of the subject is due to its ever increasing utility of its advancements. Many experiments lead to the discovery of new facts or amendment of old ideas and thoughts, and thus have a great impact on the human life. Systematic study, exploration and utilisation of natural resources of any country are among the most important activities of various communities for its proper growth and development. Amongst these, a proper place must be assigned to plants rich in edible grade proteins. Investigations on these plants, both of cultivated and wild variety is a very important but long neglected field in this country.

The present investigations were therefore taken in hand by the author to make detailed studies on proteins of various seeds and rice bran (dessi safari variety) of Raipur and its suburbs in Chhattisgarh area of Madhya Pradesh, India. Besides this, qualitative studies (using conventional
and chromatographic methods) have also been made for the presence of fatty acids, unsaponifiable matter, carbohydrates and cations in the materials investigated.

Analysis of the fats of the seeds and rice bran showed the presence of palmitic, stearic, oleic, linoleic and linolenic acids in different amounts. The following materials were investigated for the presence of carbohydrates:

- *Cucurbita maxima*, *Cucumis melo*, *Cucumis sativus*, *Sterculia foetida*, *Cassia tora*, *Bassia latifolia*, *Pongamia glabra*, *Cesalpinia bonducella* and *Rice bran*.

Glucose and fructose were the common monosaccharides present in the extracts of *Cucurbita maxima*, *Cucumis sativus*, *Cucumis melo*, *Cassia tora*, *Bassia latifolia* seeds and rice bran. Sucrose was present in all the seeds and rice bran investigated. The galactose and rhamnose spots were observed in *Cucurbita maxima*, *Cucumis melo*, *Cassia tora* and *Rice bran* extracts. The percentage of proteins in the seed meals was found to be maximum in *Cucumis sativus* (72.5%) and minimum in *Oryza sativa* Kernel (8.5%). The proteins were isolated with alkaline 10% brine solution, followed by adjustment of pH value of the protein solution between 3.5 - 4.5.

The proteins isolated from various sources have been hydrolysed using a mixture (1:1) of 6 M hydrochloric acid and 80% formic acid. It has been observed that the presence of formic acid checks the decomposition of lysine, threonine, methionine, and tryptophane (to check completely its decomposition. however, alkaline hydrolysate solution
had to be used) and at the same time catalyses the hydrolysis, which is achieved in a much shorter period than when hydrochloric acid is used alone.

The protein hydrolysates have been qualitatively analysed for amino acid by a new technique using a combination of unidimensional ascending and or descending chromatography with circular paper chromatography. A comparative study has also been made employing less effective chromatographic techniques used by previous workers. The hydrolysates have thus been found to contain 19 amino acids viz, cystine, lysine, histidine, arginine, aspartic acid, glycine, serine, glutamic acid, threonine, alanine, proline, tyrosine, valine, methionine, tryptophan, phenylalanine, leucine and isoleucine. Besides these, isatin positive spot for hydroxyproline has been observed in the hydrolysates of Momordica charantia and Linum usitatissimum. Presence of alpha amino butyric acid has been observed in the hydrolysates of Momordica charantia and Bassia latifolia seeds. Gamma amino butyric acid spot has been observed in the hydrolysate of all the seeds (except Momordica charantia) and rice bran. Asparagine and glutamine have been found absent in all the seeds and rice bran protein hydrolysates. Possibly these two amides undergo hydrolysis to form aspartic and glutamic acids.

A quantitative estimation of the amino acids present in various sources has also been made. A special technique has been evolved for resolving lysine and histidine using a combination of unidimensional ascending or descending paper chromatography with ion exchange resin (Amberlite IRC-50)
column chromatography and the results (quantitative estimation) have been found in good agreement with those obtained from the techniques used by previous workers.

A perusal of the results shows that the cucurbitaceae seeds, particularly Cucumis sativus, Cucurbita maxima and Cucumis melo, analysed by the present author have been found rich in protein contents and unsaturated fatty acids and hence the partially defatted seeds in the form of seed milk may prove superior to ground nut milk (having lesser percentage of protein and unsaturated fatty acids) as a substitute for mother's milk for feeding infants. Some of the other seed meals which possess higher percentage of lysine, methionine and tryptophane (amino acids most needed) may prove as food supplement for combating protein deficiency and thus brings out the utility of the present investigations. Prior to the use of these seeds as food materials, however, the presence of toxins in them will have to be checked, and if found present will have to be removed before use.

The much advocated idea about the utility of rice bran has also been substantiated in the present work since this contains a good proportion of essential amino acids, particularly lysine, methionine and tryptophane.
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