# STUDIES OF FREE SUGAR AND DIETARY FIBRES OF SOME LOCAL VEGETABLES



*THESIS PRESENTED*

*BY*

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# DEDICATED TO PARENTS

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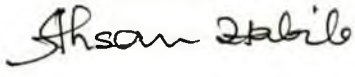

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## LIST OF ABBREVIATIONS

Ara*f* = Arabinofuranosyl residue

Ara*p* = Arabinopyranosyl residue

Gal*p* = Galactopyranosyl residue

# SUMMARY

# Summary

Eight vegetable namely, Potol (*Tricosanthes dioica*), gajor (*Daucus carota*), shalgam (*Brassica campestris* var. *turnip*), fulkopy (*Brassica oliracea* var. *botrytis*), badhakopy (*Brassica olracea* var. *capitata*), karela (*Momordica charantia*), chalkumra (*Benincaca cerifera*) and chichinga (*Trichosanthes anguina*) were taken for determintion free sugars and diteray fiber content and compositions.

Edbible parts of all the vegetables were made into small pieces, air dried and dried at 105°C in an oven. The result showed that all the vegetables centained large amount of water (89-97%). Chichinga contained highest amount of water and the lowest amount of water was present in gajor among the eight vegetables.

The edible part of the fresh vegetables was extracted with aqueous 80% ethanol after making the vegetable materials into small pieces. During addition of ethanol water content of each vegetble was taken into consideration. The residue was extracted with chloroform. From the ethanol soluble part lipid was removed by partition.

Ethanol soluble materials varied from 2-5% and lipid part varied from 0.1-0.3%. The result indicated that the vegetables did not contain much of fatty materials.

The aqueous ethanol soluble material was fractionated into acidic, basic and neutral parts. The neutral material (NLMW) was analysed for free sugar content. The NLMW was converted into their trimethyesilyl (TMS) ethers and analysed by GLC. Glucose and fructose were the two free sugars indentified and quantified in the vegetables. *Myo*-inositol was only found in chalkumra. Shalgam, gajor and badhakopy contained more free sugars than the other five vegeables.

Dietary fiber content and composition of the vegetables were determined by removing protein and starch from the extractive free powder by enzymatic treatments. All the vegetables contained high percent of dietary fiber (0.6-4.9%). Among the eight vegetables highest amount of total dietary fiber (4.9%) was present in gajor followed by fulcopy and potol.

Soluble and insoluble dietary fiber were isolated separately. Soluble dietary fibre (SDF) of all the vegetables were less than insoluble dietary fiber (IDF) except shalgam where SDF was slightly higher than IDF.

Uronic acid content and neutral sugar constituents of the SDF samples were determined saparately. All the SDF contained quite high percent of uronic acid. Among the vegetables uronic acid content of gajor and shalgom was nearly 34%.

Neutral sugar constituents of the SDF showed that galactose and arabinose were the major sugar residues present in the SDF samples. SDF of shalgom and fulkopy contained more than 34% of glucose indicated large amount of non-starch glucans were present in the SDF of the shalgam and fulkopy.

Choloroform soluble part of the vegetables were analysed for fatty acid content and composition. Only esterified fatty acids were analysed by saponifition followed by conversion of liberated free fatty acids into their methyl esters. Lauric, myristic, palmitic, stearic, oleic, arachidic and behemic acids present in varying amounts (3.68-34mg/100g) fresh vegetables in the lipid part of the vegetables.

# INTRODUCTION

# 1. INTRODUCTION

## 1.1. General background

In ancient times people survived on roasted meats, fresh fruits and vegetables. In course of time and with the progress of civilization, people have changed there food habit. Now - a - days we eat cooked food as well as fresh fruits and vegetables as diet. Vegetables are necessary ingredient of our daily food list. It is considered to be nutritious and beneficial to health all over the world. It can undoubtedly be said that people can survive on vegetables alone if they have nothing else to eat. This is true for the poor people of the villages of Bangladesh. It is a fact that a patient, especially in the case of kidney failure, ischimic heart disease and severe metabolic disorders who has restriction in almost all types of food still can take vegetables.

The importance of vegetables is unlimited because the required elements which are essential to our body are obtained from vegetables. It has been reported[1] that most vegetables contain almost all types of vitamins, minerals, iron, calcium, protein and also some amount of carbohydrates. It is also unique in guarding against many diseases caused by the deficiency of nutritional ingredients. Moreover, vegetables are good sources of dietary fiber.

Dietary fiber (DF) is defined as materials which are not digested by the endogeneous secretion of the upper gastro-intestinal tract of human[2]. These materials include polysaccharides, lignin and other organic compounds. Dietary fiber plays an important role in human growth and nutrition. The physiological importance of dietary fiber in human nutrition has been recognized in that it decreases blood sugar and also serum cholesterol of human[3]. Free sugars are also present in vegetables[4].

## 1.2 . OBJECTIVE OF THE PRESENT WORK:

Bangladesh is an agro-based evergreen country and various kinds of vegetables are grown round the year abundantly every where including kitchen garden and agricultural fields. Vegetables are essential item of our daily food list. Human being are very much dependent on vegetables as these are cheap sources of vitamins, minerals and dietary fibers. Bangladeshi people are even more dependent on vegetables as they can not afford to have enough meet or fish. Vegetable items are inseparable ingredients in their every meal. Therefore, it is very important to have detailed knowledge about the chemical compositions of the vegetables which are locally grown.

It is well known that dietary fiber plays an important in physiological action, it lowers blood glucose and serum cholesterol[2]. Patients suffering from diabetes or ischimic heart

disease are not capable to metabolize carbohydrates (starch), protein and fat like healthy persons. Fruits and vegetables are good sources of dietary fiber. More vegetables are essential in meals to control their metabolic disorders. Fruits and vegetables are not only the source of dietary fiber (DF) but they also contain free sugars. DF is very useful for diabetic patients but free sugars are restricted for them. To evaluate the food value of the local fruits and vegetables the Carbohydrate and Natural Products Group of the Department of Chemistry, University of Dhaka undertook a research project to determine the free sugar and dietary fiber content and composition of the local fruits and vegetables. A large number of local fruits were analyzed and published[5,6]. Similar studies were carried out on seven local vegetables[7].

The present work is a part of the total dietary fiber project of the group. In this studies another eight vegetables were considered to study. The main objective was to determine the free sugar and DF content and composition of the eight vegetables. The result will help to choose vegetables for diabetic patients as well as for healthy people.

Fat is another important constituent of food item. Although vegetables do not contain much of fatty material but it is good to know what fatty acids are present in the vegetables. The present study also includes the fatty acid composition of the eight vegetables.

## 1.3 Carbohydrates in vegetables.

Carbohydrates present in food play a very important role in human growth and nutrition. Although the main function of carbohydrates in the diet is to provide a ready source of energy for the body, however, many polysaccharide fractions in food take part in various important biological activities in the body.

Carbohydrates are the main constituents of all plants, vegetables and fruits of which cellulose and hemicelluloses are the major components. Many low-molecular weight carbohydrates and allied substances are also present along with polysaccharides in plants, vegetables and fruits. These low-molecular weight components are present from the very early stages of growth of plants and they take part in the biosynthesis of polysaccharides as well as other constituents of the plant kingdom. In the later stages of growth these low-molecular weight substances may also be generated by degradation of oligo- and polysaccharides and various other glycosides.

Polysaccharides include structural material cellulose, energy reserve starch and many other soluble hemicelluloses and also glycoconjugates. The starch and most of the low-molecular sugars are digested by human but others are not. The non-digestible materials are generally termed as dietary fiber (DF).

### 1.3.1 Free sugars and allied materials in fruits, vegetables and plants.

Glucose, fructose and sucrose and other common sugars present in free state in plant kingdom i.e, in fruits, vegetables and other parts, are termed as free sugars. Most of the fruits, specially which are sweet, contain substantial amounts of free sugars. Vegetables are usually not sweet in taste. So, these may be considered to contain only small amounts of free sugars. With the increasing importance of dietary fiber in human, the vegetables, the main source of DF, have become even more important. The study of DF will remain incomplete without proper evaluation of the free sugar contents of the corresponding vegetables. In recent years free sugars including alditols and cyclitols were reported by our group[5-7]. The reports are described below.

Nahar *et. al.* (1993) analysed[7] seven local vegetables namely, elongated bean, ladies finger, unripe papaya, bitter gourd, brinjal, white gourd and green banana. Total free sugars in fresh vegetables varied from 0.2 to 1.8%. Glucose, fructose and sucrose were the main free sugars identified and quantified in the vegetables. Small amounts of *myo*-inositol was found in all the vegetables.

In 1990, Nahar *et. al.* also reported free sugars in seven local fruits[5] of Bangladesh. Analysis was carried out on sweet as well as on sour fruits, namely, mango, pineapple, guava, hog plum, latkan and lukluki. Large amount of sucrose was found in the sweet fruits, mango and pineapple (~ 40-60% of ethanol extract of fresh fruit). All the fruits contained substantial amount of glucose and fructose. Small amounts of *myo*-inositol was found in all the fruits. Very small amounts of glycerol, glucitol and mannitol were found in some of the fruits.

Free sugar content and composition of the ten different fruits[6] namely, litchi, star gooseberry (horbori), Indian gooseberry (amloki), honey dew melon (bangi), water melon (tarmuj), blackberry (kalajam), jamrul, jalpai, karamcha and ripe papaya were reported by Rahman *et. al.* in 1991. Total free sugars varied from 0.7-13.0%. The sweet fruits like litchi and ripe papaya contained more free sugars (12.9% and 8.1 %, respectively) than the sour fruits like karamcha (0.7%) and jalpai (1.5%). All the fruits except karamcha (0.6%) contained substantial amout of glucose (1.1–6.3%) and fructose (0.6–5.4%), whereas sucrose was either absent or present in a small proportion (0.1–1.2%).

Free sugars present in the water of green coconut ( 1.4 to 1.9 g in 100 ml of water) of different ages (2-7 months) were reported by Mosihuzzaman *et. al.*[8] in 1993. Glucose and fructose were the

only two sugars found in the water. Unlike fruits, vegetables and other plant materials no sucrose or any cyclitol or alditol were found in the coconut water.

Low molecular weight carbohydrates including free sugars, alditols and cyclitol of the fast growing plants of Bangladesh like jute, dhanchi and pigeon pea (arahara) were analyzed and published[9-11].

Low molecular weight carbohydrates in two different varieties of jute *(Corchorus capsularis* and *Corchorus olitorius)* were determined[9]. Glycerol, erythritol, threitol, rhamnitol, arabinitol, xylitol, mannitol, fructose, mannose, galactitol, glucitol, glucose, *myo*-inositol, scyllitol and sucrose were identified and quantified in the neutral low molecular part of the unretted and retted bark and stem of both the varieties of jute plant. Jute plant contained more alditols and cyclitols than free sugars. Glycerol, erythritol, threitol, arabinitol, xylitol, glucose, fructose and sucrose were also identified and quantified in the bark, stem and leaf of pigeon pea *(Cajanus cajan)* plant[10]. Erythritol, arabinitol, fructose, glucose, galactitol, *myo*-inositol and sucrose were present in varying amounts in the bark and stem of dhanchi *(Sesbania canabina)* plant[11].

The rapeseed meal was extracted with aqueous 80% ethanol and eleven low molecular weight carbodydrates[12] were identified by chromatographic separation. Identified carbohydrates were

stachyose, raffinose, digalactosyl glycerol, melibiose, sucrose, *myo*-inositol, glucose, galactitol, galactose and fructose. In this report, the sugars were identified and quantified as their trimethylsilyl derivatives by means of gas liquid chromatography.

A series of low molecular weight components were isolated[13] from the dehulled and fat free meal of *Brassica compestris* using chromatography on a carbon celite column, and Tollier *et. al.*[14], reported the carbohydrate composition of *Brassica napus*.

Low-molecular weight components were determined[15] in stored tubers of three potato cultivars grown at four localities. Glucose (0.7-1.5%) and sucrose (0.7-1.2%) were the major components followed by fructose (0.1-0.8%) and *myo*-inositol (0.1-0.2%). In some samples negligible amounts of galactose, maltose, melibiose and raffinose were detected.

Theander *et. al.* have isolated[16,17] some low molecular extractives from the source *Pinus silvestris*. In the hydrophilic extract mainly carbohydrates and related cyclitols were found. The components included glucose, fructose, sucrose, pinitols and shikimic acid in a total yield of 1.5-2.5%. The next group of compounds present in the range of 0.1-1.0% were L-arabinose, L-rhamnose, D-mannitol, melibiose, raffinose, *myo*-inositol and sequoitol. Laminaribiose and cellobiose were found as free disaccharides among the low molecular weight carbohydrate constituents.

### 1.3.2 Methods of determination of free sugar:

The free sugars in various plant materials as discussed above are usually identified and quantified with the help of gas liquid chromatography (GLC) as their volatile derivatives like acetates, alditol acetates[18] and trimethylsilyl ethers[19]. The derivatives may also be identified by means of thin layer chromatography (t.l.c.) and the major free sugar components are also identified by paper chromatography. The free sugars may also be identified and quantified directly (without converting them to derivatives) by high pressure liquid chromatography (h.p.l.c.). From the literature survey it appears that determination of free sugar as their trimethylsilyl derivatives with the help of GLC, is the best method because these products are highly volatile and are well resolved in gas chromatograms. However, these derivatives are unstable (stable for 1-2 weeks in a refrizerator) and have to be analysed quickly after preparation. A combination of acetates and alditol acetates in gas chromatogrpahic determination of sugars often give very good results.

### 1.3.3 Dietary fiber.

Dietary fiber was defined in terms of physiology as the remnants of plant cells resistant to hydrolysis by the ailmentary enzymes of human being[20]. This implies that the plant cell walls are the main source of dietary fiber (DF) whereas polysaccharides

present in some food additives, such as plant gums, algal polysaccharides, modified starches, modified cellulose, suberin, cutin, polyphenols and pectins are not included. Therefore, the term DF has been redefined[21] to include undigested storage polysaccharides, present within the contents of the cell, as well as undigested polysaccharides and lignin present in the cell walls. For analytical purposes, the term DF refers mainly to starch free polysaccharides and lignin in the diet[22]. Hence, the major portion of our DF intake comes from the cell walls in food such as fruits, vegetables, cereal products and seeds[23]. Therefore, it may be more practical to include the indigestible plant cell wall polysaccharides, associated glycoproteins and other organic compounds in the food intake as DF rather than defining it simply on the basis of digestion.

The cell walls of the parenchymatous tissues of fruits, vegetables, the cotyledons of seeds and the endosperm of cereals are the major sources[24] of dietary fiber where it is also obtained from lignified and cutinized tissues of fruits, vegetables, cereal products and seeds.

Pectic substances, hemicelluloses (e.g. xyloglucans in parenchymatous tissues while glucuronoxylan in lignified tissues), celluloses and lignins are the main components[24] of DF. The middle lamella of the cell walls of the dicotyledonous plants

of parenchymatous tissues mainly contain pectins which bind the cells together while the primary wall is a composite of two distinct morphological phases known as micro fibrils. The walls of the parenchymatous tissues contain the bulk (90-95%) of the DF from fruits, vegetables and the endosperm of cereals and hence becomes important and thus, fruits and vegetables are major source[23] of DF.

### 1.3.4 Importance of dietary fiber.

The physiological importance of dietary fiber in human nutrition has been recognised[25]. Dietary fiber also play an important role in mammalian growth and nutrition.

Present interest in DF has grown mainly from the medical point of view. The beneficial effect of fiber-rich food is frequently reported[26]. This may be due to the intake of fiber as such, but also to the fact that high intake of dietary fiber from natural food, normally also leads to a lower intake of fat and total calories. The importance of DF is in the use of bran as a treatment in diverticular disease and the spastic kind of irritable bowel disease[27], which is characterized by the passing of a small pellety stool. In these circumstances there is a high success rate in the treatment of such individuals with high intake of DF. This is quite separate from the etiological aspect. For a long time most doctors have recommended

removing fiber from the diet in treatment of these diseases. Now it is proved that this was wrong and that fiber should be put back into the diet in the treatment of these diseases. For gastroenterology this has been a very significant improvement in patient care.

Various vegetable fibers have been shown to affect the serum cholesterol concentration. The diversity of their origin and chemistry suggests[28] that the mechanism by which they work is complex. Some may act by enhancing faecal bile acid excretion by adsorption or by increasing faecal flow. Others may act by providing surfaces which means that there is a qualitative change in the bacterial metabolic product of bile acids coming into the caecum.

It has been suggested[28] that fiber has an important role in prevention of carcinoma of the colon. It is possible, if this hypothesis is correct, that fiber could also dictate whether a solute coming into the colon is metabolised to an innocent compound or to a compound with carcinogenic properties. Such a change could be influenced by fiber having physical properties, either through surface effects or whether the fiber itself has been hydrolysed and changing the redox atmosphere in the colon.

Burkitt and Trowell[29] showed that appendicitis, diverticular disease of the colon, benign and malignant tumors of the large bowel, ulcerative colitis, varicose veins, deep vein thrombosis,

haemorrhoids, hiatus hernia, gall stones and cholecystitis, ischemic heart disease, diabetes, obesity, dental caries, peridontal disease, duodenal ulcer and possibly some other diseases are the result of "fiber deficiency".

The hyphothesis that dietary fiber may give protection against coronary heart disease by lowering plasma cholesterol was presented by Trowell[30]. Those who developed the general dietary hypothesis gave particular emphasis on wheat fiber[31], which has a reproducible effect on colonic function, Wheat fiber does not affect plasma lipids in short-term experiments but some other forms of the dietary fiber are now reported to lower plasma lipids, usually in fairly large amounts.

Besides prevention of constipation and reducing risk of colon cancer by dietary fiber, specific polysaccharides have been implicated[32] in lowering blood cholesterol. The effect has been frequently explained in terms of the binding of bile acids by the specific polysaccharides.

Dietary fiber is one of many factors which can affect the intestinal handling of glucose[26]. It has clearly been demonstrated[33] that the viscous polysaccharides such as guar, pectin, or $\beta$-glucan from oats when incorporated in the diet of patients with adult onset diabetes, significantly reduce their insulin requirements. It has been reported by Anderson[34] that

high-carbohydrate, high-fiber diets are good for patients with diabetes mellitus. A recent report[35] cites *Trigonella foenum-graecum* fibers having similar properties of reducing sugar levels.

Besides the content and type of dietary fiber, the structure of food may influence the glucose response. Thus, apples, either whole, or given as puree of juice, produced glucose responses which increased both with the disruption as well as removal of the dietary fiber[36]. After eating whole apple, the glucose levels returned to the basal level after 60 min and remained there, whereas rebound hypoglycemia appeared after administration of juice and puree.

Methods of incorporation of fiber supplements, or food naturally high in dietary fiber, seem to have an influence on the glycemic response[37,38]. Thus, the effect of guar is greatly reduced if it is taken separately and not mixed into the food[39,40,41].

Recently, it has been reported[41] that the levels of DF and pectin were determined in different types of Bulgarian diebetic food. The effects of baking on water soluble non-starch polysaccharides in white bread fraction has also been reported[42].

It appears that the non-starch character which is demonstrated by non-digestibility, and the high viscosity of the polysaccharides

in the critical segment of the intestine may offer the explanation of this effect. The understanding of the beneficial physiological effects of the non-starch polysaccharides on the molecular basis is the area for further research.

Now, it is a matter of great interest to find out the particular components of dietary fiber responsible for bioactivity.

## 1. 4. Methods of determination of dietary fiber:

There is no universal method availabe for determination of soluble and insoluble dietary fiber but a good number of methods have been developed for the analysis of DF. Some of the methods availabe are cited below.

### 1.4.1. Detergent fiber method[43, 44]

The detergent system used may be neutral or acidic giving DF products termed as neutral detergent (NDF) and acid detergent fiber (ADF). In NDF method the extraction of plant tissue is done by boiling with neutral solutions of sodium lauryl sulphate and EDTA in essential non-hydrolytic condition and removes soluble carbohydrates, proteins, pectins and lipids as detergent complexes. In the ADF method the plant fraction is refluxed for one hour with 0.5M sulphuric acid and 2% cetyltrimethyl-ammonium bromide. The soluble and insoluble materials are termed as acid detergent fiber (ADF).

### 1.4.2. The Southgate method[45]:

The Southgate method was developed for the analysis of unavailable carbohydrates (dietary fiber) in mixed diets and part of a procedure for measuring the various classes of carbohydrates in mixed diet. In this method free sugars, starch, non-cellulosic polysaccharides, cellulose and lignins are measured separately. Dietary fiber is determind using suitable corrections.

### 1.4.3. Theander and Aman method[46]:

A procedure for the analysis and chemical characterization of water-soluble and water-insoluble dietary fiber including non-starch polysaccharides and lignin from plant foods has been developed[46]. The method consists of extracted of the foodstuff with aqueous 80% ethanol and chloroform. The residue is treated with amylase at 85°C and extraction with hot water. The water soluble polymeric fraction is recovered by dialysis and freeze-drying and water insoluble fraction is obtained as the residue. Klason lignin in the water-insoluble fraction is determined gravimetrically. The amounts of sugar and uronic acid constituents calculated as polysaccharides provide a measure of the dietary fiber content in the water-soluble fraction, and these components plus klason lignin provide a measure of the content in the water-insoluble fraction. A wide variation was found in the chemical composition of the both water-soluble and water-insoluble dietary fibers.

### 1.4.4 Rapid enzymatic assay[47] of insoluble and soluble dietary fiber.

The procedure includes the following main steps :

(a) Gelatinization by boiling 15 min in the presence of a heat-stable α-amylase.

(b) Incubation with pepsin at acid pH for 1 h, and incubation with pencreatin at neutral pH for 1 h.

(c) Insoluble dietary fiber is filtered off with celite 545 as the filter aid.

(d) Soluble dietary fiber is precipitated from the filtrate with four volumes of ethanol and recovered by filtration in the same way as insoluble dietary fiber.

### 1.4.5 Prosky *et. al.* method[48] for the determinatioon of total dietary fiber.

The dried foods are extracted to remove fat and gelatinized with Termamyl (heat stabel α-amylase), then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present. Then 4 volumes of 95% ethanol are added to precipitate the soluble dietary fiber. The total residue is filtered, washed with 74% ethanol, 95% ethanol and acetone, After drying, the residue is weighed. Total dietary fiber is the weight of the residue less the weight of the protein and ash present.

### 1.4.6 Faulks and Timms procedure[44].

This method is based on the procedure of Theander and Aman[46]. In this method starch is removed by treating the gelatinized material with Termamyl at 100°C for 15 min by dispersing it in aqueous dimethyl sulfoxide (DMSO) and treating with amyloglucosidase at 37°C for 35 min. The main advantage of the process is that the removal of starch can be accomplished in a very short time.

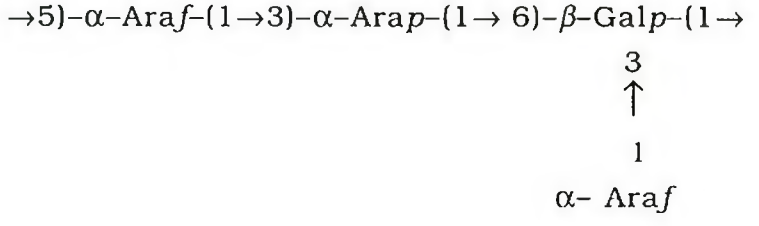
### 1.4.7 Theander and Westerlund method[50].

Homogenized and/or milled foodstuff is extracted with 80% ethanol and light petroleum ether. The extractive free residue is analysed directly for the DF and starch content (method B) or treated further with thermostable $\alpha$-amylase at 96°C for 1 h and amyloglucosidase at 60°C (16 h). Insoluble and soluble DF components are then separated by centrifugation dialysis and freeze-drying of the supernatant (method A). Alternatively, insoluble and soluble DF components are isolated together by precipitation of soluble fiber in 80% ethanol and subsequent centrifugation (method C). Starch is determined enzymatically. A satisfactory correlation between the different DF preparation methods was observed[50] for several food samples.

### 1.4.8 Composition of the soluble dietary fiber:

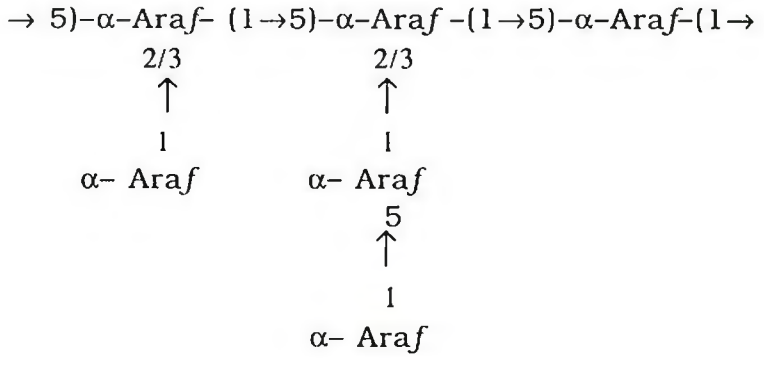
Water-insoluble dietary fiber mostly contain water–insoluble hemicelluloses, cellulose and lingin. There are not much report available on water-insoluble DF of fruits and vegetables. Water soluble dietary fiber plays important role in biological system. Therefore, composition of some of the water–soluble DF are cited below.

Water–soluble pectin, a polygalacturonide was isolated[57] from guava *(Psidium guajava)* fruit. A polysaccharide which contained substantial amount of uronic acid and neutral sugar constituents were also reported by the same workers. Structure of another polysaccharicle was determined as a acidic arabinogalactan SDF of guava was also isolated and its structure was elucidated by chemical and spectroscopic methods as an neutral arabinogalan[58]. The structure of the neutral arabinogelactan of SDF of guava is given below.



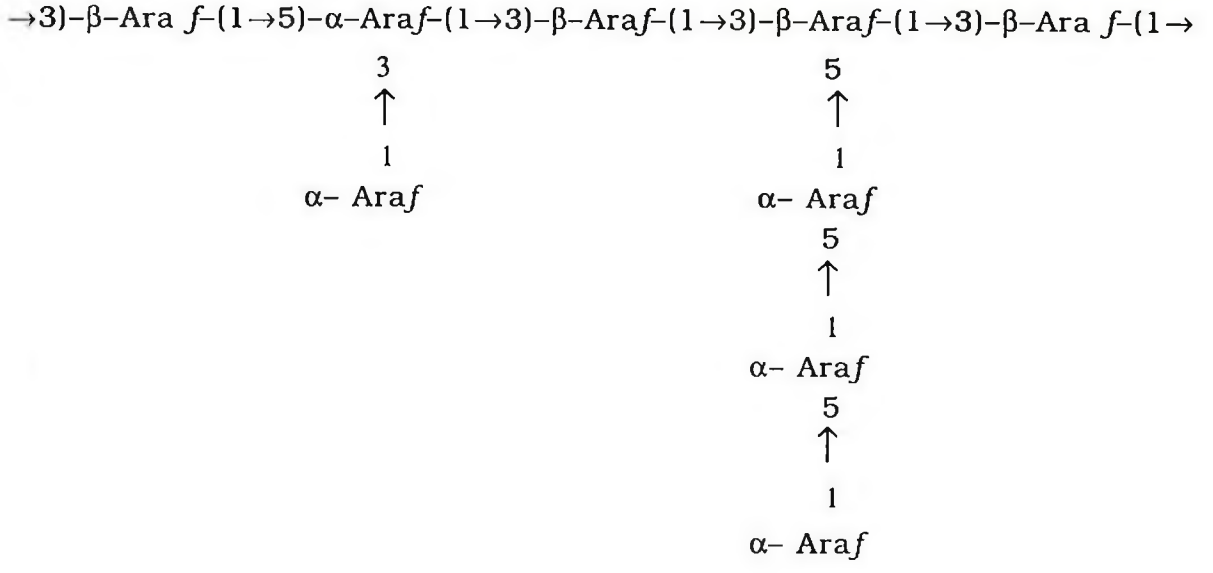

**Structure– 1**

Detailed studies were made on water-soluble DF of lukluki *(Flacuartia indica)* and boroi *(Zizyphus mauritiana)* fruits[59]. A neutral and an acidic SDF were isolated. Acidic SDF showed that they were very complex mixture of polysaccharides containing substantial amount of uronic acids. Arabinose and galactose were the two predominating neutral sugars present in the SDF. Structure of the neutral part of SDF of the two fruits were determined by sugar and methylation analyses and $^{1}H$- and $^{13}C$-NMR spectroscopic studies. Neutral SDF of lukluki was a highly branched arabinan composed of arabinofuranoside residues in the main chain as well as in the side chain (Structure 2).



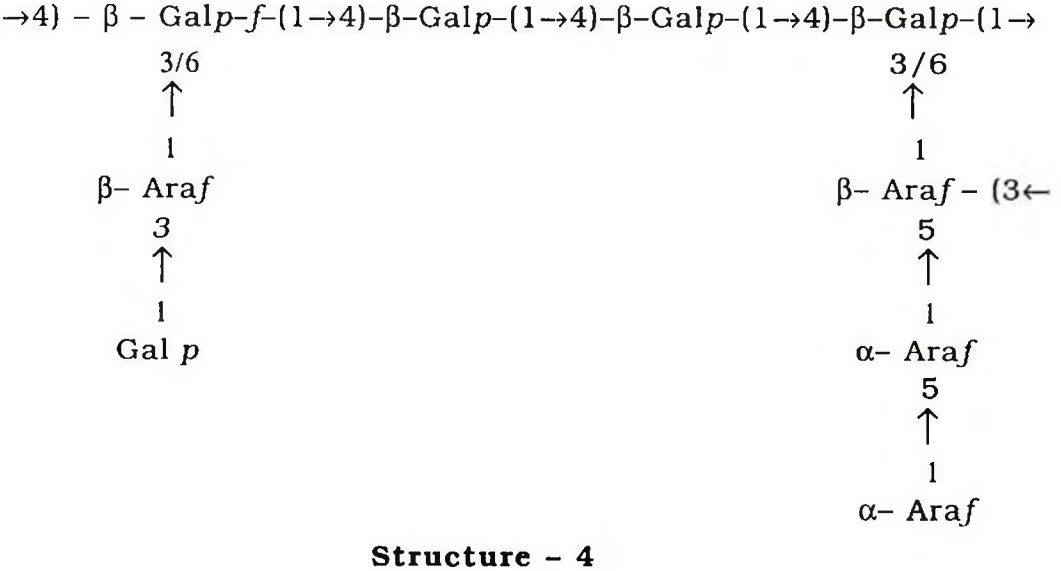

**Structure- 2**

Structure of the neutral part of the SDF of boroi was also a highly branched arabinan (Structure 3) but the anomeric linkages were different. Structure of the arabinan of boroi is given below.




**Structure - 3**

An arabinogalctan[59] from the SDF of jalpai (*Elaeocarpus robustus*) was isolated and structure of the neutral SDF was determined by chemical and spectroscopic methods (Structure-4). The main chain of the neutral SDF was composed of β-1,4 -linked galactan backbone having a side chain of arabinofuranoside at the position 6 and or 3.




**Structure - 4**

## 1.5 Fatty acids.

Fatty acid composition of common fats and oils are available[51] (Table-1). Based on the chemical compositions, fats and oils are classified as edible and non-edible. Vegetables generally do not contain much of lipid or fatty materials. Although, small amount of lipids are present in the vegetables the composition of the fatty acids are not yet reported. Therefore, studies were made to determine the fatty acid composition of the eight local vegetables.

Fatty acid composition of the unretted jute plant *(Corchorus capsularis and Corchorus olitorius)* was reported[52]. Lauric, myristic, palmitic, stearic, oleic, linoleic, arachidic and behemic acids in the jute plants were identified and quatified. Fatty acid content and their coposition in low and high yeilding varities of rice straw were also published[53]. Nonanoic, capric, undecanoic, myristic and stearic acids were found only in the traditional varieties of rice straw *i.e.* balam, kalojira and beroi in varying amounts. Palmitic, oleic, linoleic, arachidic, behemic acids were found in the high yeilding varities, BR10, BR11 and BR14 as well in the low yeilding varieties. Fatty acid present in the coconut water were also published[8]. Only three fatty acids, caprylic, capric and lauric acids were indentified and quantified in the coconut water.

## Table-1

### Fatty acid composition of the some common fats and oils

| No. of C atoms | Acid | Formula | Butter | Cotton | Soybean | Corn | Menhaden | Whale | Linseed | Coconut | Beef Tallow | Lard |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Butyric | $C_3H_7COOH$ | 4.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 5 | Caproic | $C_5H_{11}COOH$ | 2.0 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 8 | Caprylic | $C_7H_{15}COOH$ | 1.0 | ... | ... | ... | ... | ... | 8.0 | 8.0 | ... | ... |
| 10 | Capric | $C_9H_{19}COOH$ | 2.5 | ... | ... | ... | ... | ... | 7.0 | 7.0 | ... | ... |
| 12 | Lauric | $C_{11}H_{23}COOH$ | 3.0 | ... | ... | ... | ... | ... | 48.0 | 48.0 | ... | ... |
| 14 | Myristic | $C_{13}H_{27}COOH$ | 10.0 | 0.6 | ... | ... | 8.0 | ... | 17.5 | 17.5 | 3.0 | 1.0 |
| 14 | Myristoleic | $C_{13}H_{25}COOH$ | ... | ... | ... | ... | 1.5 | ... | ... | ... | ... | 0.2 |
| 16 | Palmitic | $C_{15}H_{31}COOH$ | 25.0 | 22.9 | 8.3 | 7.5 | 12.1 | 6.5 | 8.8 | 8.8 | 28.0 | 28.0 |
| 16 | Palmitoleic | $C_{15}H_{29}COOH$ | ... | ... | ... | ... | 15.0 | ... | ... | ... | ... | 3.0 |
| 18 | Steatic | $C_{17}H_{35}COOH$ | 11.0 | 2.2 | 5.4 | 3.5 | 2.3 | 4.5 | 2.0 | 2.0 | 25.0 | 13.0 |
| 18 | Oleic | $C_{17}H_{33}COOH$ | 28.5 | 24.7 | 24.9 | 46.3 | 33.4 | 20.9 | 6.0 | 6.0 | 42.0 | 46.0 |
| 18 | Linoleic | $C_{17}H_{31}COOH$ | 2.5 | 49.7 | 52.7 | 42.0 | ... | 17.4 | 2.5 | 2.5 | 2.0 | 6.0 |
| 18 | Linolenic | $C_{17}H_{29}COOH$ | ... | ... | 7.9 | ... | 9.0 | 50.6 | ... | ... | ... | 0.7 |
| 20 | Arachidic | $C_{19}H_{39}COOH$ | ... | ... | 0.9 | 0.5 | ... | ... | ... | ... | ... | 2.0 |
| 20 | Arachidonic | $C_{19}H_{35}COOH$ | ... | ... | ... | ... | 8.2 | 0.1 | ... | ... | ... | ... |
| 22 | Clupanadonic | $C_{21}H_{35}COOH$ | ... | ... | ... | ... | 10.5 | ... | ... | ... | ... | ... |
| 24 | Lignoceric | $C_{23}H_{37}COOH$ | ... | ... | ... | 0.2 | ... | ... | ... | ... | ... | ... |

## 1.6 Classification of vegetables.

**Vegetables are mainly classified into three general classes:**

i) Earth vegetables, ii) Herbaceous vegetables and iii) Fruit vegetables.

**i) Earth Vegetables:** Earth vegetables include all forms of plants in which food is stored in the undergound part. Some are true root such as 'carrot' and others represent modified stem such as potatoes. Some other earth vegetables are sweet potatoes, beet, raddish, khamalu etc.

**ii) Herbaceous Vegetables:** The plants whose stem, leaves and flowers are taken as vegetables are known as herbaceous vegetables. These inlcude, asparagus, cabbage and cauliflower, *(Ipomoea aquatica, Spinacia Oleracea etc.)*

**iii) Fruit Vegetables:** The fruits, mostly unripe, which are used as vegetables are known as fruit vegetables. Some of the fruit vegetables are, brinjal, lady's finger, pumkin, bitter gourd, water gourd, all types of beans etc.

Vegetables are also classified into two general groups according to their growing seasons such as winter vegetables and summer vegetables.

Many varieties of vegetables are grown in Bangladesh both the seasons. But the number of varieties and also the quantity of production are more in the winter season than the summer season in the tropical countries like ours. In the cold countries much less vegetables are grown and that mostly in their summer season.

## 1.7. Food value of vegetables.

The food value of vegetables are comparatively low owing to the large amount of water content. But it plays an important role in the human diet, supplying some of the ingredients which are not available from other food stuff. Green vegetables are important source of minerals, calcium and iron needed by the body.

Vegetabldes are also a valuable source of vitamin. Green and yellow vegetables are important source of 'vitamin A'. Vegetables also supply ascorbic acid and appreciable amounts of thiamin, niacin and folic acid, which are all very essential for our body growth.

Although, vegetables in general are not considered as a source of carbohydrates, proteins and fats, some of them such as dried seed of beans, peanuts etc. are rich in protein. Other vegetables such as green banana, potatoes, sweet potatoes are important source of carbohydrates.

# EXPERIMENTAL

# EXPERIMENTAL

## 2.1 GENERAL METHODS.

### 2.1.1 Evaporation.

All evaporations were earried out under reduced pressure with the help of rotary vacuum evaporator (Buchi; Switzerland) at bath temperature not exceeding 40°C.

### 2.1.2 Freeze-drying.

All freeze-drying were carried out with the help of a HETOSICC CD 52 (HETO LAB EQUIPMENT, DENMARK) and a VARIAN-801, Model LY-3-TT (Germany) freeze-dryer. The samples were pre-frozen in round bottomed Quickfit flasks in a HETOFRIG methanol cooling bath (HETO BIRKERD DENMARK) at - 40°C.

### 2.1.3 Solvents, reagents and chemicals.

All the solvents, reagents and chemicals used in the experimental work were analytical reagent grade and were procured from Sigma, E. Merck or BDH. Ethanol (absolute alcohol) was obtained from Carew and Co. Darsana, Kushtia, Bangladesh, and was used after distillation.

### 2.1.6 Preparation of buffers.

#### (a) Phosphate buffer.

Sodium hydrogen phosphate ($NaH_2PO_4$. $2H_2O$; 31.2 g) was dissolved in water to make 1000 ml of solution. Disodium hydrogen phosphate ($Na_2HPO_4$. $12H_2O$; 71.6 g) was dissolved in water to make 1000 ml solution. Both the solution was 0.2M in concentration. Sodium dihydrogen phosphate solution (610 ml; 0.2M) and disodium hydrogen phosphate solution (390 ml; 0.2M) were mixed together and was diluted to 2000 ml with water to make the solution overall 0.1M. The buffer had a pH of 7.0.

#### (b) Acetate buffer.

Sodium acetate ($CH_3$-COONa, $3H_2O$; 27.2 g) was dissolved in water to make 1000 ml of solution. The resulting solution was 0.2M. Acetic acid (12 ml) was poured in water to make 1000 ml of solution. The resulting solution was 0.2M. Then 794 ml of 0.2M sodium acetate solution was mixed with 206 ml of 0.2M acetic acid solution, and the mixture was diluted to 2000 ml with water to make the solution overall 0.1M. The buffer had a pH of 5.0.

### 2.1.7 Preparation of ion-exchange column.

#### (a) Cation exchange column:

At first some glass wool was pressed at the bottom of a clean and dry glass column (70 x 2.3 cm) with a clean glass rod. The column was half-filled with a slurry of Dowex 50Wx8 $H^+$ resin in water. During filling the column with slurry of the resin it was continuously tapped for homogeneous packing of the resin.

When the column was packed, 2M hydrochloric acid (twice the volume of the column) was passed through it to ensure the resin to be fully in the $H^+$ form. The column was then washed with distilled water until it was acid free. The eluent from the column was tested with pH paper and silver nitrate solution to make sure that the column was acid free. The cation exchanger was then ready for use.

**(b) Anion exchange column:**

Anion exchange column was prepared in the same way as the cation using amberlite CG 400 $OH^-$ resin. The column (70 × 2.3 cm) was packed with the resin and aqueous 20% ammonia solution was passed through the column for complete coversion of the resin to the $OH^-$ form. The column was again washed with distilled water to remove excess alkali. The eluent form the column was tested with pH paper to make sure that the column was alkali free. Then the column was ready for use.

### 2.1.8 Measurement of pH

All pH were measured by a METHOHM 632/pH meter with an accuracy of ± 0.01 pH. The pH meter was calibrated by using standard buffers of pH 7.0 and 5.0.

### 2.1.9 Identification of sugars:

#### (a) By paper chromatography:

All paper chromatograms were run on Whatman No.1 paper by the descending development technique, using different solvent systems described below:

i) *n*- Butanol : ethanol : water (40 : 11: 19) ; V/V

ii) *n*- Butanol : pyridine : water (10 : 3 : 3) ; V/V

iii) Ethyl acetate : acetic acid : water (3 : 1: 1) ; V/V

The irrigated papers were dried and then these were developed with two methods given below:

**i) Spraying with, *p*-anisidine phthalate solution. (O.1M *p*-anisidine + 0.1M phthalic acid, 1:1)**

*p*-Anisidine-phthalate spray reagent was prepared in the following way.

a) *p*-Anisidine (0.35 g) was dissolved in ethanol (100 ml).

b) Phthalic acid (1.66 g) was dissolved in ethanol (100 ml)

The two solutions were mixed in equal proportion (*p*-anisidine phthalate). The dried chromatographic papers were sprayed with the reagent with the help of a sprayer. The paper was then heated at 105°C in an oven for 30 min. The presence of sugars were indicated by colored spots, (orange, red, yellow or greenish yellow).

**ii) Dipping in aqueous acetonic silver nitrate followed by aqueous alcoholic sodium hydroxide.**

Dipping reagents were made in the following procedure.

Saturated aqueous solution of silver nitrate (1 ml) was mixed with acetone (200 ml) and water was added dropwise till the precipitate formed was redissolved.

Aqueous alcoholic sodium hydroxide solution (2%) was prepared by dissolving 40 g of sodium hydroxide in 100 ml of water and then diluting it to 2000 ml with absolute alcohol.

The irrigated paper was air-dried and then it was dipped in silver nitrate solution and air-dried again. The dried paper was dipped in sodium hydroxide solution. A few minutes later the paper was washed with aqueous 10% sodium thiosulphate solution followed by water and finally air-dried. The presence of sugars was indicated by black spots on the paper.

**(b) By gas-liquid chromatography (GLC)**

**i) As trimethylsilyl (TMS) derivatives:**

Neutral LMW fraction (5 mg) was dissolved in water (10 ml) containing 1 mg of ribose as internal standard. It was evaporated to dryness with added methanol. The dried material was dissolved in pyridine (2 ml). The flask was flashed with

nitrogen gas to remove air. In nitrogen atmosphere, hexamethyl disilazane (HMDS, 1 ml) and trimethyl chlorosilane (TMCS, 0.5 ml) were added to it. The mixture was shaken for 5 min in a ultrasonic bath. It was then heated for 20 min in an oven at 60°C. The mixture was again shaken in an ultrasonic bath for 2 min and then evaporated with added toluene to remove last traces of pyridine. The TMS derivative was dissolved in *n*-hexane (1 ml) and analysed by GLC.

Speration of trimethylsilyl (TMS) derivatives were carried out with a packed column of CP-Sil 5 (100 cm ×0.2 cm, i.d.) using a PYE UNICAM GCD gas chromatograph at 185°-220°C, 2°C $min^{-1}$. Quantification of the peaks were done manually by using formula, peak area = peak height × width at $^1/_2$ height of the peak.

**ii) As alditol acetates:**

The sample neutral LMW fraction or hydrolysate of a polysaccharide material (5 mg) was taken in a pear shaped flask and treated with sodium borohydride (50 mg). The solution was allowed to stand for 2 hours with occasional shaking. Cation exchange resin DOWEX ($H^+$) was added to acidify the solution. It was filtered and the filtrate was evaporated to dryness by adding methanol. The residue was then dissolved in pyridine (1 ml) and acetic anhydride (1 ml).

The resulting mixture was heated on a boiling water bath for half an hour and was evaporated with ethanol to remove last traces of pyridine and acetic anhydride. The product was then dissolved in chloroform and analysed by GLC.

Gas-liquid chromatography (GLC) was conducted with a packard 427 gas chromatograph fitted with flame ionization detector and quartz WCOT capillary column. Separation of acetates and alditol acetates were perfomed on OV-225 (2300 cm × 0.2 cm, i.d.) and CP Sil-88 (1250 cm × 0.2 cm, i.d.) at 160° - 220°C, 4°C $min^{-1}$. For quantitative evaluation a LKB 2230 recording integrator (LKB, Bromma) was used.

### 2.1.10. Gas-liquid chromatography (GLC) technique used in fatty acid analysis.

Fatty acids containing sample (50 mg) of vegetables (Table-3) and benzoic acid (5mg) as internal standard were taken in pear-shaped flask (25 ml). Then the mixture was dissolved in 0.5M methanolic sodium hydroxide solution and refluxed it for 10 min. The mixture was evaporated to dryness and $BF_3$. $CH_3OH$ complex ( 5 ml) was added to it. The resulting mixture was heated on a boiling water bath for 2 min. The hot mixture was washed into a small separatory funnel with petroleum ether (3 ml) and distilled water (2 ml) was added. The funnel was shaken vigorously and the layers were allowed

to separate. The petroleum ether layer was separated from aqueous-methanol layer and was dried by treating with anhydrous sodium sulphate, filtered into another Quickfit flask, concentrated to a small volume by using vacuum evaporator and transfered to a vial. Finally the petroleum ether containing mixture of methyl ester of acids was concentrated to a few drops by blowing nitrogen gas and analysed by GLC.

Gas-liquid chromatography was conducted with a PYE UNICAM GCD gas chromatograph fitted with a flame ionization detector and glass column (1500 cm x 0.2 cm, i.d.). For separation of fatty acids a column packed with 10% Silar 10C ( Supelco, Bellefonte Pennsylvania, USA) coated on Gas-chrom Q 100-120 mesh was used. Temperature program of 120°-220°C, 4°C $min^{-1}$ with 2 min initial holding time was used. The nitrogen flow rate was 40 ml $min^{-1}$.

### 2.1.11. Determination of water content.

Water content of vegetables were determined by heating the edible part of the vegetables in an electric oven at 105°C until a constant weight was obtained. The water content of the vegetable was obtained from the deference in weight and from that the percentage of water content and the dry matter of the vegetable was calculated.

### 2.1.12. Determination of uronic acid (Carbazole method).

**i) Sulphuric acid reagent;**

Sodium metaborate (2.38 g) was dissolved in concentrated sulphuric acid (250 ml).

**ii) Carbazole reagent.**

Carbazole (125 mg) was dissolved in ethanol (100 ml).

Sample solution ( 500 µl ) and a solution of sodium metaborate in sulphuric acid (3 ml) was taken in a test tube. The mixture was shaken well with a test tube shaker and heated on a boiling water bath for 20 min. The solution was cooled and an ethanolic solution of carbazole ( 100 µl ) was added to it and the mixture was heated again on a boiling water bath for 15 min and cooled. Appearance of pink colour indicated the presence of uronic acid. The uronic acid content of the sample was then determined colorimetrically by measuring the absorbance of the coloured solution at 530 nm in a UV spectrophotometer.

## 2.2 Experiments

### 2.2.1 Collection of vegetables.

Good quality, green and fresh vegetables were bought from Mohammadpur market, Mohammadpur, and Kawran bazer market, Kawran bazer, Dhaka.

Only the edible parts of the vegetables (Table-2; Photograph 1-8) were used for analytical purposes. About 150 g fresh edible parts of each vegetable was used for extraction with aqueous 80% ethanol.

### 2.2.2 Determination of dry matter in different vegetables.

A previously washed, dry and weighed weighing bottle was used for determination of dry matter of vegetables. Fresh vegetable (100 g) was taken in it and was then dried at 105°C in an oven. The bottle was cooled in a dessicator and weighed. The drying, cooling and weighing were repeated until a constant weight was obtained. The water content of the vegetable was obtained from the deference in weight and from that the percentage of water content and the dry matter of the vegetable was calculated. The same procedure was followed for all the eight vegetables and the results are presented in Table -2 and graphics- 1-8.

**Photograph 1:** A photograph of fresh Parbal (Potol)

**Photograph 2:** A photograph of fresh Bitter gourd (Karela)

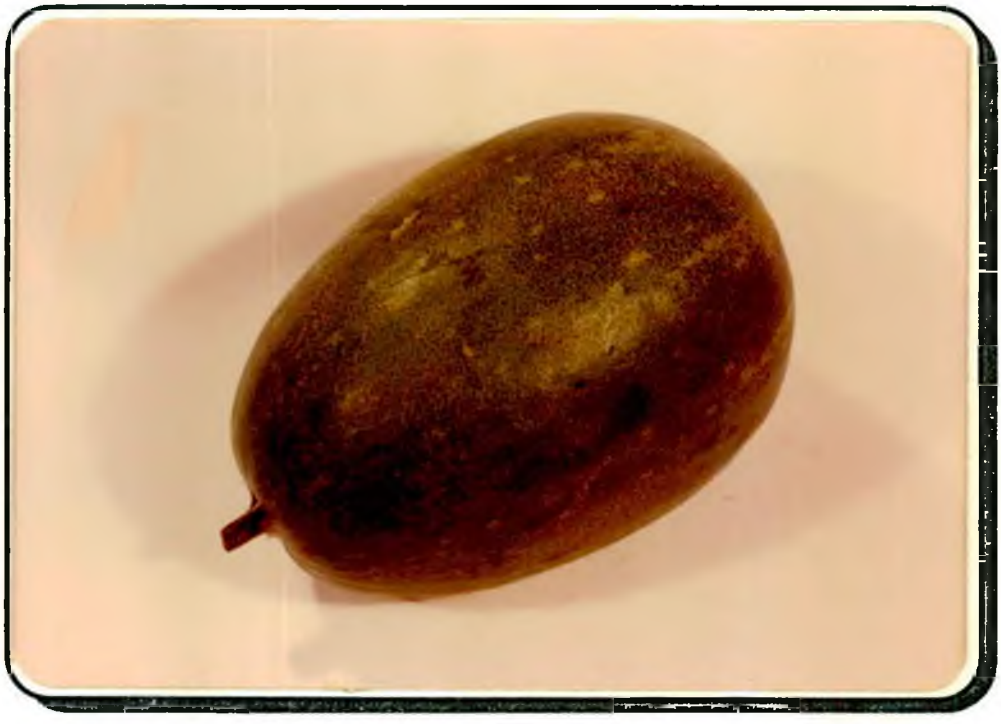
**Photograph 3:** A photograph of fresh Cauliflower (Fulkopy)

**Photograph 4:** A photograph of fresh Cabbage (Badhakopy)

**Photograph 5:** A photograph of fresh Snake gourd (Chichinga)

**Photograph 6:** A photograph of fresh Carrot (Gajor)

**Photograph 7:** A photograph of fresh Turnip (Shalgam)



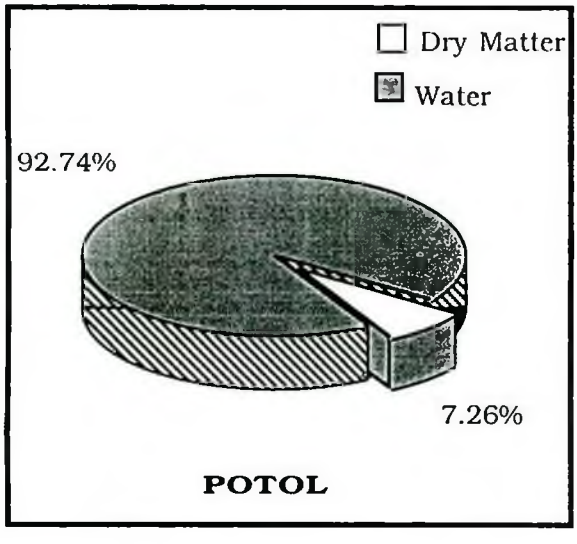
**Photograph 8:** A photograph of fresh Pumpkin (Chalkumra)

## Table- 2

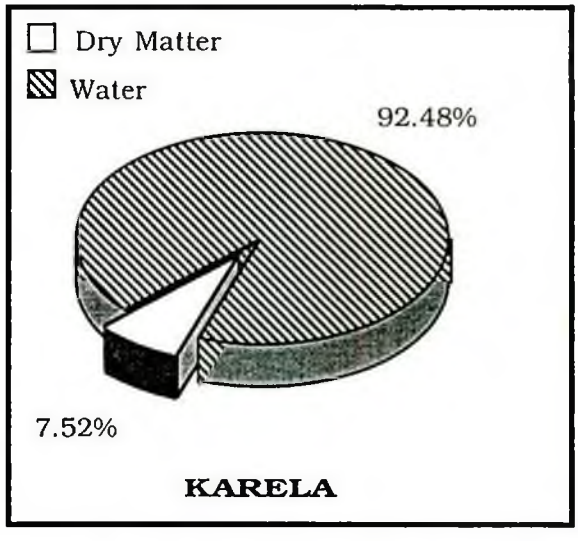
### Dry matter in defferent vegetables

| Botanical name of vegetable | English name/ Common name | Local name* | Dry matter content g/100 g fresh vegs. |
|---|---|---|---|
| 1. *Trichosanthes dioica* | Parbal | Potol | 7.26% |
| 2. *Momordica charantia* | Bitter gourd | Karela | 7.52% |
| 3. *Brassica oliracea* var. *botrytis* | Cauliflower | Fulkopy | 7.14% |
| 4. *Brassica* oliracea var. *capitata* | Cabbage | Badhakopy | 6.09% |
| 5. *Trichosanthes anguina* | Snake gourd | Chichinga | 3.13% |
| 6. *Daucus carota* | Carrot | Gajor | 10.88% |
| 7. *Brassica campestris* var. *turnip* | Turnip | Shalgam | 5.84% |
| 8. *Benincasa cerifera* | Pumpkin | Chalkumra | 4.35% |

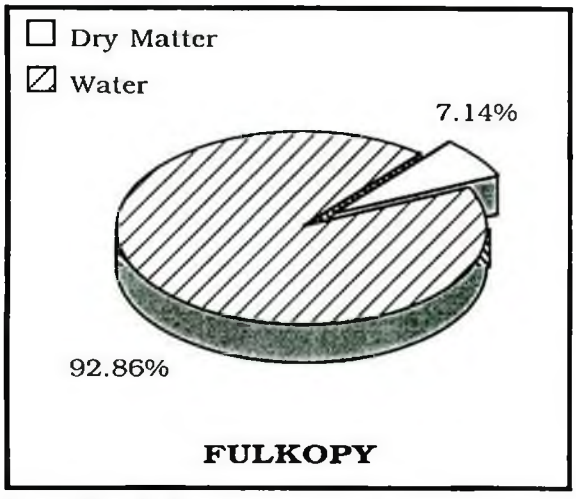
*** In the rest of the Tables only the local names are used.**



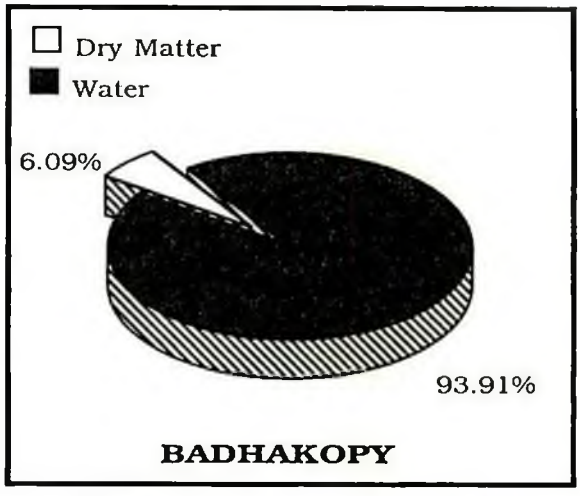

**Graphic- 1** Dry matter and water content in potol.



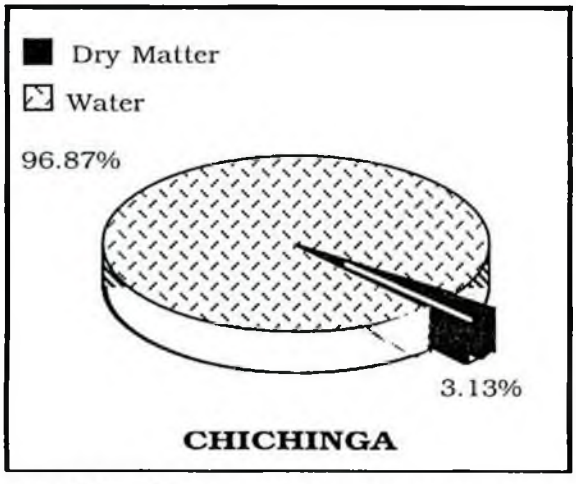

**Graphic- 2** Dry matter and water content in karela.



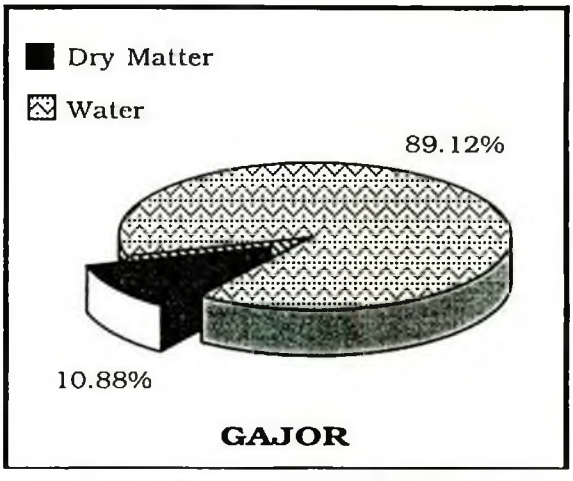

**Graphic- 3** Dry matter and water content in fulkopy.



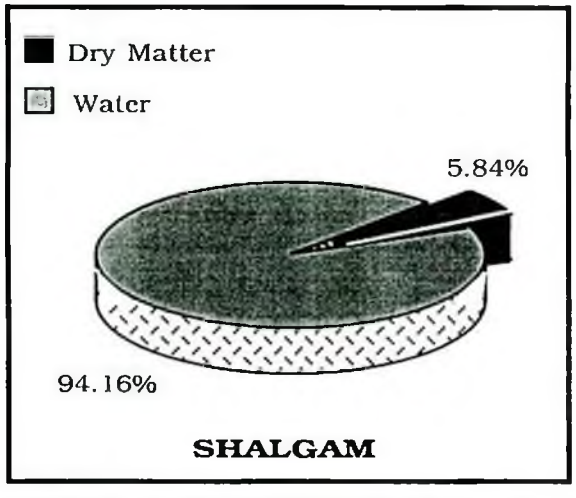

**Graphic- 4** Dry matter and water content in badhakopy.



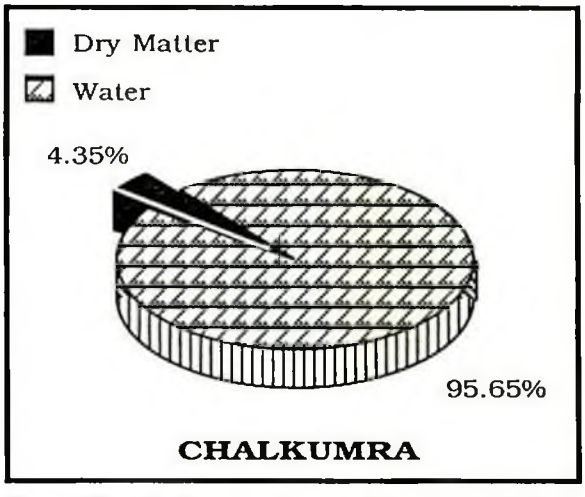

**Graphic- 5** Dry matter and water content in chichinga.




**Graphic- 6** Dry matter and water content in gajor.




**Graphic- 7** Dry matter and water content in shalgam.




**Graphic- 8** Dry matter and water content in chalkumra.

### 2.2.3 Extraction of fresh vegetables (Scheme-1).

The green, fresh and chopped or crushed edible parts of vegetables (100 g) were taken in a Quickfit flask and then sufficient amount of absolute alcohol was added to it so that solvent composition became aqueous 80% alcohol. The proportion of ethanol was adjusted taking into consideration the water content of the vegetables. The mixture was refluxed for 1 h on a boiling water bath and then filtered through a Buckner funnel. The residue was extracted with aqueous 80% ethanol in the same way three to four times for each vegetable. The residue was air-dried and was suspended in distilled chloroform and refluxed for 1 h on a boiling water bath. The solvent was filtered through a Buckner funnel. The residue was extracted with chloroform three more times in the same way as before. The extractive-free residue was air-dried, weighed and was used for dietary fiber (DF) determination.

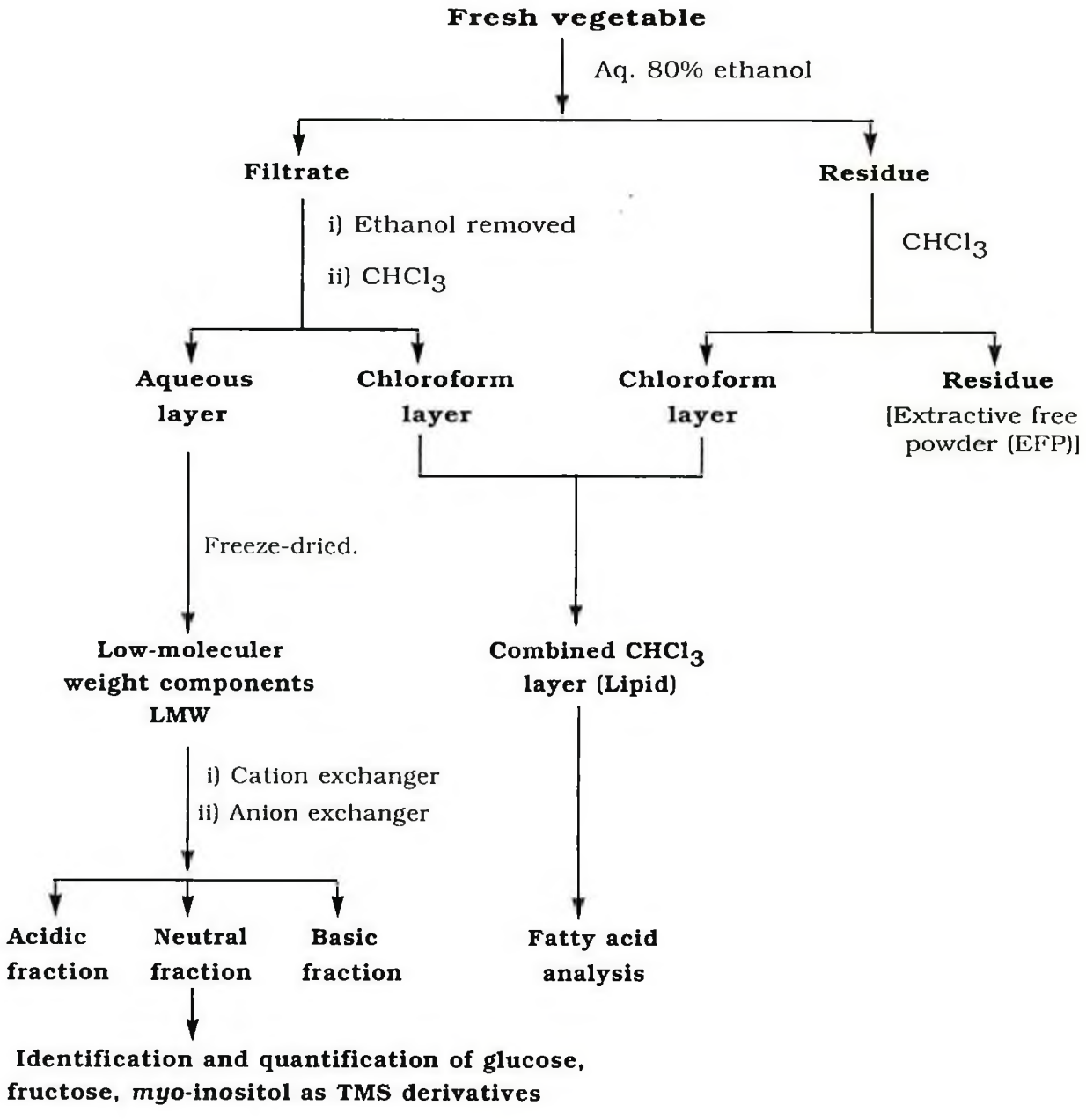
The aqueous ethanolic extracts were combined and the ethanol was removed by rotary evaporation. The resulting alcohol-free aqueous solution was extracted with distilled chloroform two to three times (Section 2.2.4). The chloroform extracts were combined and dried with sodium sulphate, filtered, concentrated to dryness by rotary evaporation and weighed. The results are presented in Table-3. This fraction was termed as lipid (liphophilic materials) and was used for fatty acid analysis.

The aqueous layer was concentrated to dryness and after weighing the sample was dried in a vacuum oven at 40°C to constant weight for determining the amount of aqueous ethanol extract. The results are presented in Table-3 and Bar graph-1. This fraction was termed as low-molecular weight components (LMW) and used for free sugar analysis as TMS derivatives by GLC.

The same procedure was followed for all the eight vegetables.

## Extraction of fresh vegetable

### Scheme-1



### 2.2.4 Removal of liphophilic material from aqueous 80% ethanol with chloroform.

The aqueous 80% ethanol soluble fraction was concentrated to 100 ml with added water (to remove alcohol). The water solution was transfered to a cleaned seperating funnel (500 ml) with glass stopper and chloroform was added to it. The mixture was shaken well. Then the solution was allowed to settle for complete seperation into chloroform and aqueous layers. The greenish colored chloroform extract was collected from the bottom of the seperating funnel. The procedure was repeated for another two to three times. To the chloroform layer, anhydrous sodium sulphate was added to remove any residual water. The mixture was filtered and the chloroform solution was added with the chloroform layer which was obtained from chloroform extraction of residue. The chloroform solution was concentrated to dryness in a previously weighed pear shaped flask and the weight of the chloroform extract was determined. The amount of chloroform extracts are presented in Table-3 and Bar graph-1.

### 2.2.5 Fractionation of low-molecular weight components.

The low-molecular weight component (LMW; 200 mg) was dissolved in distilled water (10 ml) by ultrasonication. The solution was applied to a cation exchange column (Section 2.1.7) with a syringe. The column was eluted with distilled

water. After passing two column volumes of water, a small amount of eluent was analyzed by phenol-sulphuric acid method which indicated the absence of sugar.

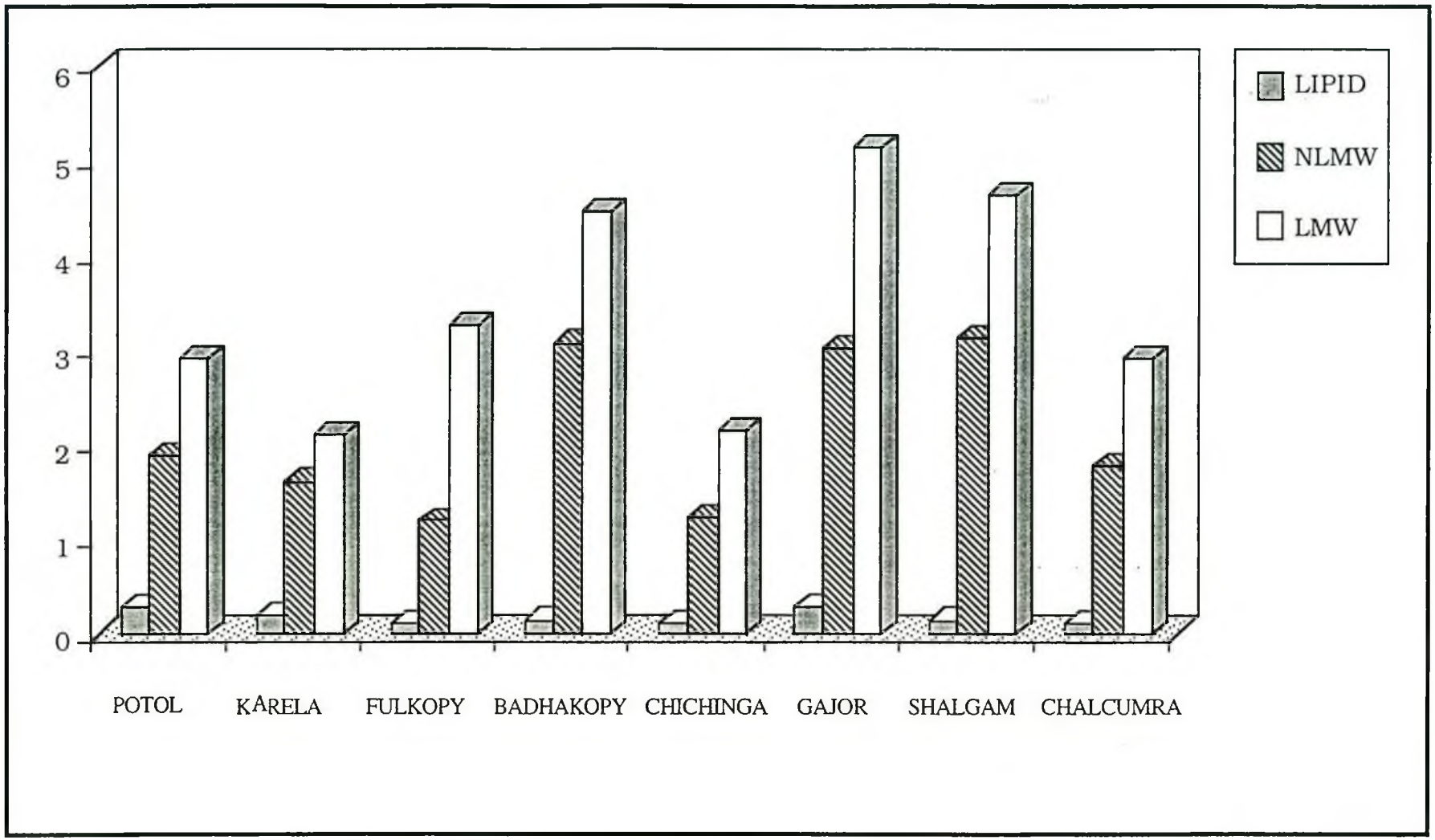
All the eluent coming out from the cation exchange column was put on to the top of an anion exchange column (Section 2.1.7). The anion exchange column was eluted with water. The neutral water soluble carbohydrates coming out from the anion exchange column was concentrated to a small volume (50 ml) and freeze-dried in a previously weighed pear shaped flask.

The same procedure was followed for all the eight vegetables and the amount of neutral LMW components are presented in Table 3 and Bar graph 1.

**Table-3**

**Amount of LMW, neutral LMW and chloroform extract in g/100 g fresh vegetable.**

| Name of the vegetables | Aq. 80% ethanol extract (LMW) | Chloroform extract (lipid) | Neutral LMW |
|---|---|---|---|
| Potol | 2.91 | 0.27 | 1.89 |
| Karela | 2.11 | 0.19 | 1.61 |
| Fulkopy | 3.28 | 0.12 | 1.49 |
| Badhakopy | 4.47 | 0.14 | 3.09 |
| Chichinga | 2.15 | 0.12 | 1.23 |
| Gajor | 5.15 | 0.28 | 3.02 |
| Shalgam | 4.67 | 0.15 | 3.13 |
| Chalkumra | 2.92 | 0.11 | 1.78 |




**Bar graph -1** Constituents of the extract of the vegetables

## 2.2.6 Analysis of neutral LMW

**Determination of neutral sugars as trimethylsilyl derivatives.**

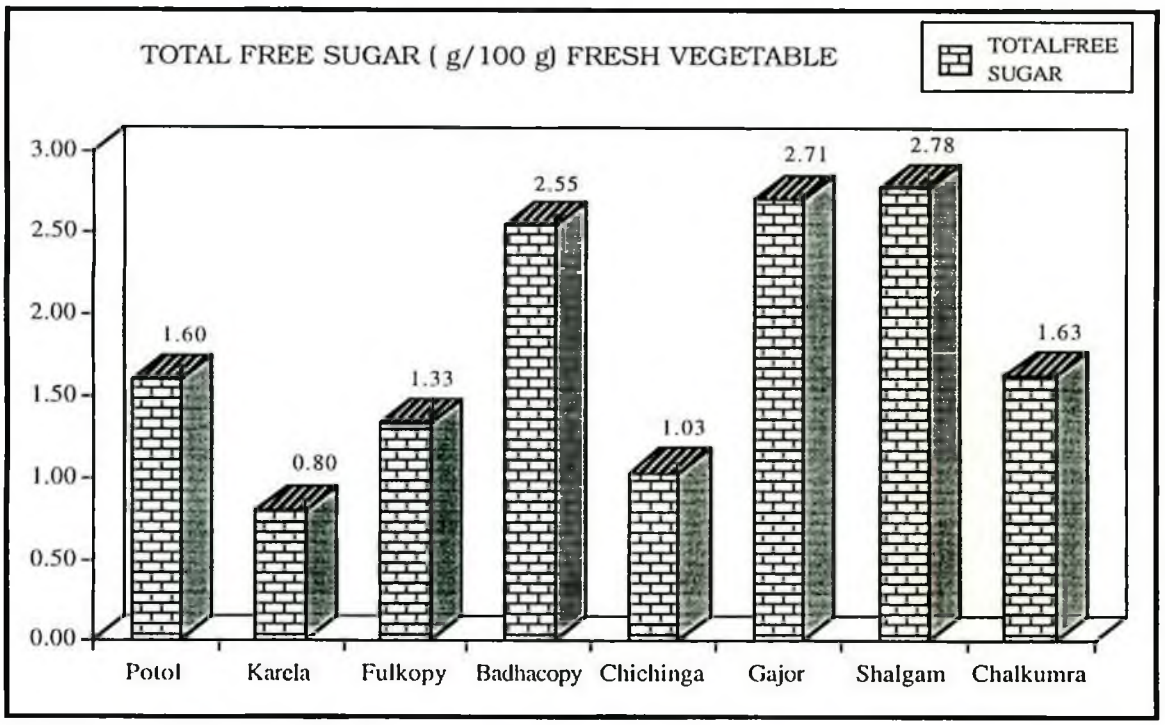
Neutral LMW fraction ( 5 mg) was dissolved in 10 ml water, containing 1 mg of ribose as internal standard. It was evaporated to dryness with added methanol. The dried mass was dissolved in pyridine (2 ml). The flask was flashed with nitrogen to remove air. In nitrogen atmosphere hexamethyl disilazane (HMDS; 1 ml) and trimethyl chlorosilane (TMCS; 0.5 ml) were added to it. The resulting mixture was shaken for 5 min in a ultrasonic bath. It was then heated at 60°C for 20 min in an oven. Then the mixture was again shaken in an ultrasonic bath for 2 min and then evaporated to dryness by adding toluene to remove pyridine completely. The TMS derivative was dissolved in *n*-hexane (1 ml) and transfered to a vial by passing through a cotton column. The sample was concentrated to a few drops by blowing air and analysed by gas liquid chromatography (GLC).

The same procedure was followed for all the eight vegetables . The results are presented in Table-4 and graph-2.

## Table-4

**Analysis of low-molecular weight components of vegetables as TMS derivative.**

| Name of the vegetable | Sugar component in g/100 g fresh vegetable | | | |
|---|---|---|---|---|
| | Glucose | Fructose | *myo*-Inositol | Total free sugar |
| Potol | 1.12 | 0.48 | - | 1.60 |
| Karela | 0.58 | 0.22 | - | 0.80 |
| Fulkopy | 1.06 | 0.27 | - | 1.33 |
| Badhakopy | 1.86 | 0.69 | - | 2.55 |
| Chichinga | 0.82 | 0.21 | - | 1.03 |
| Gajor | 1.91 | 0.80 | - | 2.71 |
| Shalgam | 2.00 | 0.78 | - | 2.78 |
| Chalkumra | 1.18 | 0.45 | 0.05 | 1.63 |




**Bar Graph-2:** A compratative graph of total free suger of eight vegetables

### 2.2.7 Determination of dietary fiber content of the vegetables (Seheme-2).

The extractive-free powder (2-3 g) [extracted with 80% aqueous ethanol and chloroform (Section 2.2.4)] was taken in a well stoppered conical flask and suspended in phosphate buffer (0.1M; pH 7.0). Protease (0.5 mg) was added to the suspension and it was agitated in a ultrasonic bath followed by shaking in an incubator at 60°C for 3 h. The suspension was then dialysed for 16 h. The dialysate was concentrated and freeze-dried.

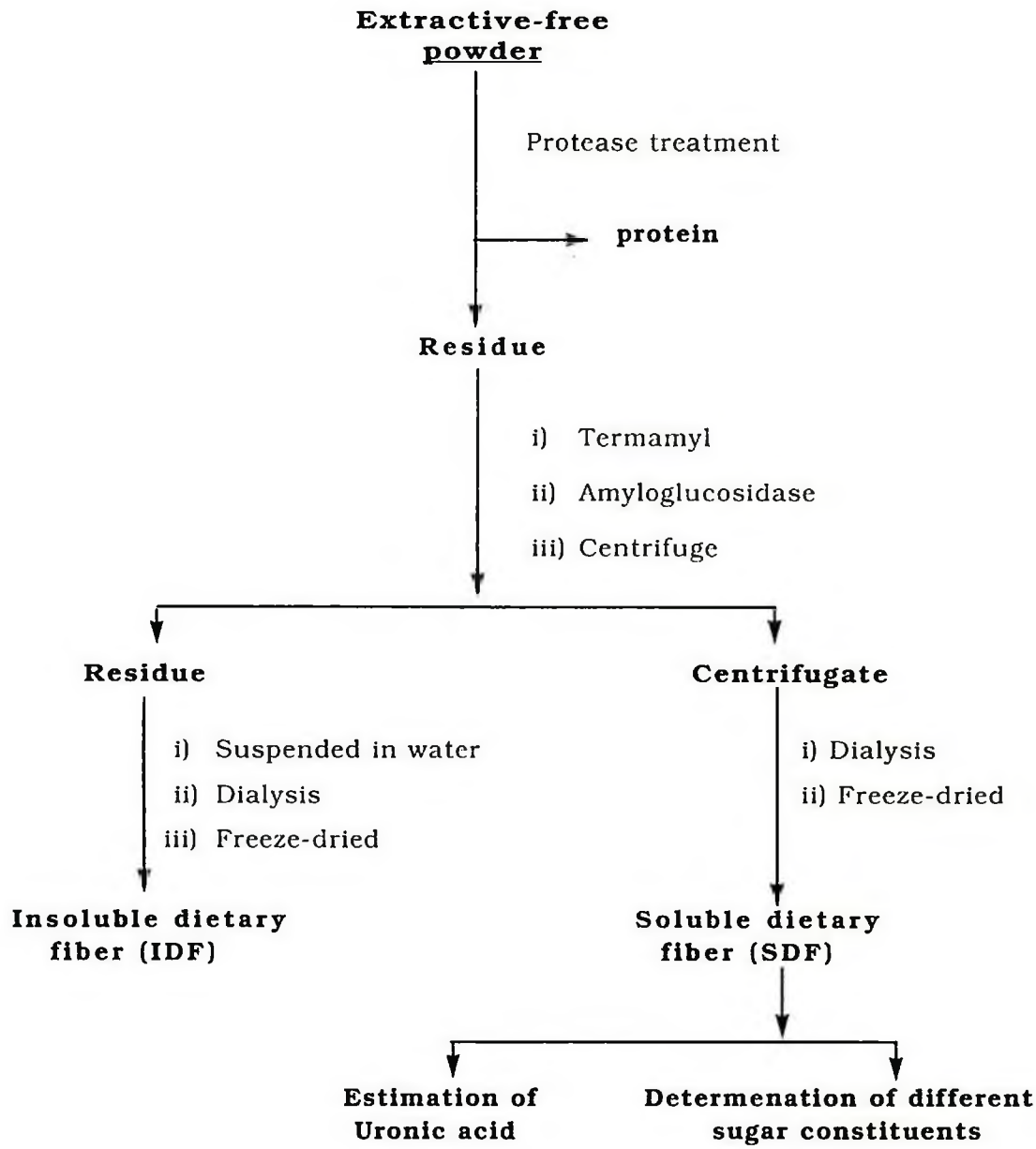
The freeze-dried sample was again suspended in acetate buffer (0.1M; pH 5.0). Termamyl (100 µl) was added to it and the enzyme-treated mixture was heated at 96°C for 1h. Amyloglucosidase (300 µl) was then added to it and the mixture was kept for 16 h in a constant temperature shaker (GFL England) at 60°C.

After cooling the enzyme-treated mixture was centrifuged at 2500 rpm for 15 min. After removing the water suspension part by decantation, water (50 ml) was added to the residue and the suspension was ultrasonicated for 5 min followed by centrifugation and the residue was washed with water once more. The water suspension from the original mixture and washings were combined and dialysed for 48 h in distilled water by changing the water several times. The dialysate was concentrated and freeze-dried. This freeze-dried material was termed as soluble dietary fiber (SDF).

The residue after water washings was further washed with ethanol (50 ml) followed by acetone (50 ml). The final residue was dried and this fraction was termed as insoluble dietary fiber ( IDF).

The same procedure was repeated for all the eight vegetables. The amount of SDF and IDF are presented in Table-5 and Bar graph-3.

**Scheme of dietary fiber isolation fromextractive free powder of vegetables,**




**Scheme-2**

**Table-5**

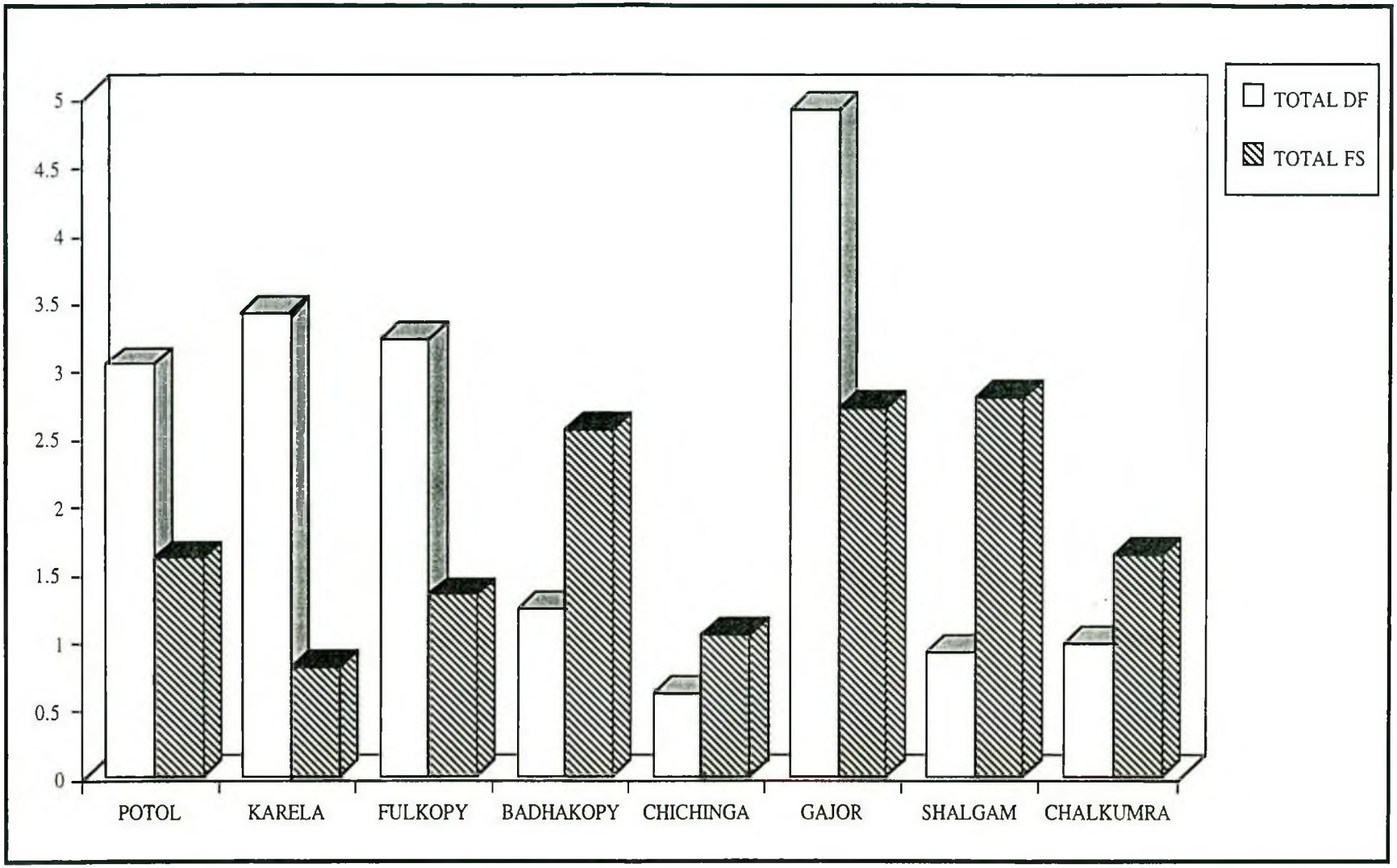
**Dietary fiber contents of the vegetables**

| Name of vegetables | Soluble dietary fibre (SDF) g/100 g fresh vegetable | Insoluble dietary fiber (IDF) g/ 100 g fresh vegetable | Total dietary fiber (DF) g/100 g fresh vegetable | % Total free suger |
|---|---|---|---|---|
| Potol | 0.11 | 2.92 | 3.03 | 1.60 |
| Karela | 0.33 | 3.13 | 3.46 | 0.80 |
| Fulkopy | 0.28 | 2.94 | 3.22 | 1.33 |
| Badhakopy | 0.18 | 1.05 | 1.23 | 2.55 |
| Chichinga | 0.06 | 0.55 | 0.61 | 1.03 |
| Gajor | 0.72 | 4.19 | 4.91 | 2.71 |
| Shalgam | 0.11 | 0.79 | 0.90 | 2.78 |
| ChalKumra | 0.15 | 0.83 | 0.98 | 1.63 |

### 2.2.8 Analysis of sugar components in soluble dietary fiber.

The soluble dietary fiber (SDF; 0.5 mg) was taken in a pear-shaped flask. It was dissolved in 2M TFA (2 ml) solution and hydrolysed in an oil bath at 120°C for 2 h.

The mixture was cooled and the TFA was removed by evaporation with added water. After complete removal of TFA, the hydrolysate was dissolved in water and treated with sodium borohydride (50 mg) for reduction. The solution was allowed to stand for 2 h with occassional shaking. Cation exchange resin Dowex ($H^+$) was added to acidify the solution. It was filtered and the filtrate was evaporated to dryness by adding methanol.




**Bar graph-3** Dietary fiber and total free sugar of eight vegetables.

The dried and reduced material as alditol was dissolved in pyridine (1 ml) and acetic anhydride (1 ml). The mixture was heated on a boiling water bath for half an hour. The mixture was then evaporated with added ethanol to remove the last trace of pyridine and acetic anhydride. The product was then dissolved in chloroform and it was filtered by a cotton column to a vial. The sample was then concentratied to a few drops by blowing air and analysed by GLC.

The same procedure was repeated for all the eight vegetables and the results as relative percentage are presented in Table-6.

**Table-6**

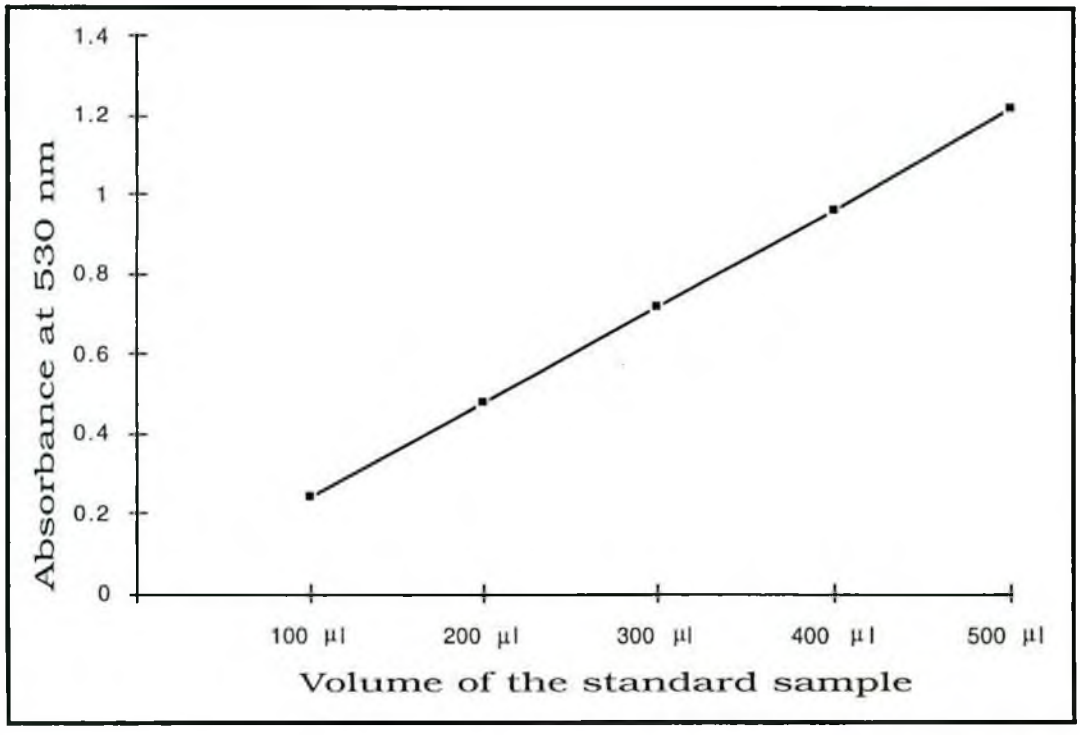
Sugar constituents in soluble dietary fiber presented as relative percentage (analysed by GLC as alditol acetate).

| Name of the vegetables | **Sugars:** relative percentage | | | | | |
|---|---|---|---|---|---|---|
| | **Rhamnose** | **Arabinose** | **Xylose** | **Galactose** | **Mannose** | **Glucose** |
| Potol | 2.77 | 34.08 | 4.75 | 38.41 | 10.89 | 9.10 |
| Karela | 3.15 | 18.82 | 2.97 | 54.34 | 9.86 | 10.86 |
| Fulkopy | 18.23 | 11.24 | 8.26 | 12.74 | 16.10 | 33.43 |
| Badhakopy | 3.13 | 56.14 | 1.28 | 24.77 | 10.86 | 3.82 |
| Chichinga | Trace | 16.64 | 6.68 | 71.16 | 5.52 | Trace |
| Gajor | 6.50 | 38.45 | 2.91 | 38.46 | 9.42 | 4.26 |
| Shalgam | 8.93 | 5.53 | 5.15 | 19.99 | 24.88 | 35.52 |
| Chalkumra | 3.77 | 14.72 | 3.42 | 63.52 | 8.39 | 6.18 |

### 2.2.9 Estimation of uronic acid in soluble dietary fiber.

**a) Preparation of standard curve of uronic acid.**

Glucuronic acid (10 mg) was dissolved in water (100 ml) in a volumetric flask. Measured amount of the glucuronic acid solution (500, 400, 300, 200, 100 µl) was taken in different test tubes and was diluted with water to make the volume 500 µl in each of the test tube. Water (500 µl) was taken into another test tube as a blank experiment. The solutions were seperately treated with sodium metaborate in sulphuric acid (3 ml) followed by carbazole (100 µl) as described in Section 2.1.12 . The absorbance of the resulting coloured solutions were measured at 530 nm and a standard curve (straight line) was prepared by plotting absorbance agianst the amount of glucuronic acid (Graph-4)




**Graph-4:** Standard carve of uronic acid determination in SDF of vegetables

### b) Uronic acid in soluble dietary fiber.

Soluble dietary fiber (10 mg) was dissolved in a volumetric flask (10 ml). The SDF solution (500 µl) was taken in three different test tubes. The solution (500 µl) was treated with sodium metaborate in sulphuric acid (3 ml) and carbazole solution (100 µl) as described earlier.

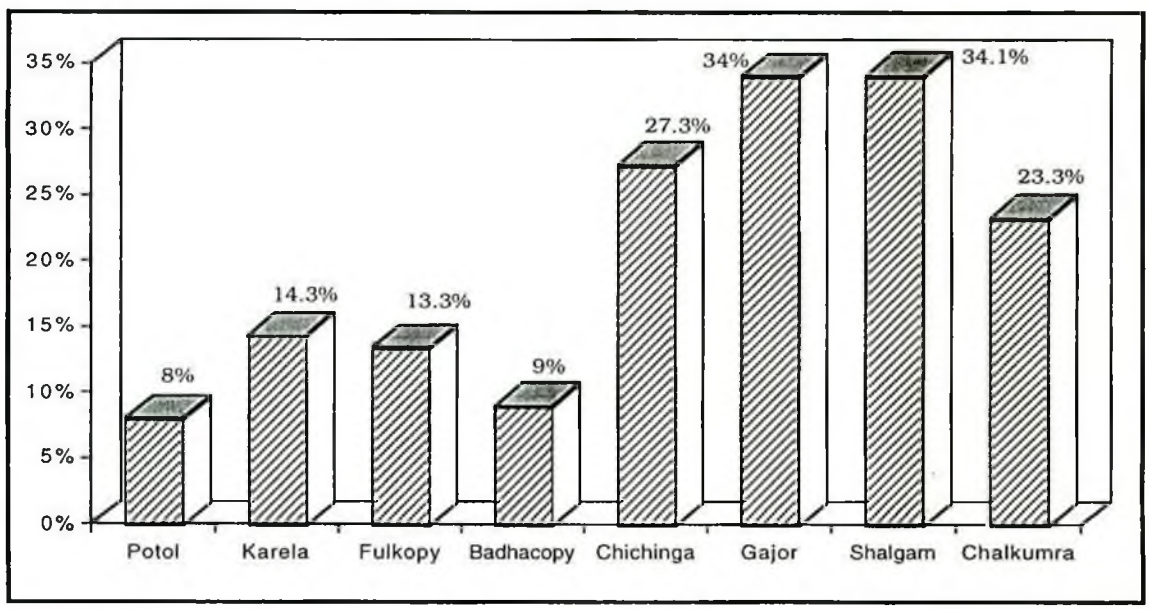
The absorbance of the coloured solutions were measured at 530 nm. The amount of uronic acid in the samples were estimated by comparison with the standard glucuronic acid curve.

The uronic acid of eight different vegetbles were measured according to the same procedure. The uronic acid content in different vegetabls are given in Table -7 and Bar graph-5.

**Table - 7**

Amount of uronic acids in SDF of different vegetables.

| Potol | Karela | Fulkopy | Badhakopy | Chichinga | Gajor | Shalgam | Chalkumra |
|---|---|---|---|---|---|---|---|
| 8% | 14.3% | 13.33% | 9% | 27.33% | 34% | 34.1% | 23.3% |




**Bar graph-5:** A comprtative graph of uronic acid in SDF of eight vegetables

### 2.2.10 Preparation of methyl esters of standard fatty acids with benzoic acid as internal standard.

Standard fatty acids (Caprylic, Capric, Lauric, Myristic, Palmitic, Stearic, Oleic, Arachidic and Behemic acids, 2 mg of each) were taken with benzoic acid (2 mg) as internal standard in a pear shaped flask.This mixture was dissolved in 0.5M methanolic sodium hydroxide solution and was then refluxed for 10 min. The mixture was evaporated and $BF_3$-$CH_3$.OH complex (5 ml) was added to it and the resulting mixture was heated on a boiling water bath for 2 min. The hot mixture was washed into a small separating funnel with petroleum ether (3 ml) and distilled water (2 ml) was added. The funnel was shaken vigorously and the layers were allowed to separate. The aqueous-methanol layer was drained off and discarded. The petroleum ether layer was dried by treating with anhydrous sodium sulphate, filtered into an another Quickfit flask, concentrated to a small volume by using vacuum evaporator and tramsfered to a vial. Finally the petroleum ether containing mixture of methyl ester of fatty acids was concentrated to a few drops by blowing nitrogen gas and stored in a refrigerator before analysis by GLC.

### 2.2.11 Gas chromatographic analysis of prepared standard sample.

The prepared methyl esters of standard samples of fatty acids with benzoic acid as internal standard (Section 2.2.10) were analysed by GLC using a column packed with Silar 10C (Section 2.1.10). The peak areas were calculated by multiplying the height of the peak with the width at the half height of the peak ( peak area = peak height × width at half-height of peak). Area response factores of the given acids were determined by dividing their peak area by that of benzoic acid which was taken as unit for convenience. The retention times and response factors of the analyzed samples are presented in Table-8.

**Table-8**

Gas chromatographic analysis of standard fatty acids with benzoic acids as internal standard.

Determination of response factor of the standard fatty acids by GLC.

| Name of acids | Retention time | Peak area ($mm^2$) | Wt. of acids (mg) | Peak area per mg | Response factor |
|---|---|---|---|---|---|
| Caprylic | 3.7 | 28.00 | 2.0 | 14 | 4.66 |
| Capric | 5.4 | 128.75 | 2.0 | 64.37 | 21.45 |
| Lauric | 7.6 | 207.00 | 2.0 | 103.50 | 34.5 |
| Myristic | 12.6 | 551.25 | 2.0 | 275.62 | 91.87 |
| Benzoic (I S) | 15.6 | 6.0 | 2.0 | 3.0 | 1.0 |
| Palmitic | 17.8 | 678.00 | 2.0 | 339.00 | 113.0 |
| Stearic | 21.7 | 231.00 | 2.0 | 115.50 | 38.5 |
| Oleic | 22.5 | 369.6 | 2.0 | 184.80 | 61.33 |
| Arachidic | 25.9 | 167.5 | 2.0 | 83.75 | 27.9 |
| Behemic | 30.5 | 129.5 | 2.0 | 64.75 | 21.58 |

### 2.2.12. Preparation of methyl ester of fatty acid in chloroform extract (lipid) and their analysis by GLC.

Fatty acids containing chloroform extractive part (50 mg) of vegetables (Table-2) and benzoic acid (5 mg) as internal standard were taken in pear-shaped flask (25 ml). Then the mixture was dissolved in 0.5M methanolic sodium hydroxide solution and refluxed it for 10 min. Methanol was evaporated and $BF_3$-$CH_3OH$ complex ( 5 ml) was added to it. The resulting mixture was heated on a boiling water bath for 2 min. The hot mixture was washed into a small separatory funnel with petroleum ether (3 ml) and distilled water (2 ml) was added. The funnel was shaken vigorously and the layers were allowed to separate. The petroleum ether layer was separated from aqueous- methanol layer and was dried by treating with anhydrous sodium sulphate, filtered into an another Quickfit flask, concentrated to a small volume by using vacuum evaporator and transfered to a vial. Finally the petroleum ether containing mixture of methyl ester of acids were concentrated to a few drops by blowing nitrogen gas and analysed by GLC.

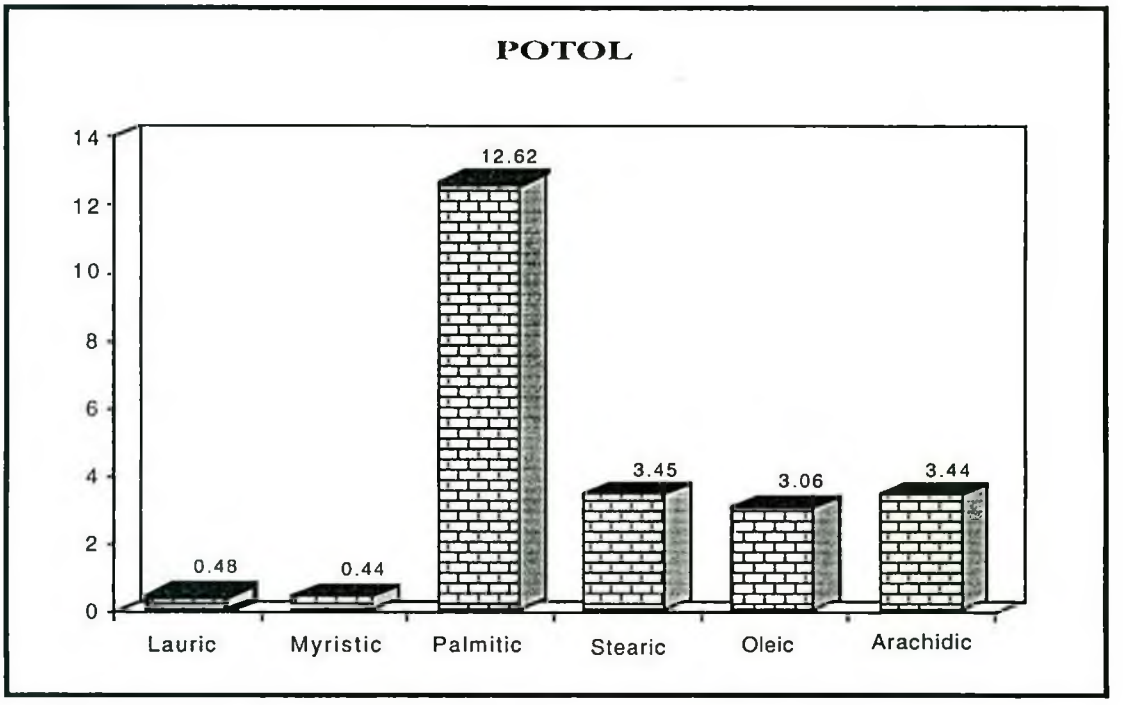
The same procedure was used for all the five vegetables. Results are presented in Tables- 9 to 13 and Bar graph-6 to10.

## Table-9

**Fatty acid analysis of chloroform extractive part of potol.**

* In the folowing Tables I.S. indicate internal standard

| Name of acids | Retention time | Peak area $mm^2$ | Response factor | Fatty acid content mg/ 100 g fresh vegetables |
|---|---|---|---|---|
| Lauric | 7.7 | 8.0 | 34.5 | 0.48 |
| Myristic | 12.6 | 19.6 | 91.87 | 0.44 |
| Benzoic | 15.1 | 10.0 | 1.00 | I. S. |
| Palmitic | 17.6 | 687.0 | 113.00 | 12.62 |
| Stearic | 21.4 | 41.0 | 38.5 | 3.45 |
| Oleic | 22.3 | 90.6 | 61.33 | 3.06 |
| Arachidic | 26.9 | 46.25 | 27.9 | 3.44 |

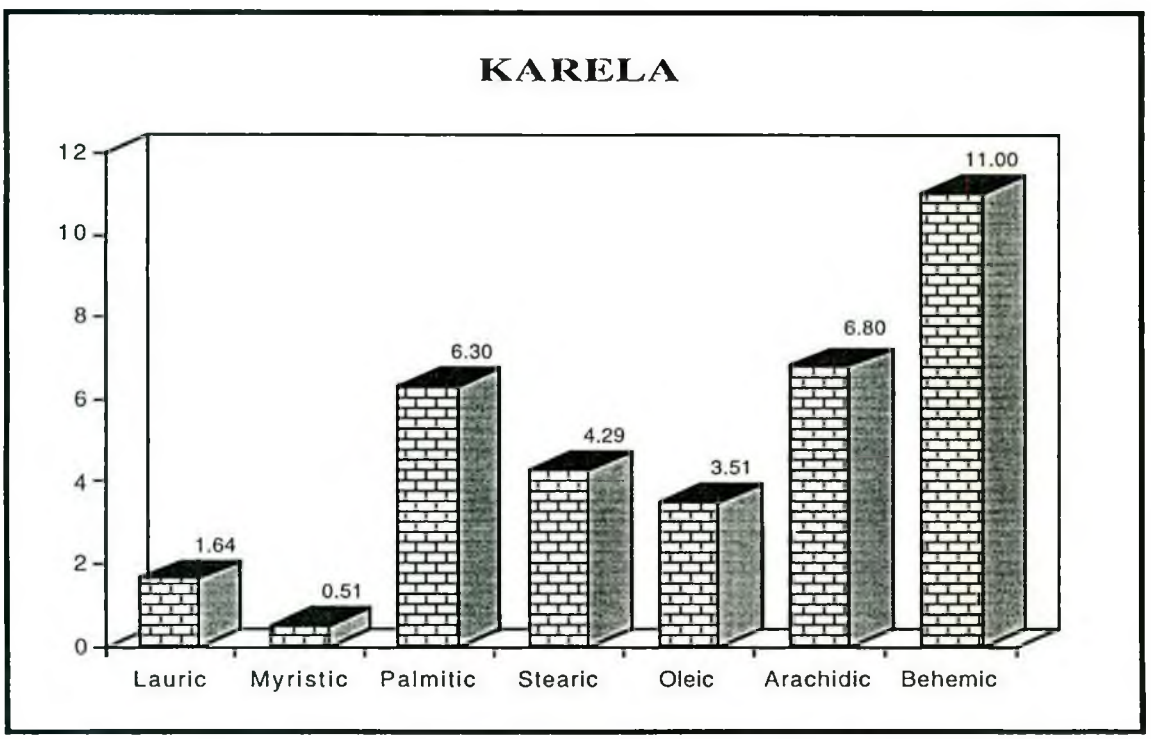



**Bar graph- 6:** A compratative graph of different fatty acids of Potol.

## Table-10

**Fatty acid analysis of chloroform extractive part of Karela.**

| Name of acids | Retention time | Peak area $mm^2$ | Response factor | Fatty acid content mg/ 100 g fresh vegetables |
|---|---|---|---|---|
| Lauric | 8.4 | 18.00 | 34.50 | 1.64 |
| Myristic | 12.8 | 15.00 | 91.87 | 0.51 |
| Benzoic | 15.3 | 6.00 | 1.00 | I. S. |
| Palmitic | 17.7 | 225.00 | 113.00 | 6.30 |
| Stearic | 21.6 | 52.25 | 38.50 | 4.29 |
| Oleic | 22.5 | 68.25 | 61.33 | 3.51 |
| Arachidic | 26.9 | 60.00 | 27.90 | 6.80 |
| Behemic | 31.4 | 75.00 | 21.58 | 11.00 |

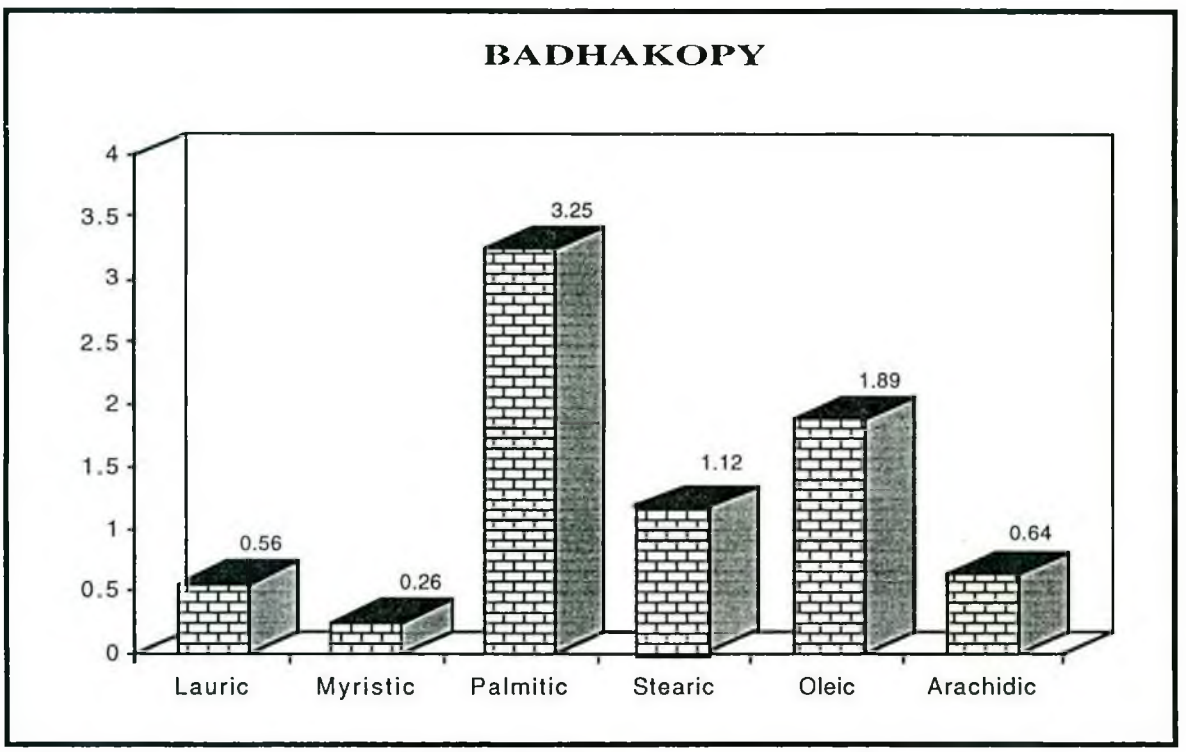



**Bar graph-7:** comparative graph of different fatty acids of karela.

## Table-11

**Fatty acid analysis of chloroform extractive part of Badhakopy**

| Name of acids | Retention time | Peak area $mm^2$ | Response factor | Fatty acid content mg/ 100 g fresh vegetables |
|---|---|---|---|---|
| Lauric | 6.8 | 62.5 | 34.50 | 0.56 |
| Myristic | 11.8 | 80.0 | 91.87 | 0.26 |
| Benzoic | 14.1 | 45.0 | 1.00 | I. S. |
| Palmitic | 16.7 | 1182.0 | 113.0 | 3.25 |
| Stearic | 20.8 | 476.0 | 38.50 | 3.84 |
| Oleic | 21.5 | 373.0 | 61.33 | 1.89 |
| Arachidic | 25.9 | 58.0 | 27.9 | 0.64 |

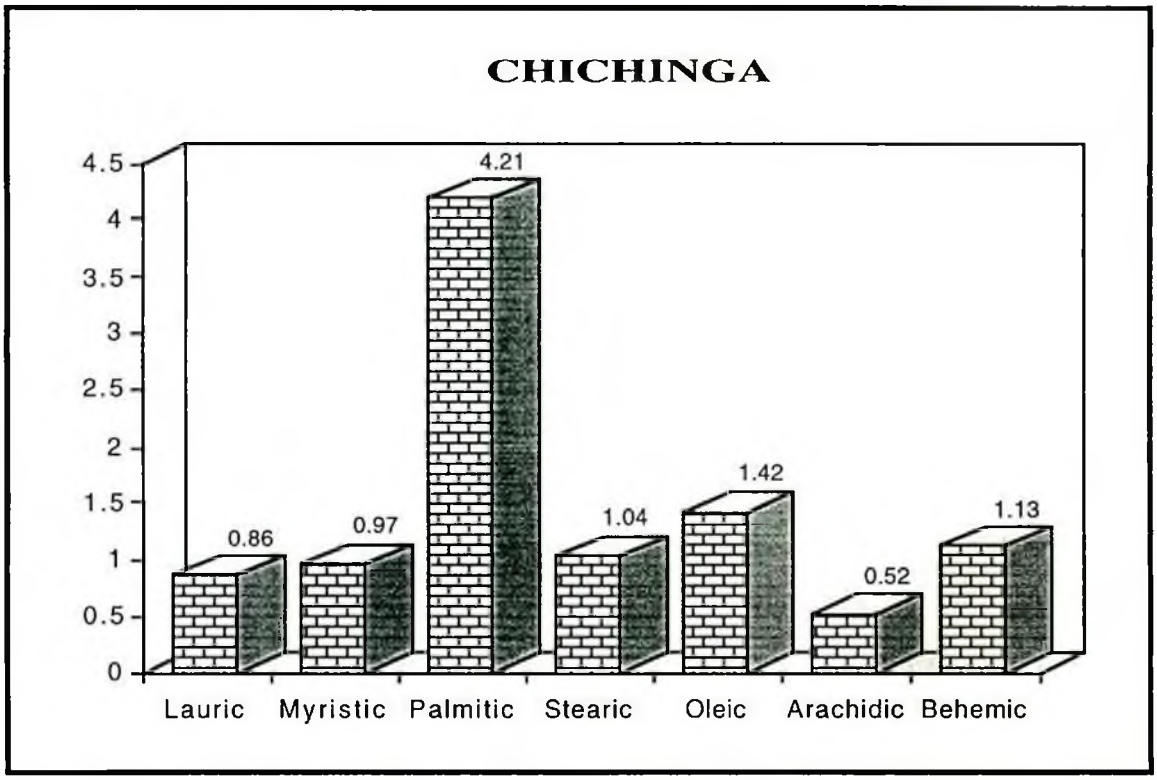



**Bar graph-8:** comparative graph of different fatty acids of badhakopy

## Table-12

**Fatty acid analysis of chloroform extractive part of chichinga.**

| Name of acids | Retention time | Peak area $mm^2$ | Response factor | Fatty acid content mg/ 100 g fresh vegetables |
|---|---|---|---|---|
| Lauric | 9.1 | 22.40 | 9.82 | 0.86 |
| Myristic | 15.5 | 31.50 | 12.28 | 0.97 |
| Benzoic | 19.5 | 31.50 | 1.00 | I.S. |
| Palmitic | 21.8 | 130.95 | 11.83 | 4.21 |
| Stearic | 26.7 | 30.00 | 10.89 | 1.04 |
| Oleic | 27.6 | 26.60 | 7.14 | 1.42 |
| Arachidic | 32.7 | 21.00 | 15.25 | 0.52 |
| Behemic | 38.0 | 31.50 | 10.53 | 1.13 |

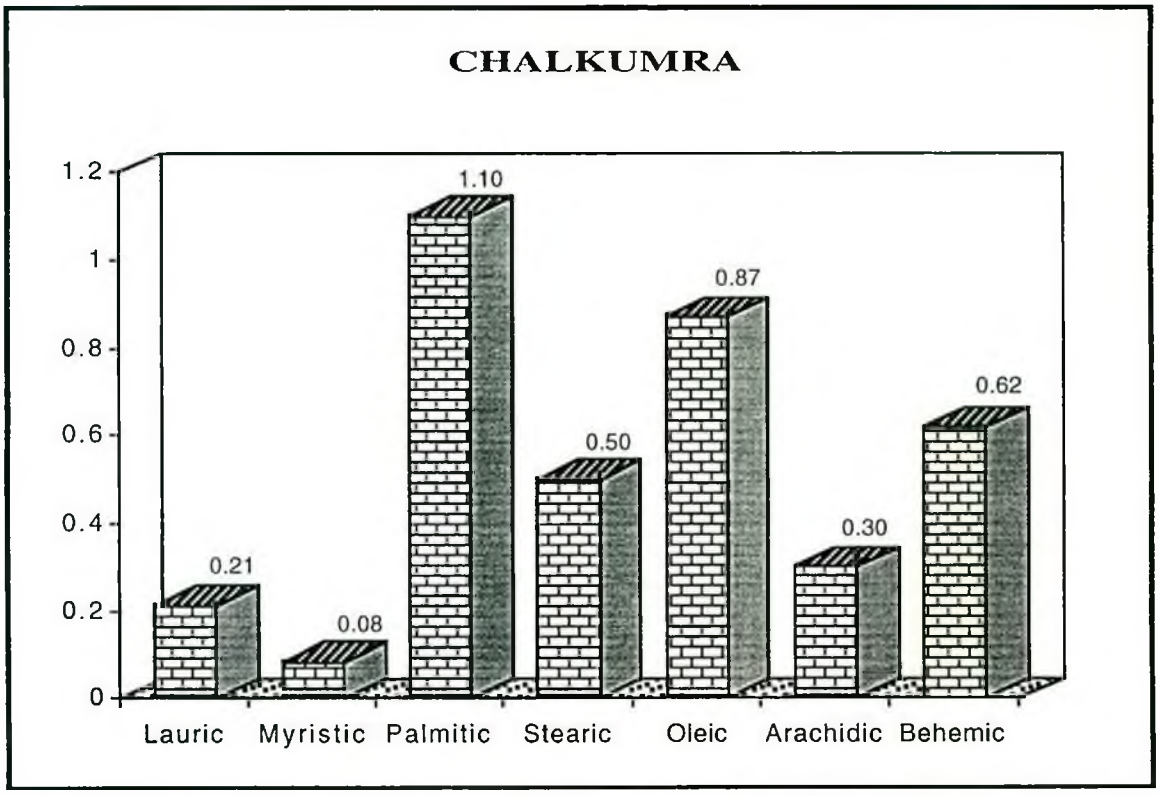



**Bar graph- 9:** comparative graph of different fatty acids of chichinga

## Table-13

**fatty acid analysis of chloroform extractive part of Chalkumra.**

| Name of acids | Retention time | peak area $mm^2$ | Response factor | Fatty acid content mg/ 100 g fresh vegetables |
|---|---|---|---|---|
| Lauric | 7.9 | 42.60 | 34.50 | 0.21 |
| Myristic | 12.7 | 46.20 | 91.87 | 0.08 |
| Benzoic | 15.6 | 63.75 | 1.00 | I. S. |
| Palmitic | 17.7 | 724.75 | 113.0 | 1.10 |
| Stearic | 21.5 | 113.47 | 38.50 | 0.50 |
| Oleic | 22.5 | 310.50 | 61.33 | 0.87 |
| Arachidic | 26.6 | 49.30 | 27.90 | 0.30 |
| Behemic | 31.7 | 78.60 | 21.58 | 0.62 |

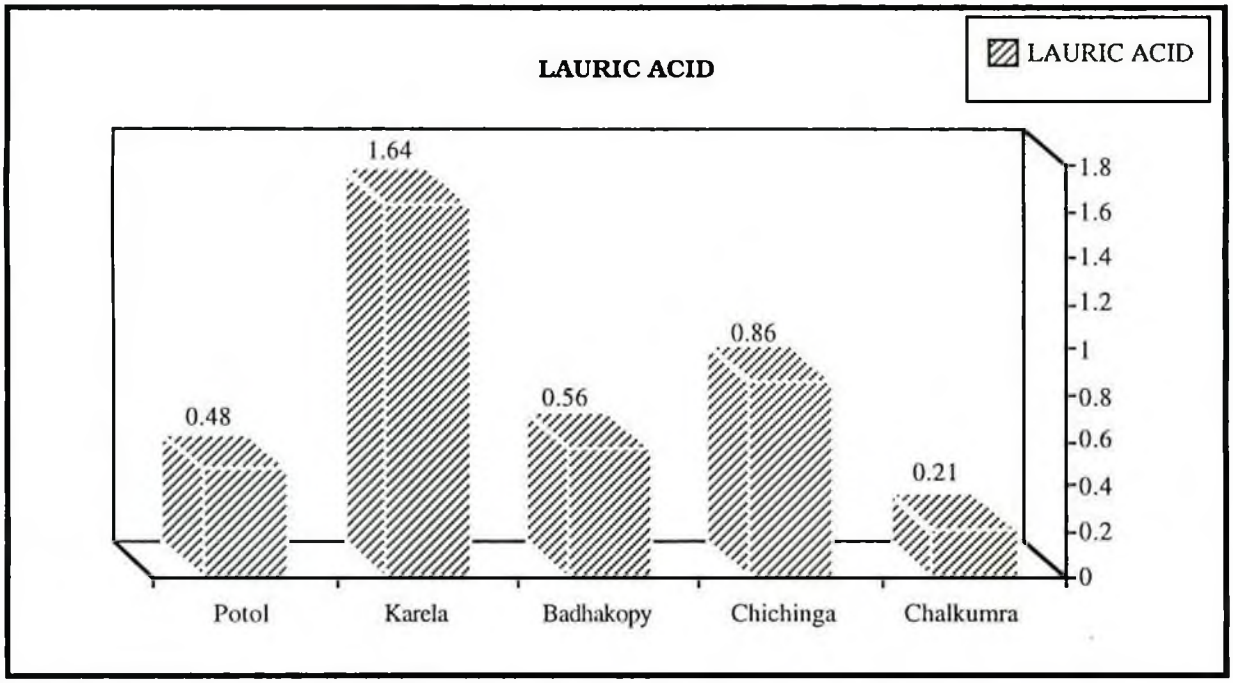



**Bar graph-10:** A compratative graph of different fatty acids of chalkumra.

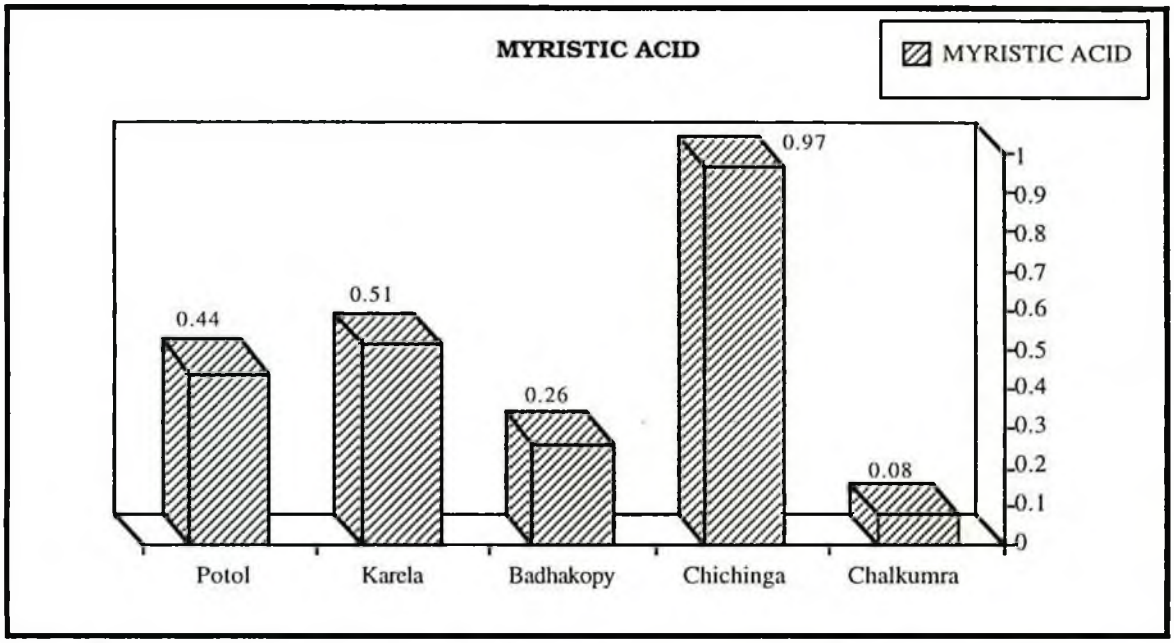
## Table-14

**A comparative table of different fatty acids of above five vegetables in mg per 100 g fresh vegetable.**

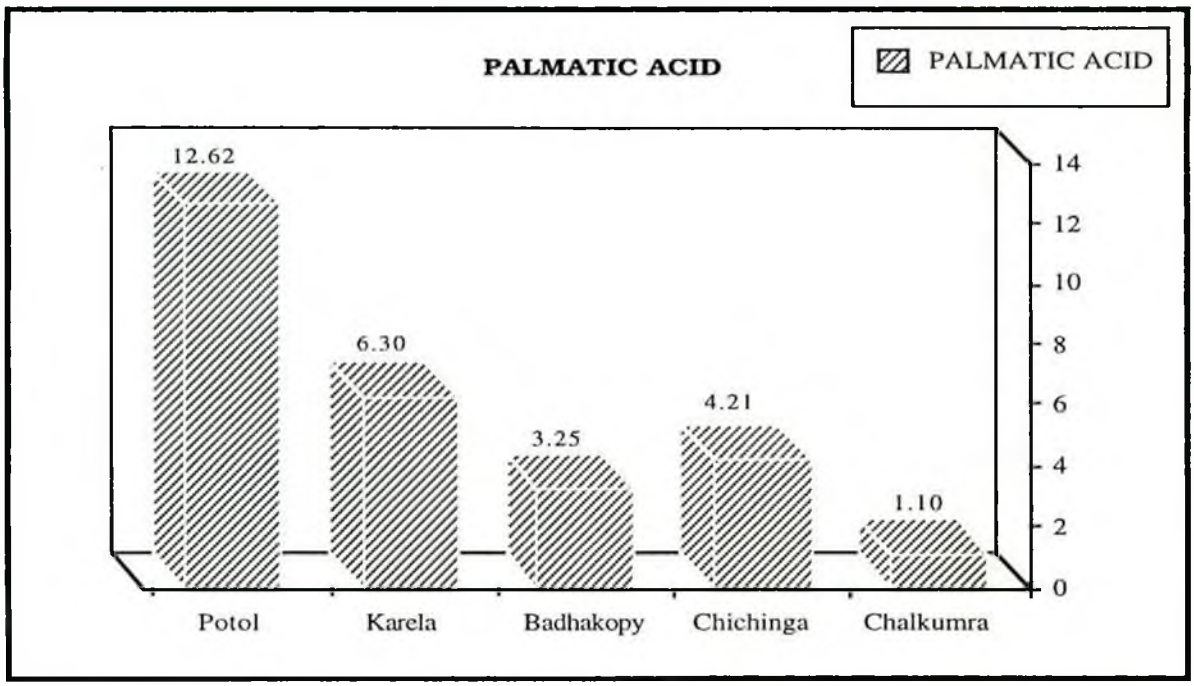
| Name of the vegetables | Laurie acid | Myristic acid | Palmatic acid | Stearic acid | Oleic acid | Arachidic acid | Behemic acid |
|---|---|---|---|---|---|---|---|
| Potol | 0.48 | 0.44 | 12.62 | 3.45 | 3.06 | 3.44 | - |
| Karela | 1.64 | 0.51 | 6.30 | 4.29 | 3.51 | 6.80 | 11.00 |
| Badhakopy | 0.56 | 0.26 | 3.25 | 3.84 | 1.89 | 0.64 | - |
| Chichinga | 0.86 | 0.97 | 4.21 | 1.04 | 1.42 | 0.52 | 1.13 |
| Chalkumra | 0.21 | 0.08 | 1.10 | 0.50 | 0.87 | 0.30 | 0.62 |



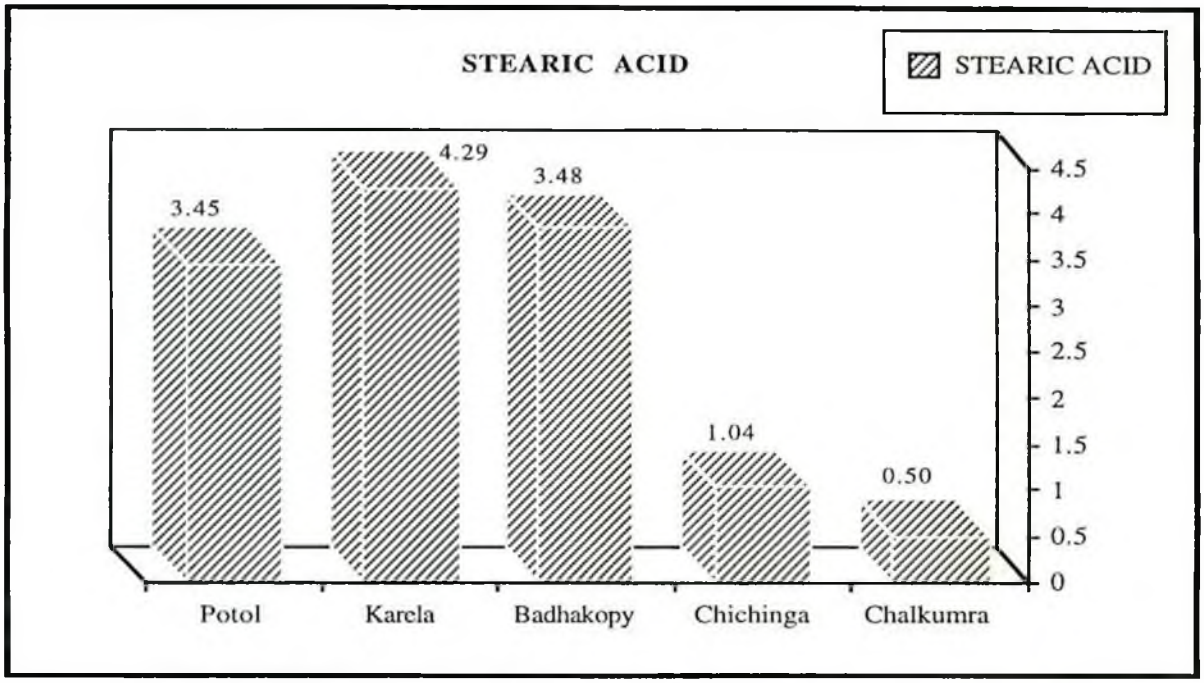

**Graph- 11:** A comp arative graph of Lauric acid in five vegetables



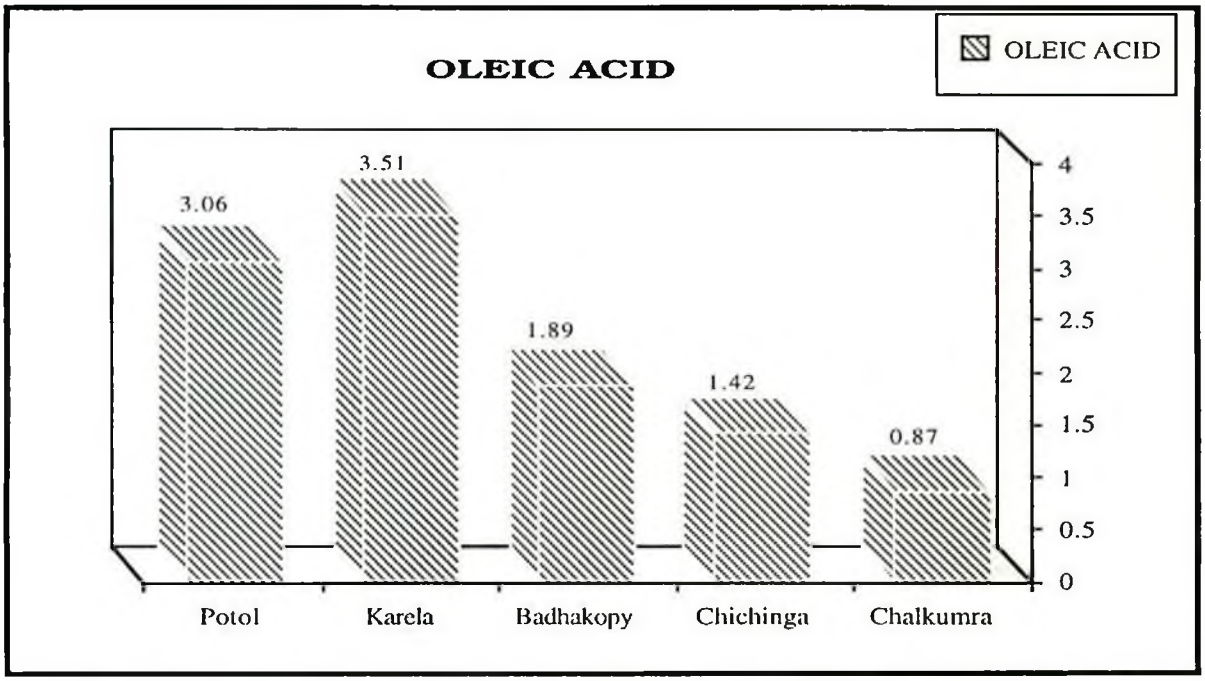

**Graph- 12:** A comparative graph of Myristic acid in five vegetables



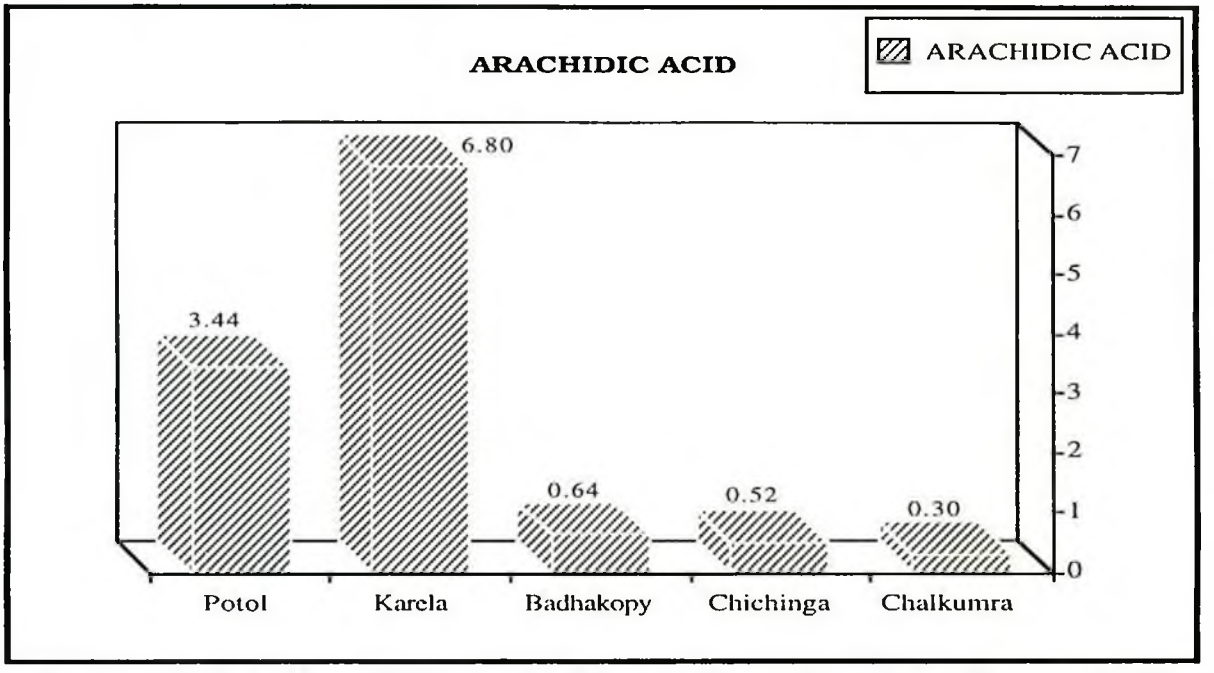

**Graph- 13:** A comparative graph of Palmatic acid in five vegetables



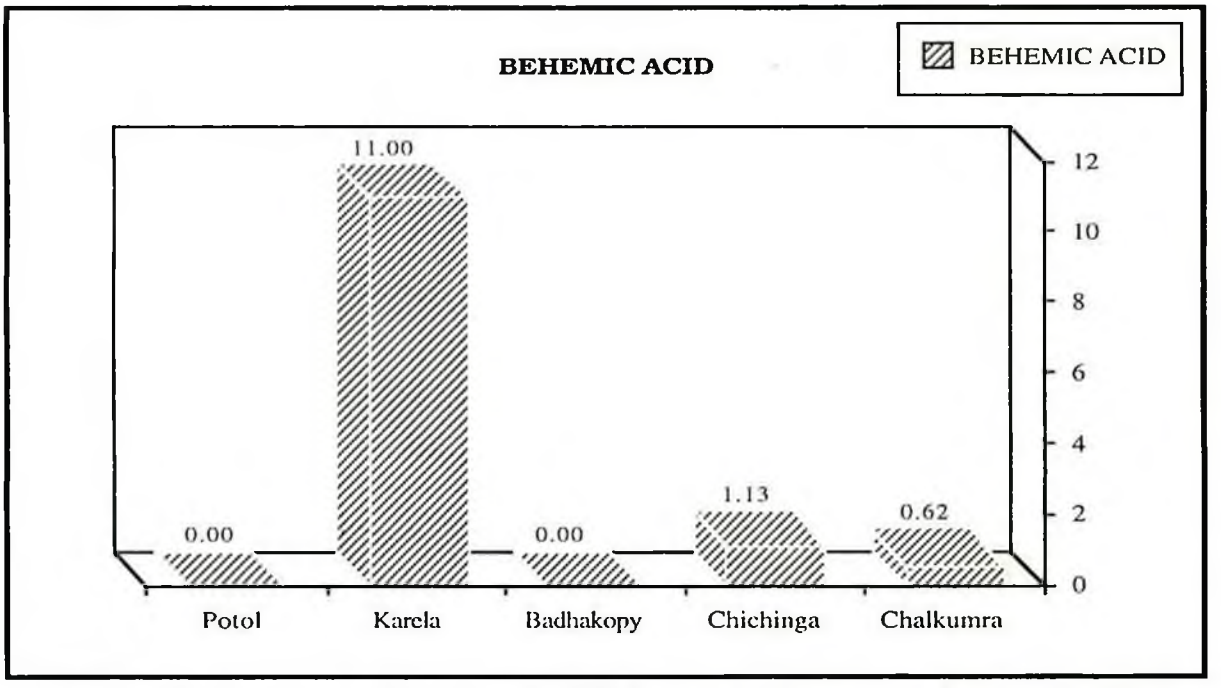

**Graph- 14:** A comparative graph of Stearic acid in five vegetables




**Graph- 15:** Comparative graph of Oleic acid in five vegetables




**Graph- 16:** A comparative graph of Arachidic acid in above five vegetables




**Graph- 17:** A comparative graph of Behmic acid in above five vegetables

# RESULTS AND DISCUSSIONS

# 3. RESULTS AND DISCUSSIONS

Bangladesh being an agricultural country and having very fertile flat land, produces a lot of different kinds of vegetables round the year in its every corner. Vegetables are inseparable item in our daily food. Vegetables are considered to be the source of vitamins, minerals, dietary fibers and other unknown constituents which are essential for every human being. These are even more essential for the patients who are suffering from diabetes, ischimic heart disease and high blood pressure. Although, vegetables are so common and essential item of our food but very little information about our locally grown vegetables are available except a few constituents reported by Ahmed *et. al.* [1]. Very recently, free sugars and dietary fiber content and composition of seven local vegetables were reported by Nahar. *et. al*[7]. In the present work similar studies were made on eight other common vegetables. Summer and winter vegetables parbal, (potol), pumkin (Chalkumra), cabbage, cauliflower, carrot, snakegourd and turnip (Shalgam) were taken for the analyses. Three cultiver varieties of bitter gourd are grown in Bangladesh. One type is elongated shape (like small size of chichinga), the other one is medium sized. The smallest sized is called uchche. Uchche was studied earlier. To compare the presence of constituent in the two cultiver varieties, the medium size of karela was included in the present work. Photographs of all the vegetables are given in the photographs 1-8.

Good quality and sufficient amount of all the vegetables were purchased from the local market of Dhaka city. Only edible parts of the vegetables were taken for all the purposes.

## 3.1 Dry matter content of the vegetables:

Fresh vegetables naturally contained large amount of water. Water content of each of the vegetable was determined after making edible part of each of the vegetable into small pieces and drying the pieces in an oven at 105°C until a constant weight was obtained. Water content of the eight vegetables varied from 89-97% (Table-2 and graphic 1-8 ). Snake gourd (chichinga) contained the highest amount of water (97%) followed by pumpkin (chalkumra, 95.5%). Carrot contained the lowest amount (89%) of water among the eight vegetables. Water content of chalkumra and chichinga were very close to the water content of lao[7]. Large amount of water content of the vegetables indicated that even after processing for food substantial amount of water is coming to the body fluid from the vegetables.

## 3.2 Extraction of the vegetables :

For determination free sugar and dietary fiber content and composition of the vegetables the Theander and Westerlund[54] method was followed. In this procedure, edible part of the fresh vegetables were directly made into very small pieces and were extracted with aqueous ethanol. Amount of ethanol was added to the vegetable materials after considering the water content of each of the vegetable. Extraction was

carried on the fresh materials because more free sugar may be released from the polysaccharides part present in the vegetables during drying either by enzymatic degradation or by microbial attack.

The residue after aqueous ethanol extraction was further extracted with chloroform to isolate fatty materials completely. Ethanol was removed from the aqueous ethanolic extract by evaporation with added water and chloroform soluble part from the aqueous extract was isolated by partition with chloroform. The combined chloroform extract was evaporated to dryness followed by drying in high vacuum using freeze-dryer. The dried chloroform part was considered as total lipid content of the vegetables and was taken for further studies.

Ethanol soluble materials of the vegetables varied from 2-5% whereas chloroform soluble part (lipid) varied from 0.1-0.3% of the fresh vegetables. The result indicated that vegetables did not contain much of oily materials.

### 3.3 Fractionation of the aqueous ethanol soluble part:

Vegetables contained vitamins and minerals[1]. Like other plant materials it may also contained phenolc acids[10, 55], polyphenols and other allied components. To obtain better result these interfering materials were removed by passing the low molecular weight ethanol soluble materials through the ion exchange columns, cation exchanger followed by anion exchanger. Acidic, basic and neutral parts were collected but only neutral material was considered for free sugar

determination. From Table-3 it is clear that aqueous ethanol soluble material LMW did not contain much of acidic and basic components in comparison with neutral materials. In other words most of the LMW contained nutral matrials.

### 3.4 Analysis of neutral LMW:

Neutral low molecular weight (NLMW) component was analysed for free sugar content. Free sugars were quantified by converting the known amount of NMLW into its trimethylsilyl (TMS) derivatives by adding known amount of internal standard, ribose. The TMS ethers were analysed immediately by GLC using CP-Sil 5 column. For each sample replicate analyses were carried out (Table-4 and Bar graph-2 ).

From the previous work of the group[5-11] it was revealed that acetates and TMS ethers give similar results. Di- and trisaccharides are not possible to detect by GLC as acetate derivatives using standard column (due to less volatility). Therefore, only TMS ethers were made for quantification of the free sugars including di- or oligosaccharides ( if any) present in the vegetables.

### 3.5 Free sugars and vegetables.

Total free sugar content of the eight vegetables which were analysed in the present study was much more (0.8-2.8%) than the free sugar content (0.1-1.8%) of the seven vegetables[7] reported earlier. Glucose and fructose were only two sugars identified and quantified in all the

vegetables. Like coconut water[8] but unlike fruits[4-7] no sucrose was found in any of the vegetable. *Myo*-inositol was found only in chalkumra. Total free sugar contents (Table-4, Bar graph-2) of the vegetables (1-3%) were much lower than the fruits [5-6] but higher than the other plant materials[9-12]. Among the eight vegetables shalgam, gajor and badhakopy contained more free sugars (2.78, 2.71 and 2.55%, respectively) than the other five vegetables. Karela contained lowest amount of free sugar (0.8%). It was also evedent that two cultiver varieties of karela contained more or less the same amount of free sugars.

### 3.6 Isolation of dietary fiber (Scheme 2):

For determination of dietary fiber (DF) aqueous ethanol and chloroform extractive free residue of the vegetable was made into powder. Protein was removed from the vegetable powder by the treatment with protease[50]. In this procedure vegetable material was suspended in phosphate buffer and incubated in an thermostatic shaker after adding small amount of protease. All the protein present in the vegetables is supposed to be brokendown into corresponding amino acids. The protein free material was collected by dialysis and freeze-drying. The protein free material was further treated with Termamyl (α-amylase) followed by amyloglucosidase to remove starch[63] present (if any) in the sample. The sample was suspended in acetate buffer and treated with the enzyms. As we know α-amylase hydrolyses only linear α-1,4-linked glucan amylose, therefore, it is necessary to use amyloglucosidase

to hydrolyse α-1,4-linked glucan having branching at the 6-position (amylopectin). In this treatment all the starch present in the vegetable sample was completely broken down into glucose molecules. The soluble dietary fiber (SDF) was collected by centrifugation of the material and dialysis and freeze-drying of the supernatent. The insoluble dietary fiber (IDF) was collected by suspension of the centrifugate in water, dialysis and freeze-drying (Scheme-2; Table-5 ).

Dietary fiber content of the vegetables varying between 0.6-4.9% (fresh weight basis) indicated high percentage of dietary fiber present in the vegetables. The solid material content of the vegetables were not very high due to large amount of water present in the vegetables. Among the eight vegetables gajor contained the highest amount of total DF (4.9%). Substantial amount of DF were also found in fulkopy and potol (3.25 and 3.03%). Shalgam and chalkumra contained nearly the same amount of DF (0.9%) (Table-5).

Soluble DF content of all the vegetables were lower than the insoluble DF. But badhacopoy contained little higher amount of SDF (1.80%) than the IDF (1.05%).

### 3.7 Free sugars vs dietary fiber:

Total dietary fiber and total free sugar contents of the eight vegetables are presented in the Bar graph-3. One of the main objective of the present work was to compare the free sugars and dietary fiber content of the vegetables which will help the diabetic

or other patients as well as healthy people to choose vegetables in thier food list. From the graph it is clear that karela had more positive value followed by gajor, fulkopy and potol in consideration of fiber content. Shalgom and badhakoy had on the other hand large negative value which means that they contained more free sugars than the dietary fiber indicated that these two vegetables are not at all good for the diabetic patients. Chichinga and chalkumra had also negative fiber value i.e. these vegetables are not also good for the patients. From earlier studies[7] on the vegetables, elongated beans, ladies finger, green papaya, karela, brinjal, lao and green banana it was found that all of the vegetables had positive fiber value which means that they contained more dietary fiber than the free sugars. Among them elongated bean (barbati) was the best.

### 3.8 Analysis of soluble DF:

As the insoluble DF was composed of mainly some water-insoluble hemisellulose, cellulose and lignin, these were not further analysed. Although the role of IDF and SDF may not be distinguished, ploysaccharides in SDF may be more likely to take part in different biological and metabolic activities. Therefore, small amount SDF obtained from the vegetables were further studied for its polymeric carbohydrate constituents.

Uronic acid content of the soluble dietary fiber (SDF) of each of the vegetable was determined by Carbazole method[56]. In this method

a standard curve of galacturonic acid was made. Estimation of uronic acid of each of the sample was done with referene to the standard curve (Graph-4.) of galacturonic acid. From the Table-7 and Bar graph-5 it is evident that gajor and shalgom contained the highest amount of uronic acid (35%) followed by chalkumra (23.3%). Nearly the same amount of uronic acid was present in SDF sample of karela and fulcopy (13.14%). Badhacopy and potol contained the lowest amount uronic acid (8-9%).

Neutral sugar constituents of the SDF samples of the eight vegetables were determined by acid hydrolysis followed by conversion of the hydrolysate into their alditol acetates. The acetates were analysed by GLC using more polar Cp-Sil 88 column. Rhamnose, arabinose, xylose, mannose, galactose and glucose were the different sugars present in varying proportions in all the SDF samples of the vegetables. Uronic acid and neutral sugars were determined by two different methods. For that reason among the relative proportions of neutral sugars uronic acid was not included and expressed separately.

Gajor contained the highest amount of galactose (39%), followed by arabinose (34%). Other minor sugars present in the SDF samples were mannose, rhamnose, glucose and xylose. From the composition of the neutral sugars and uronic acid content it was revealed that arabinose, galactose and uronic acid were the major sugar residues of SDF of gajor. The result also indicated that pectic

substances i.e. uronans and arabinogalactans were the major polysaccharides of the SDF which were present in the parenchymatous tissue[60] of gajor. Presence of small proportions of rhamnose, xylose, glucose and mannose indicated that small amount of rhamnoglacturonans and xyloglucan were also present in the parenchymatous tissues of gajor. Similar composition of SDF of pea[61] was reported by Brillouet and Carre in 1983.

Uronic acid and neutral sugar constituents of SDF of badhacopy was very similar to potol and karela. In these samples more of neutral sugar residues (arabinose and galactose) were present than the uronic acid in the SDF samples. Therefore, more of neutral pectic polysaccharides were present than the uronans in the SDF of the three vegetables. Sugar constituents of immatured badhacopy[62] was analysed by Stevens ans Selvendran in 1948. They reported that uronic acid (27.8%), glucose (22.7%) and arabinose (12.3%) were the major sugar constituents present in the SDF of cabbage. Such variation may occur due to the change of condition of cultivation, harvesting time, weather and soil condition and the country of origin. Sugar composition of potol and cabbage were different from the other six vegetables and also different from the other seven vegetables reported earlier[7].

SDF of chichinga contained the highest amount of galactose (71.16%) among the eight vegetables. Arabinose and uronic acids were the other two constituent sugar residues of SDF of chichinga.

The result indicated that pectic arabinogalactan and uronans were the major polysaccharides present in the sample. Glucose and rhamnose, the other two minor sugar residues present in the SDF of chichinga indicated that minor amount of rhamnogalauronan was also present in the parenchymatous tissue of chichinga.

Other than uronic acid, glucose, mannose and galactose were the three major neutral sugar residues present in the SDF sample of shalgom indicating the presence of more non-strach glucan (xyloglucan), galactans and uronan present in shalgom. The presence of small amount a galactomannan is also possible.

## 3.9 Fatty acid composition of the vegetables.

Lipid part of the five vegetables were analysed for fatty acid content and composition. It is known[52] that in plant materials only a small percent of fatty acids exist in free state in comparison to esterified fatty acids. Therefere, analysis were carried out to determine only esterified fatty acids of the vegetables.

For isolating the esterified fatty acids, the chloroform extract was saponified with methanolic sodium hydroxide 0.5M followed by esterification and extraction of the liberated fatty acid with petroleum ether.

The petroleum ether extract was esterified with borontrifluoride–methanol complex[55] and benzoic acid was used as internal standard. From literature[52-53] it was found that methyl ester of benzoic acid does not overlap with the methyl esters of standard lipid in gas chromatograms. Total conversion of the fatty acids into corresponding methyl esters were also checked by t.l.c.

As the total lipid content of the vegetables were not very high much of the fatty acid were not expected in the five vegetables. Total fatty acid present in the vegetables varied from 3.68-34.05 mg/100 g of fresh vegetables (Table-14). Karela contained the highest amount of fatty acid (34.05 ) followed by potol (23.49 ) and chichinga and badhakopy contained nearly 10% of fatty acid. Fatty acid content of chalkumra was lowest among the five vegetables.

Lauric, myristic, palmitic, stearic, oleic, arachidic and behemic acids were the seven fatty acids were identified and quantified in the vegetables. Comparison of the fatty acids is presented by Bar graph 11-17. The lowest amount of lauric acid was present in chalkumra whereas korela contained the highest amount. Potol contained the highest amount of palmitic acid followed by stearic, arachidic and oleic acid. Nearly the same amount of lauric and myristic acids were present in potol. In karela, behemic acid content was 11 mg/100 g fresh sample (vegetables). Arachidic and palmitic acids content were

nearly the same (~7 and 6 mg/100 g). The lowest amount of myristic acid was present in karela followed by lauric, oleic and stearic acids. In badhakopy palmitic and stearic acid content were the same (~ 3-4 mg/100 g ) vegetables. Other identified acids in bathakopy were lauric, myristic, oleic and arachidic. Like potol behemic acid was not found in badhakopy. Behemic acid was also found in chalkumra. Chichinga contained the highest amount of palmitic acid followed by olelic, behemic and stearic acids. Small amount of lauric and arachidic acids were found in chichinga. Although fatty acid content of chalkumra was the lowest, but it contained small proportions of all the seven identified fatty acids. From all the data of fatty acid composition of the five vegetables it appeared that the composition were more or less similar to fatty acid composition of rice straw[53] and jute plant[52] but they were very much different from the fatty acid composition of the common fats[51] (Table- 1). Due to shortage of time fatty acid composition of the other three vegetables were not determined .

## 3.10 Concluding Remarks:

The eight vegetables which were analysed in the present studies for their free sugar and dietary fibre content and composition showed that karela contained more of dietary fiber than the corresponding free sugars. Gajor, fulkopy and potol also contained more fiber than the free sugars. Shalgom and badhakopy had on the other hand large negative value indicating more free sugar than DF which indicated that these two vegetables are not good for the

diabetic patients. Chichinga and chalkumra had also negetive fiber value *i.e.* these vegetables are also not good for the patients. From earlier studies[7] on the vegetables, elongated beans, ladies finger, green papaya, karela, brinjal, lao and green banana it was found that all the vegetables contained more dietary fiber than the free sugars. Among them elongated bean (barbai) was the best. From folkloric reputation it is said that the vegetables which grows under the soil are not good for the diabetic patients. Shalgom fits the folkloric statement but the same is not true for gajor.

The results obtained should not be taken as absolute statement in respect of vegetables and with reference to diabetic patients. Sometimes one pateint may have several complications. From digestive point of view or in other words a patient having gastric problem may have to choose the vegetables with less fiber content.

Soluble dieary fiber composition of vegetables showed that they contained different types of pectic substances i.e. they were mixtures of different polysaccharides. Isolation and structure elucidation of the individual polysaccharides will have great potential of further chemical and biological studies.

All the vegetables contained very small amount of fatty meterials. The composition of the fatty materials showed that they contained only seven different fatty acids. The composition also indicated that they were similar to the compostion of the edible oils.

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