CONCEPTUAL ANALYTICAL PRODUCTION OF HIGH POWER FUEL AND HIGH FIBER PROTEIN THROUGH SENSIBLE SOURCE

A dissertation submitted in partial fulfilment of the requirements for the degree of

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IN

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by

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Under the Supervision

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D E C L A R A T I O N S

This is to certify that this dissertation submitted by MR. MUHAMMAD ARSHAD is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, as satisfying the dissertation requirements for the degree of **Master of Philosophy in Chemistry.**

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D E D I C A T I O N

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I dedicate my humble

work

to my beloved Parents,

Sisters and Brother

ACKNOWLEDGEMENT

My deepest gratitude to Almighty ALLAH, Who enabled me to complete this research work.

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MUHAMMAD ARSHAD

ABSTRACT

The agricultural material (corn,wheat,barley,oat , rye and molasses) was subjected to fermentation to produce high fiber protein (HFP). The product could be suitable for human consumption. Plus the liquid protein by-product could be used for animal feed and recovery of carbon dioxide from the fermentation vapour. This could be sold as a gas or further processed into dry ice. Ethanol produced was further converted into high power alcohol and compared its characteristic properties with that of the natural fuel. The high power alcohol was transformed into high power fuel (gellified material) by the interaction of potassium salts of the derivative of carbohydrates with high power alcohol. The potassium salt of the derivative of carbohydrate was prepared at two stages. First, derivative of carbohydrates was synthesised by the interaction of carbohydrates with acetone. The derivative so produced was treated with chlorosulphonic acid in presence of potassium carbonate to produce potassium salts of the derivative of carbohydrates. The high power fuel could be used for house hold and defense purposes. The fermentation liquid remaining as residue could be consumed as non-alcoholic drink and used for export.

C O N T E N T S

EXPERIMENTAL

CHAPTER 1

INTRODUCTION

INTRODUCTION

The sensible source of high power fuel, high-dietary fiber and protein is obtained from corn, wheat oats, rye, barley and molasses.

Part 1 of this chapter contains a description of the chemistry of fermentation and conversion of ethanol to high power and gellified fuel as a renewable sources of energy.

Part 2 include a discussion on the chemistry and biotechnology of the high fiber protein obtained after the fermentation. HFP is available for human consumption. This part is also furnished to account the chemistry of liquid protein obtained after the fermentation. LP is normally consumed in preparation of animal feed.

Part 3 contains a description of the recovery of carbon dioxide from the fermentation vapours. This can be sold as a gas or processed into dry ice.

Part 4 will include the discussion on the recovery of the non-alcoholic drinks.

1. HISTORICAL REVIEW

Records have shown that man has brewed malt beverages for centuries. The nature of the earliest fermented beverages is uncertain; they may have been derived from barly, dates, grapes or honey. Archaeologists have found hieroglyphic accounts of how to brew beer[1]. They also have found jugs that were used for beer more than 5,000 years ago.

Chemical analysis of the jugs have uncovered the barley and yeast cells by which the beer was produced. Brewing seems to have originated in Babylon where, as in Egypt, barley grew wild, and there is some evidence that beer made from malted grain was being brewed[2] in Mesopotamia by 6000 B.C. By the fourth or fifth millenium B.C. brewing was well established and evidence exists of the various types of beer extant in Babylon about 1800 B.C. Brewing in Egypt began at a later date than in Babylon but probably developed independently. It was said to have been a gift of the god Osiris, or his wife Isis, about 2000 B.C. although it seems that several different types of beer were brewed in Egypt a thousand years before than, and there is a reference to its use as a mortuary offering in the 5th dynasty, about 2800 B.C.[3,6].

The Greeks learned brewing from the Egyptians and also

grew hops, (origin of the use of hops was unknown; one suggestion is that the Hebrews learned the use of hops during the Babylonian captivity in the 8th and 9th centuries B.C.). The Romans learned about beer from the Greeks. The northern European races probably discovered the technique of brewing long before the Christian era, the earliest Teutonic and Celtic beverages being made from a mixture of corn and honey and hence approximating mead. Various alcoholic beverage were made by the Indians long before the advent of Europeans but the history of American brewing really begins in 1584. When the British brewed beer from corn (maize) during their first attempt to colonize Virginia[7].

Until the mid 19th century only "British" types of beer were brewed in North America but about 1840 German brewers introduced the newer methods of making larger beer, thus laying the foundations of the vast modern American industry. Inspite of the long history of this important chemical transformation, its exact nature remained unknown before 1830[4,8].

In late 1830's, Schwann and Cagnaird-Latour showed that the alcoholic fermentation was caused by organized living beings today known as micro-organism. Pasteur in 1860's subsquently confirmed this idea and showed that the transformation of sugars into ethyl alcohol was caused by yeasts.

The malt beverages have been produced continuously in Europe and other countries ever since[5]. In 19th century alcohol produced by different raw materials was frequently used in some countries for burning purpose. Solidified alcohol[9] (a jelly formed by colloidal calcium acetate in alcohol) was an example used as a fuel for spirit lamps.

During the first half of the twentieth century, industrial alcohol was produced on a large scale by fermentation. The rising cost of petroleum during the 1970s and the resulting efforts towards greater reliance on naturally renewable resources created an unusually active interest in fermentation ethanol, because it was not only an attractive current alternative to fossil fuel, but also a potential feedstock for the chemical industry. Accordingly, UNIDO (United Nation Industrial Development Organization) organized the first international workshop on fermentation alcohol for use as a fuel and chemical feedstock in developing countries at Vienna, Austria on 26-30 March, 1919. The use of alcohol for internal combustion engine was promoted as far back as 1894 by Hartman in Leipzig, and even Henry Fort I gave much thought to it as an alternative to fossil petrol in the 1930s. This resulted in the Dearborn Conference on what is now called "gasohol" and the establishment of a major fuel alcohol plant in Kansas around 1936; during the 1930s and

1940s several developed countries used alcohol blends and unblended alcohol in many vehicles[10,11].

Brazil has been the leader; as far back as 1925, an alcohol powered Model A Ford was demonstrated in Brazil, but the huge Brazilian Government Programme (Proalcohol) was established as late as 1975 and US Government initiated a substantial programme in 1978. About 40 countries (including) developed as well as developing countries like Pakistan) are in the process of drawing up power alcohol programmes. Pakistan during 1980s would have to base its strategy on molasses as a source of making alcohol.

1.1 FERMENTATION AND ITS CHEMISTRY

1.1.1 Fermentation

The term 'fermentation' is derived from the Latin 'fervere' meaning 'to be boiling', and originally signified the gentle bubbling or boiling condition observed in the spontaneous transformation of fruit juices, as in wine or cider[9,17]. The meaning was changed through Gay-Lussaes study of alcoholic fermentations to indicate the conversion of sugar into carbondioxide and alcohol. Pasteur's researches as to the cause of fermentative transformation led him to define fermentation as 'life without air'. Now the work has become more closely associated with the microorganisms and the enzymes secreated by them, which catalyze the fermentative changes[15,16].

The fermentation is a process by which complex organic material is broken down into simpler substances, are brought about the action of living organisms (yeast) which secrets the enzyme-catalyst appropriate to the process. It is an incomplete and slow oxidation of the substances such as carbohydrates[14] and carbohydrate like compounds, associated with evolution of carbondioxide and production of ethanol plus a number of by-products.with respect to oxygen supply,

two types of fermentation are recognized. Aerobic fermentation (oxybionic process; respiration) is the form of dissimilation which requires free oxygen to act as a hydrogen acceptor. The acetic acid and citric acid fermentations are examples. Anaerobic fermentation is dissimilation in which atmospheric oxygen is not involved, but other substances, such as aldehydes or pyruvic acid, serve as hydrogen acceptors. Examples are the alcoholic, butyl alcohol-acetone, and lactic acid fermentations[12,13].

Fermentation is largely depend upon three basic parameters, namely; the wort composition (nutrients for the yeast); the yeast itself; and the processing conditions (such as time, temperature, volume, pressure vessel shape and size, agitation and currents in the fermenting wort). Fermentable sugars can be obtained not only from sucrose (molasses, sugar cane, beet), but also from starch (cereals; corn, barley, wheat, rye, oat, rice), as a matter of fact, by far the most important raw material for the manufacture of alcohol and recovery of other products such as protein.

(a) Bottom Fermentation

The temperature of the wort at pitching is 6-9^{o}C and the yeast is added in the form of a slurry. A certain amount

of aeration may be employed at the earliest stage to encourage yeast growth. The sooner the yeast begins to grow, the less danger there is from bacterial infection. Within a few hours a fine fluffy white head appears and the fermenting wort is often pumped or transferred to another preferably closed, vessel, leaving behind much of the unwanted protein, hops and resins. Activity increases and the stage known as 'Cauliflower', is reached after two days. After continues for three days, during which the temperature must not be allowed to rise above $8^{o}C$. The head then starts to collapse and the temperature is allowed to fall gradually to about $6^{o}C$. After about eight days fermentation is in all and the evolution of carbondioxide is too slow to buoy up the yeast cells and these, therefore remain at the bottom of vessel or vat. This is known as bottom fermentation', and is made use of in Germany in the production of certain beers.

(b) Top Fermentation

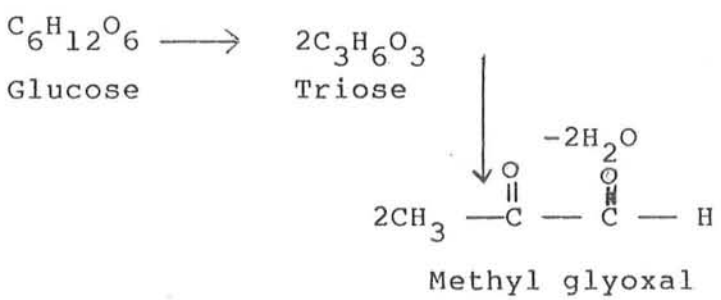
When fermentation is allowed to take place at a temperature of about $15^{o}C$ or $60^{o}F$. During five to seven day fermentation period, a light froth appears in the first few hours, giving place to small 'cauliflower'

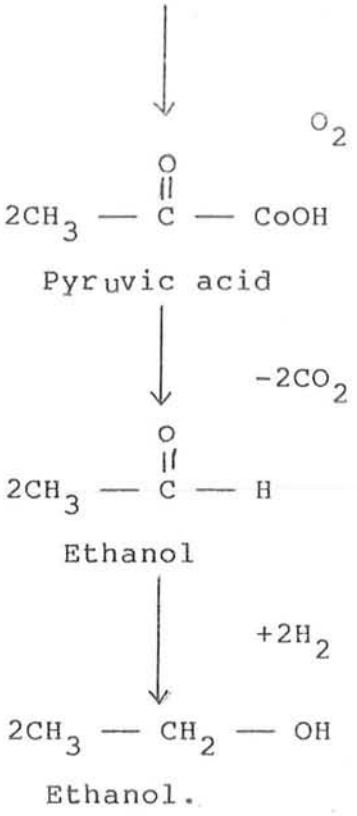
and then to 'rocky head'. On the second day a high rocky head develops, fermentation is vigorous and heat generation is at its greatest. On the third day the head gradually collapses and the blanket of yeast is skimmed off. The head then forms pleats or folds as the beer is cooled for a second skimming. During whole process a turbulent effervescence is produced and the evolution of carbon dioxide is so rapid that the yeast cells are carried to the surface of the liquid and these form a thick forth. This is known as top fermentation and is the process which is mostly used in England[4,6].

1.1.2 Fermentation Mechanisms

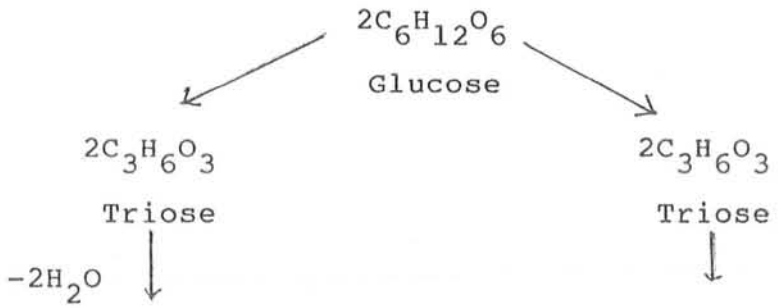
Various schemes have been proposed to elucidate the pathways involved during the fermentation of the carbohydratic materials to ethyl alcohol[18-21].

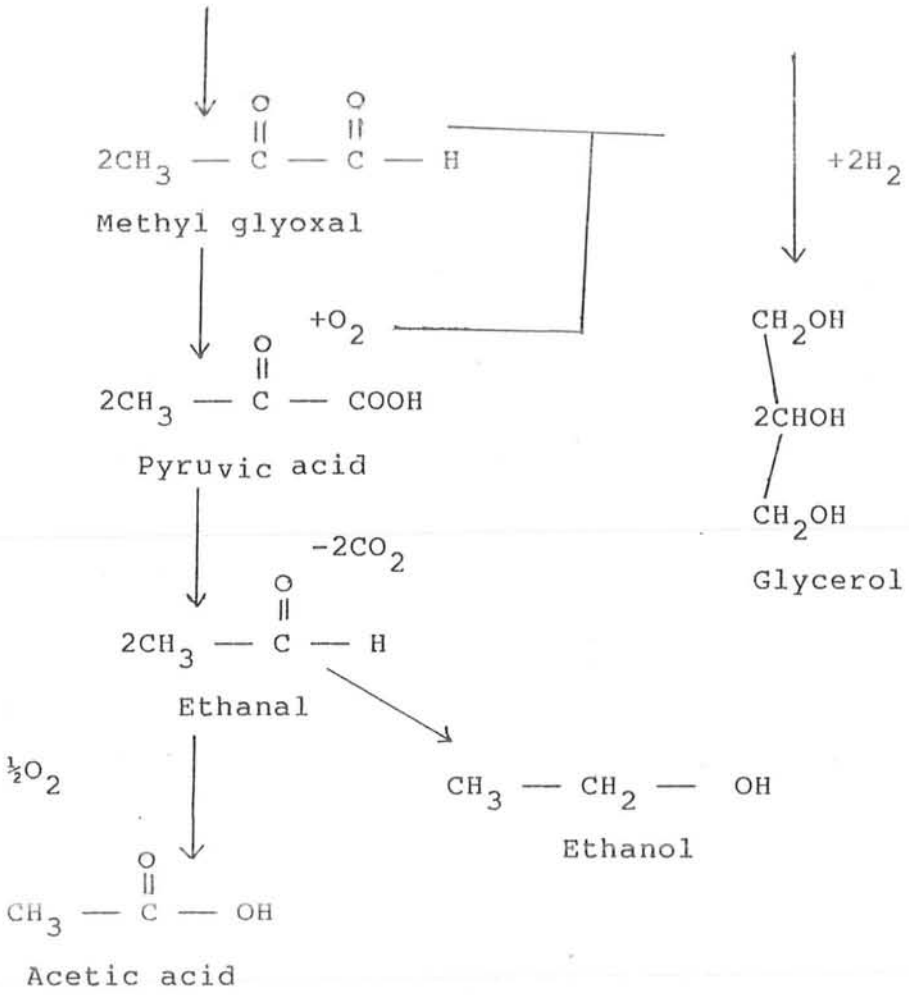
Neuberg's Schemes





(i) The reaction proceeds in acidic conditions. The glucose molecule is broken into two triose molecules which are the hydrated forms of methyl glyoxal. The triose loses a water molecule to form methyl glyoxal which in turn is oxidized to form methyl glyoxal which in turn is oxidized to pyruvic acid. The pyruvic acid is decarboxylated to acetaldehyde which is ultimately reduced to ethanol.

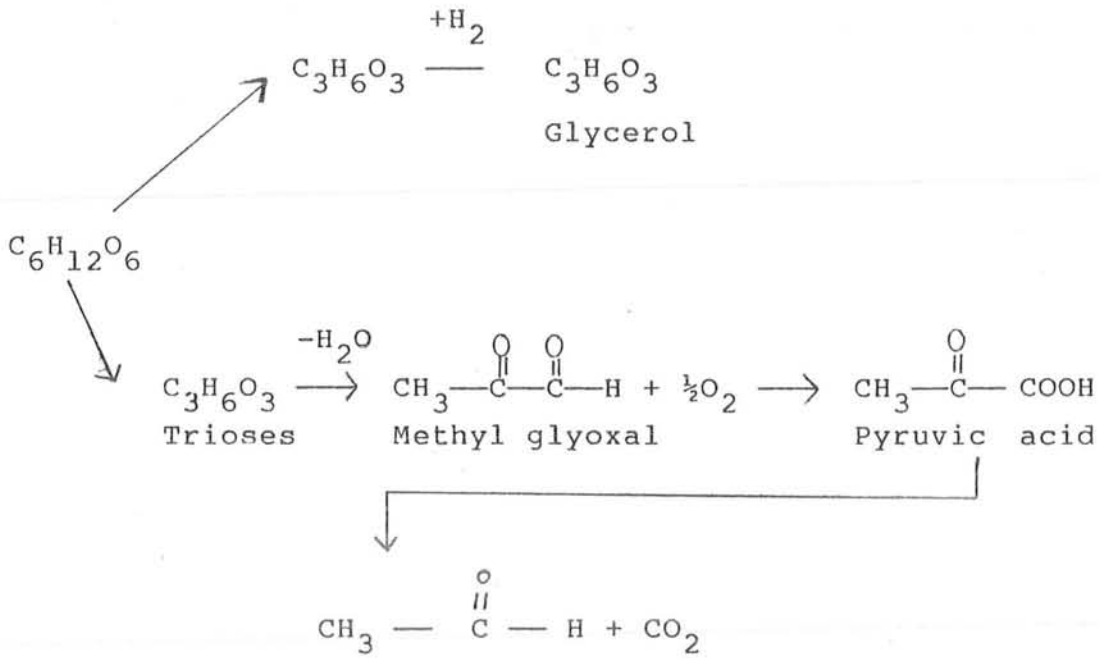




(ii) The reaction proceeds in the alkaline conditions. Here the triose produced becomes unstable and thus accepts hydrogen to form glycerol. The pyruvic acid decarboxylates to acetaldehyde after its formation as in scheme (i). The acetaldehyde is oxidized to acetic acid at the expense of the molecular oxygen.

(iii) The reaction proceeds after addition of sodium sulfite to the fermentation mixture. Sodium sulfite fixes the acetaldehyde produced and blocks its

reduction to ethanol. As a result of this fixing, the yield of glycerol increases while that of ethanol decreases.



Kluyver's Scheme

This involves the formation of hexose monophosphate (HMF) as an active intermediate. The mechanism involves the following steps:

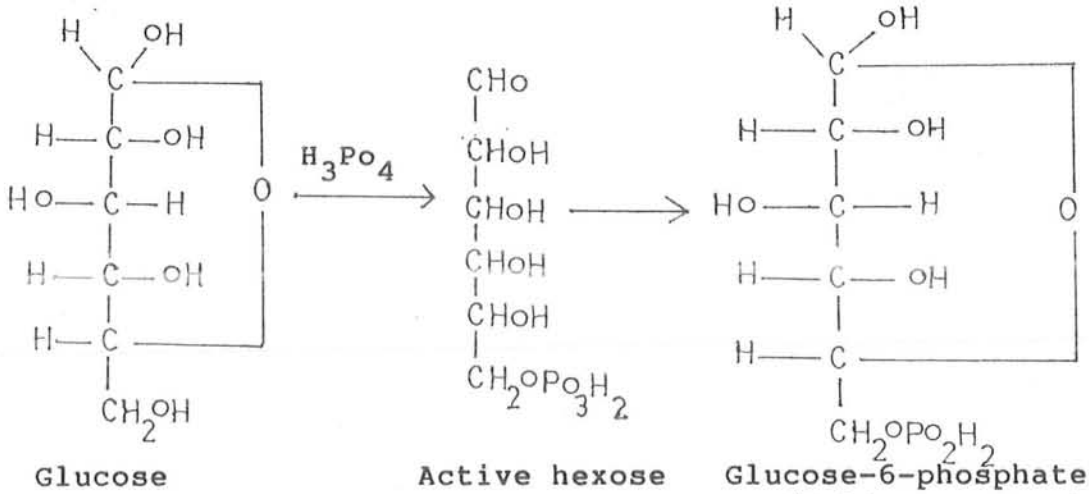
(a) Initial phosphorylation

The initial phosphorylation of glucose occurs by combination with an inorganic phosphate molecule. The

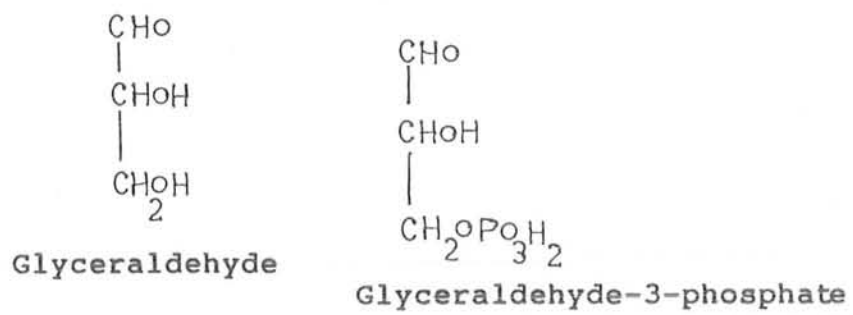
hexose monophosphate formed as a result of initial phosphorylation is partly converted into glucose-6-phosphate (Robinson ester).

(b) Oxidoreduction of HMP and cleavage

The hexose monophosphate cleaves to form a molecule of glyceraldehyde and a molecule of glyceraldehyde monophosphate.

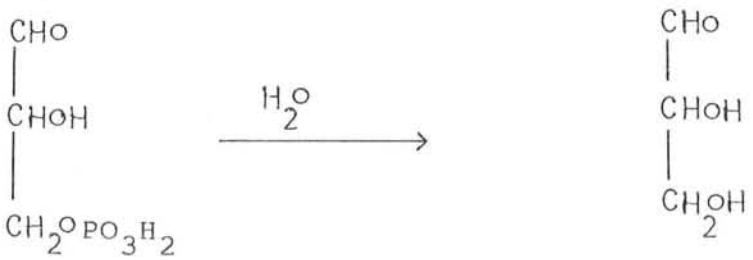


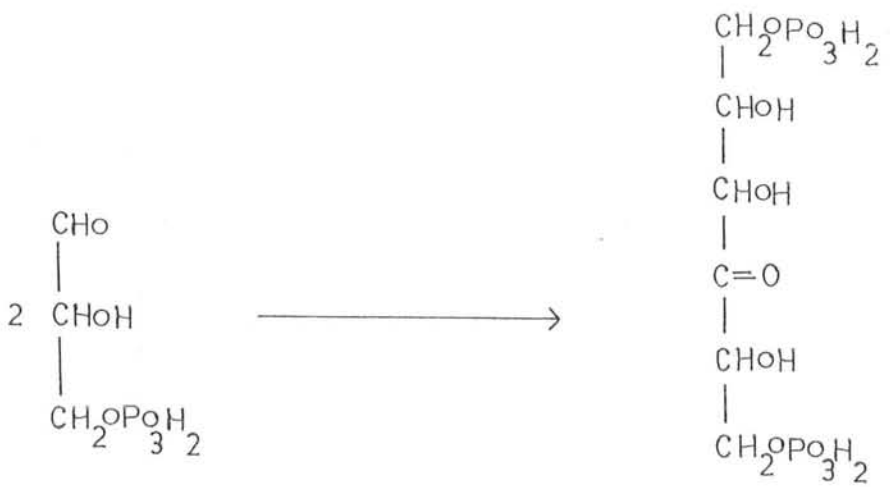
oxidoreduction + cleavage



(c) Hydrolysis and condensation of Glyceraldehyde-3-phosphate

The glyceraldehyde-3-phosphate hydrolyses to form glyceraldehyde and its two molecules condense to form fructose-1,6-diphosphate

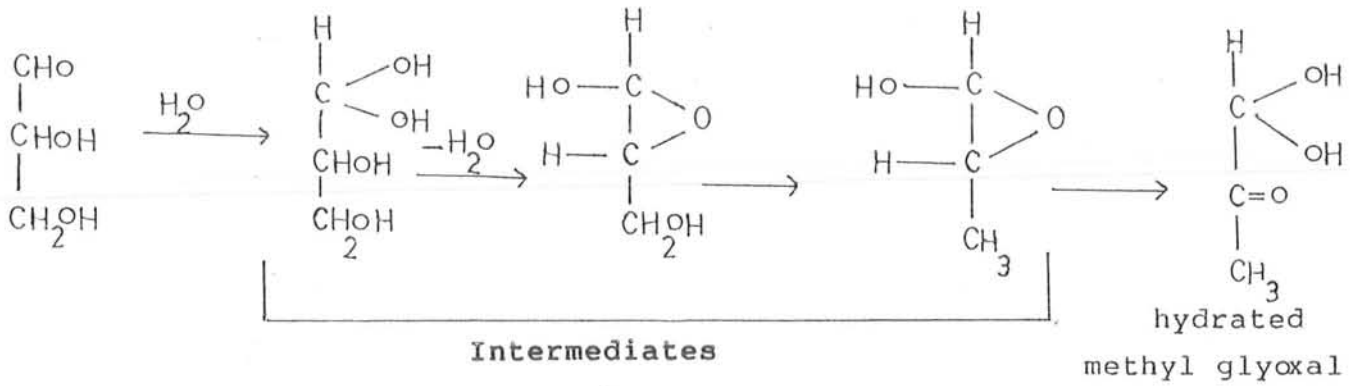


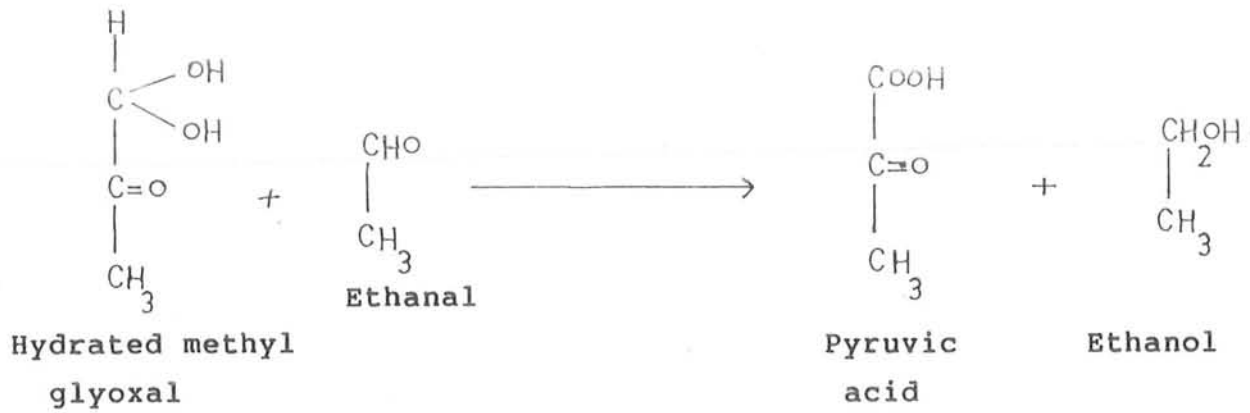


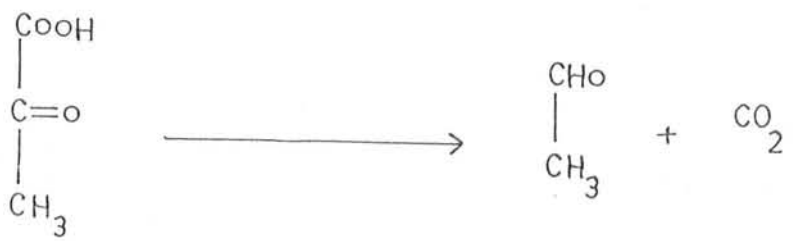
Fructose-1,6-diphosphate

(d) Final oxidoreductions

The final oxidereductions of glyceraldehyde product yield glyoxal, pyruvic acid and ethanol.



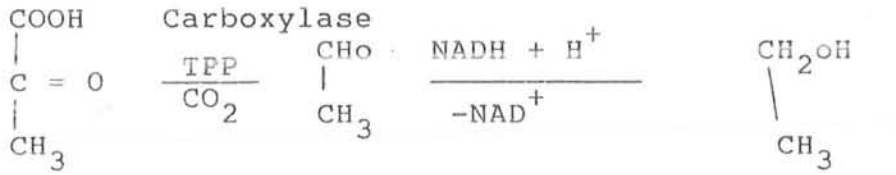




Embden-Meyerhof Scheme

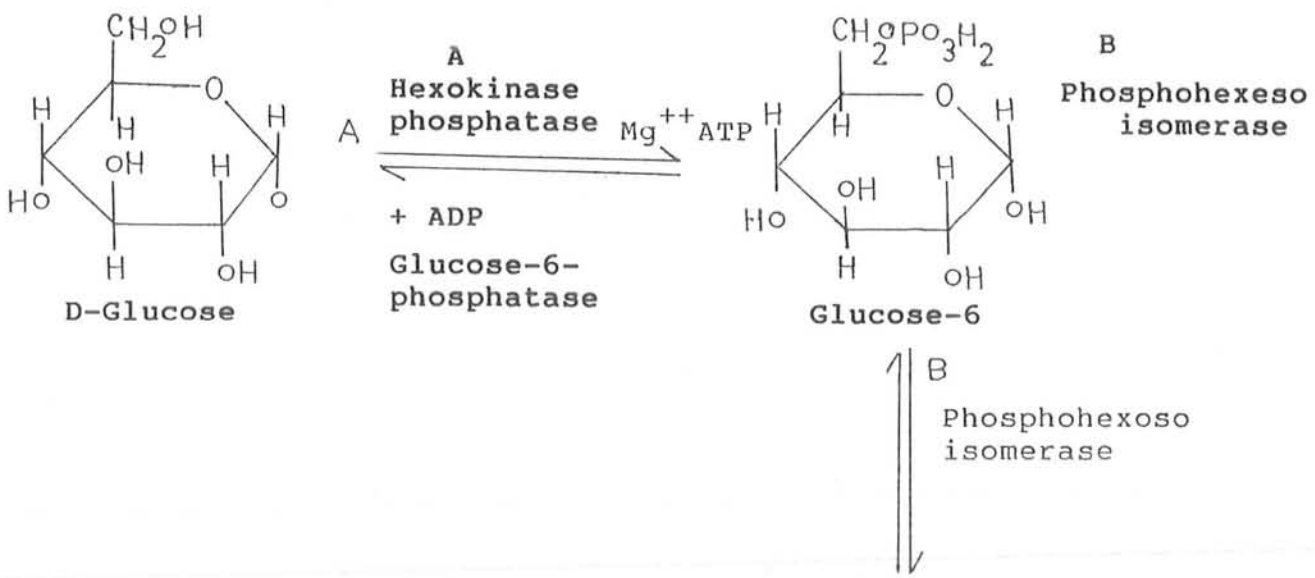
The fermentation of glucose could be carried out by cell free extract of yeast. The final product is ethanol. The metabolic pathways were elucidated by the use of inhibitors during the course of fermentation. The specific inhibitors blocked the sequence by inhibiting specific enzymes at specific stages of reaction sequence. The blocking resulted into the accumulation of products behind the block which could be isolated as intermediates. The sequence was later mapped by comparison of the various observations. Iodoacetate, for example, resulted into the accumulation of fructose 1,6-diphosphate and two triose phosphates. Similarly, the addition of fluoride resulted into the accumulation of glycerate-3-phosphate and the glycerate-2-phosphate. Different intermediates were isolated by Embden and Meyerhof and the sequence-mapped as described above. Harden and Young subsequently separated the cell free extract into the heat-labile and heat-stable fractions by dialysis. The heat labile fractions contained enzymes and the heat-stable contained co-enzymes such as NAD, ADP, ATP, etc. The splitting of a glucose molecule into two halves is accompanied by the loss of free energy sufficient to synthesize two molecules of ATP. The

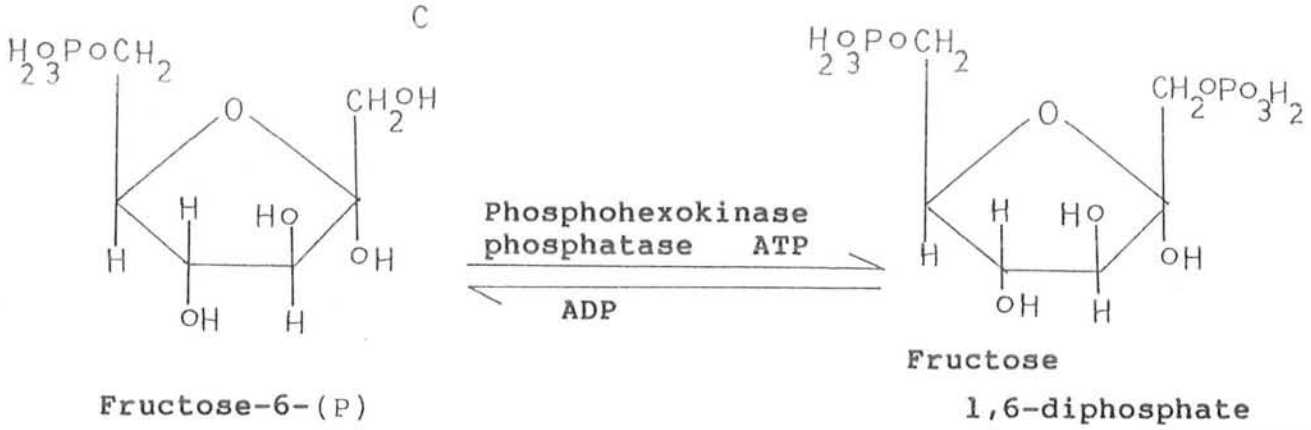
breakdown of glucose in glycolysis involves a large number of reactions catalysed by the enzymes produced by the yeast. The alcohol is formed by the change of the reaction course by yeast cells one step before the production of lactic acid. The reactions involved in the alcoholic fermentation is upto the formation of pyruvic acid. The pyruvic produced as a result of glycolysis is decarboxylated and the acetaldehyde produced is reduced at the expense of $NADH_2$ formed of glycolysis to form ethanol.

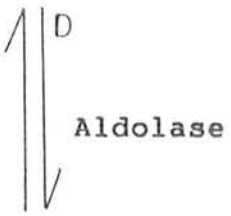


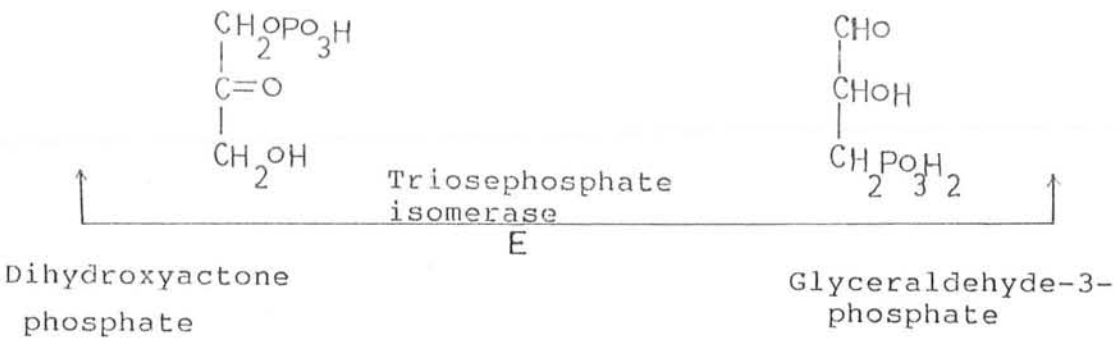
Pyruvic acid — Acetaldehyde — Ethanol

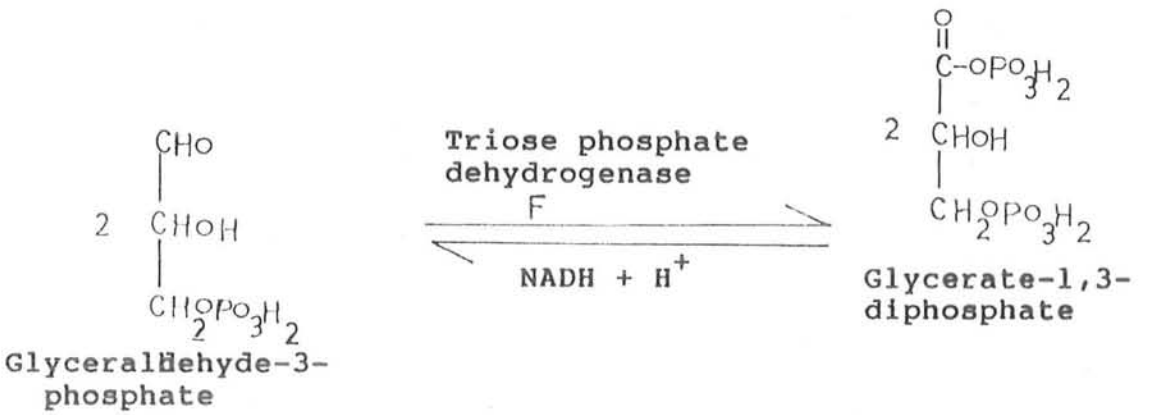
THE EMBDEN-MEHYRHOF SCHEME

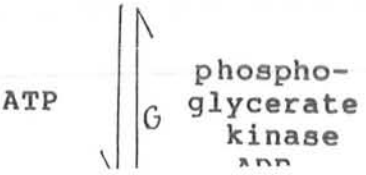


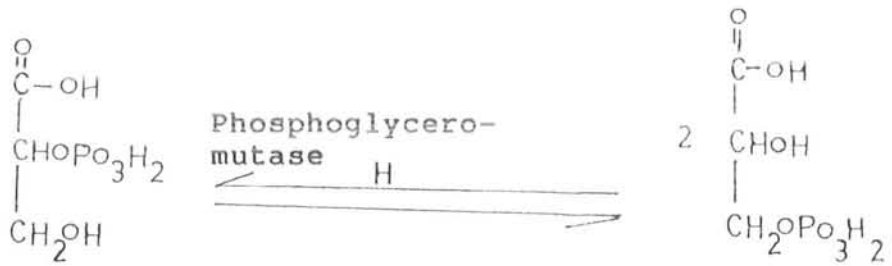

C
Phosphohexokinase
phosphatase ATP
ADP
Fructose-6-(P)
Fructose
1,6-diphosphate


D
Aldolase


Triosephosphate
isomerase
E
Dihydroxyactone
phosphate
Glyceraldehyde-3-
phosphate

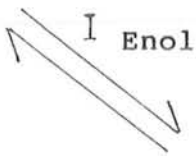

Triose phosphate
dehydrogenase
F
NADH + H+
Glyceraldehyde-3-
phosphate
Glycerate-1,3-
diphosphate

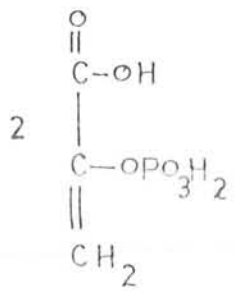

ATP
G
phospho-
glycerate
kinase



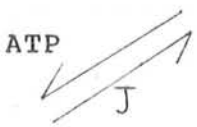
Glycerate-3-phosphate

Glycerate-2-phosphate

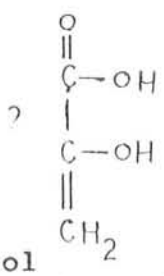




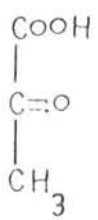
Enolpyruvic acid phosphate



Pyruvate kinase
ADP

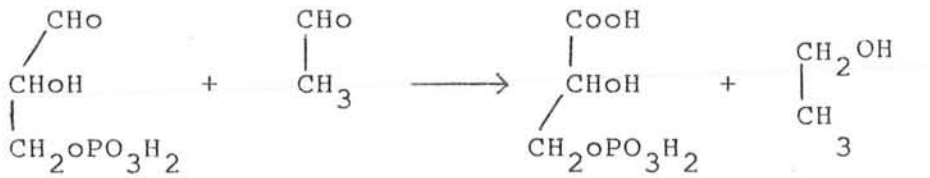


Enol pyruvic acid



Pyruvic acid

The oxidized NAD^+ reacts at step (F) of glycolysis and is reduced. The process is repeated again and again. The stage (F) and (G) can be linked with acetaldehyde and alcohol in the following reaction:

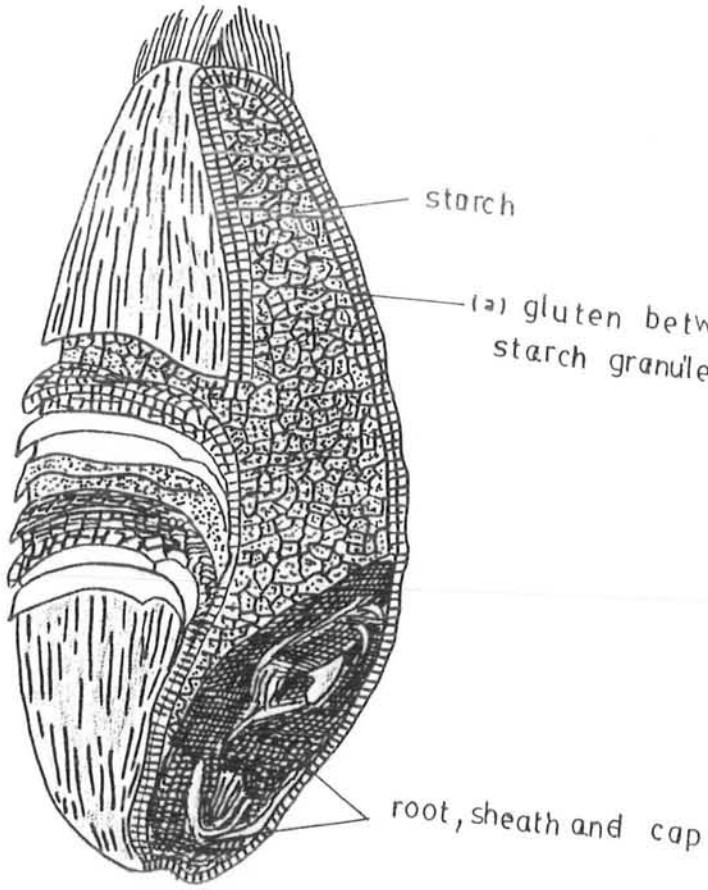


Glyceraldehyde -3-phosphate | Acetal-dehyde | Glycerate-3-phosphate | Ethanol

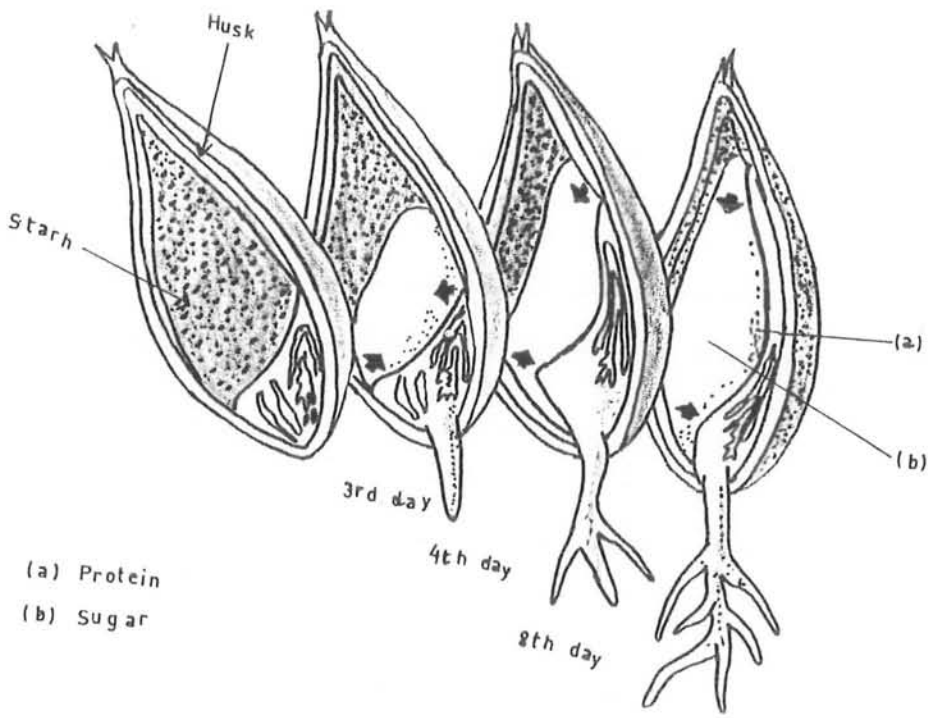
Thus NAD^+ acts as a hydrogen carrier and the above reaction occurs only in the stationary phase of the fermentation. The glyceric acid formed in the above reaction undergoes the subsequent glycolytic reactions to produce more acetaldehyde to produce more phosphoglyceric acid. The cycle is repeated again and again.

1.1.3 Malt production

During malting, profound changes occur in grains, the most important being the development of certain enzymes. The two most important groups are; (a) amylolytic enzymes: which can convert starch to carbohydrates of lower molecular weight[23]. (b) Proteolytic types; which can break down the protein consituents of the grain to simpler nitrogenous compounds. Before brewing



Longitudinal section of a grain



Phases of germination of grain.

Fig. 4

the starchy material is malted in a three-step process of steeping, germination and kilning[24].

The grains are steeped for some days in water and then spread out, or 'couched' to a certain depth on the floor. Under favourable conditions of humidity and temperature, soon the moist grains begin to sprout (Fig. A). During germination proteins and starches are broken down and diastase (amylytic enzymes; principally alpha and beta amylase) and other enzymes are produced. After germination the malt is dried in a kiln until the moisture content is between 3-5%. The shriveled rootlets drop off and are collected and sold as animal feed[25].

1.1.4 Brewing Process

The three main stages of the brewing process are: mashing, boiling and fermentation. (Fig. 1).

(a) Mashing

The malt, which contains a considerable amount of starch and a small amount of sugar, together with the amylytic enzymes, is crushed and mixed with hot water and a quantity of hop (raw grain) is added. The

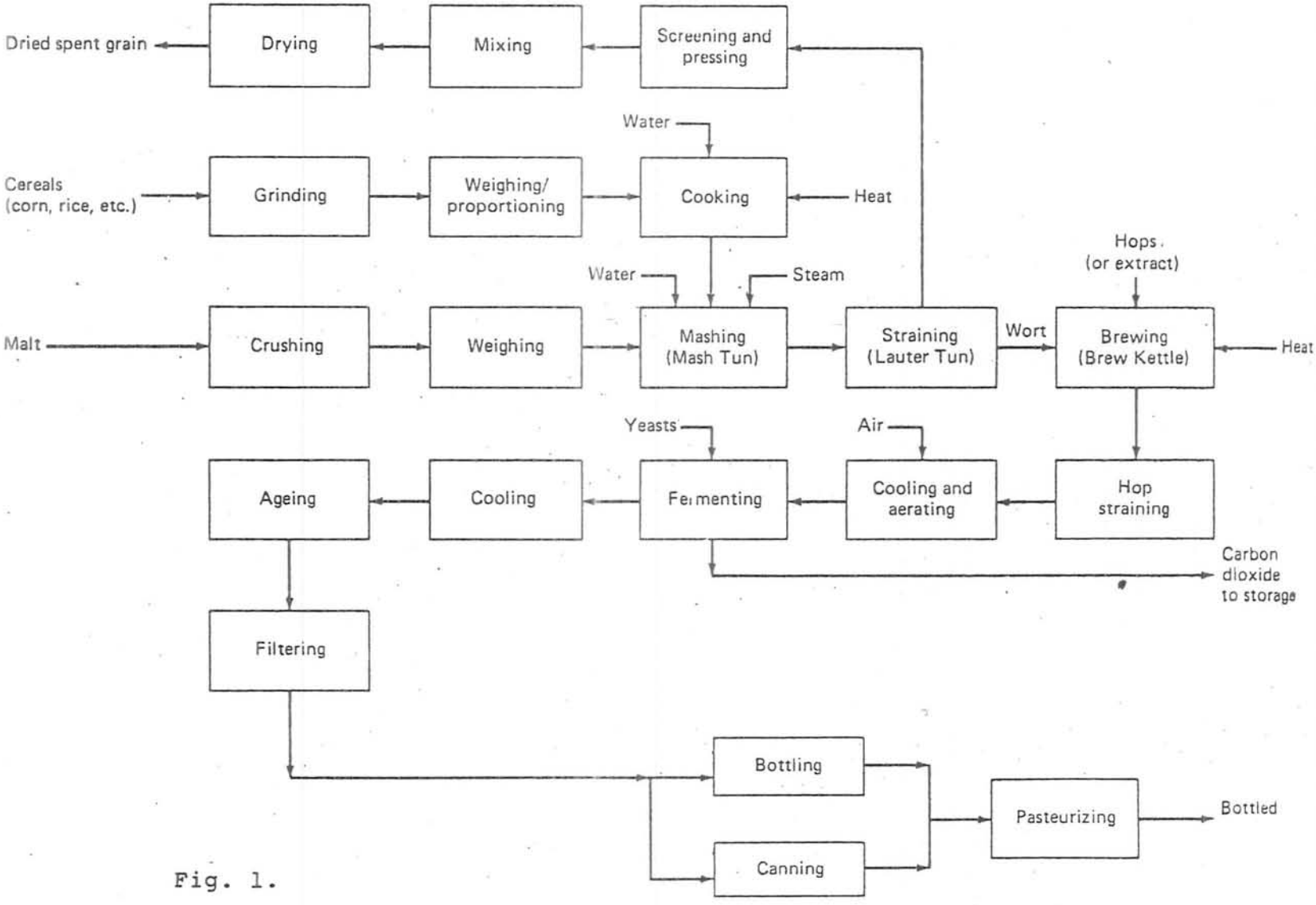


Fig. 1.

Simplified flowsheet of a typical brewing process.

"striking heat" of water is of 150°F. The principal reaction of this cooking involves the partial breakdown of proteins or starches (carbohydrates) by means of heat and hydrolysis (Fig. B) in the endosperm of the malted grain[22]. Starch is converted into soluble maltose and other malt sugars and

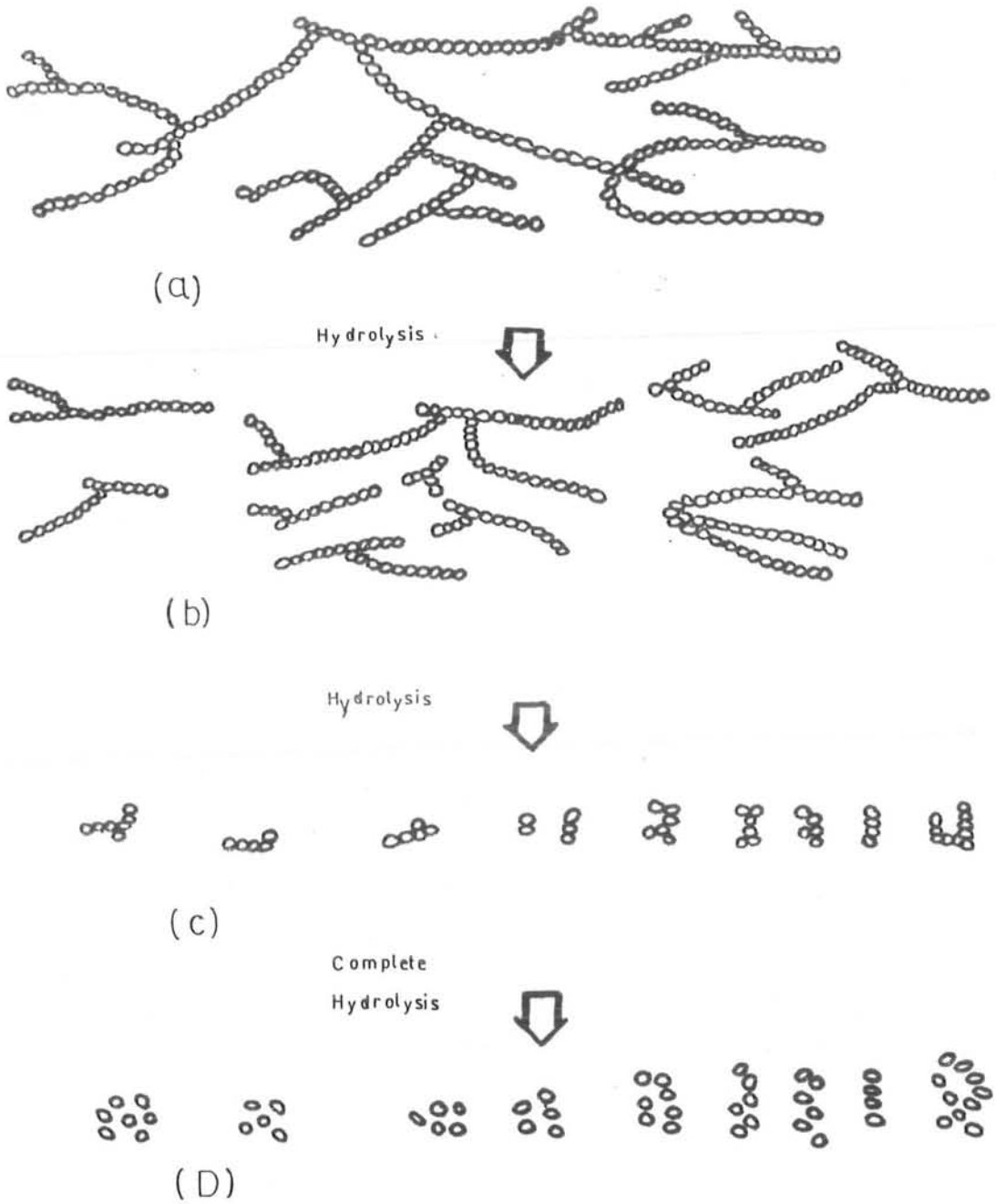
$$2(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{diastase}} n\,C_{12}H_{22}O_{11}$$

Starch → Maltose

dextrins by the amylolytic enzymes, α and β -amylase, which are present in the malt and which work best at a temperature between 40°C and 60°C (104° or 140°F). This is where the amylases (enzymes) enter the picture by cleaving the amylose molecules into maltose (two glucose units looked together) and a single glucose units) (Fig. C). When this process of mashing is completed, the liquid is boiled to destroy the diastase and sweet liquor or wort (solution of converted carbohydrate: sugar) is filtered out leaving the malt husks behind. This wort is ready for fermentation.

(b) Boiling

After mashing and sometimes after filtering, the wort is boiled. This prevents any further enzyme action



(a) Partial schematic amylopection structure

(b) Dextrins from incomplete hydrolysis of a

(c) oligosaccharides from hydrolysis of dextrins

(d) Final hydrolysis product: D-glucose.
Each circle represents a glucose unit.

Fig. B

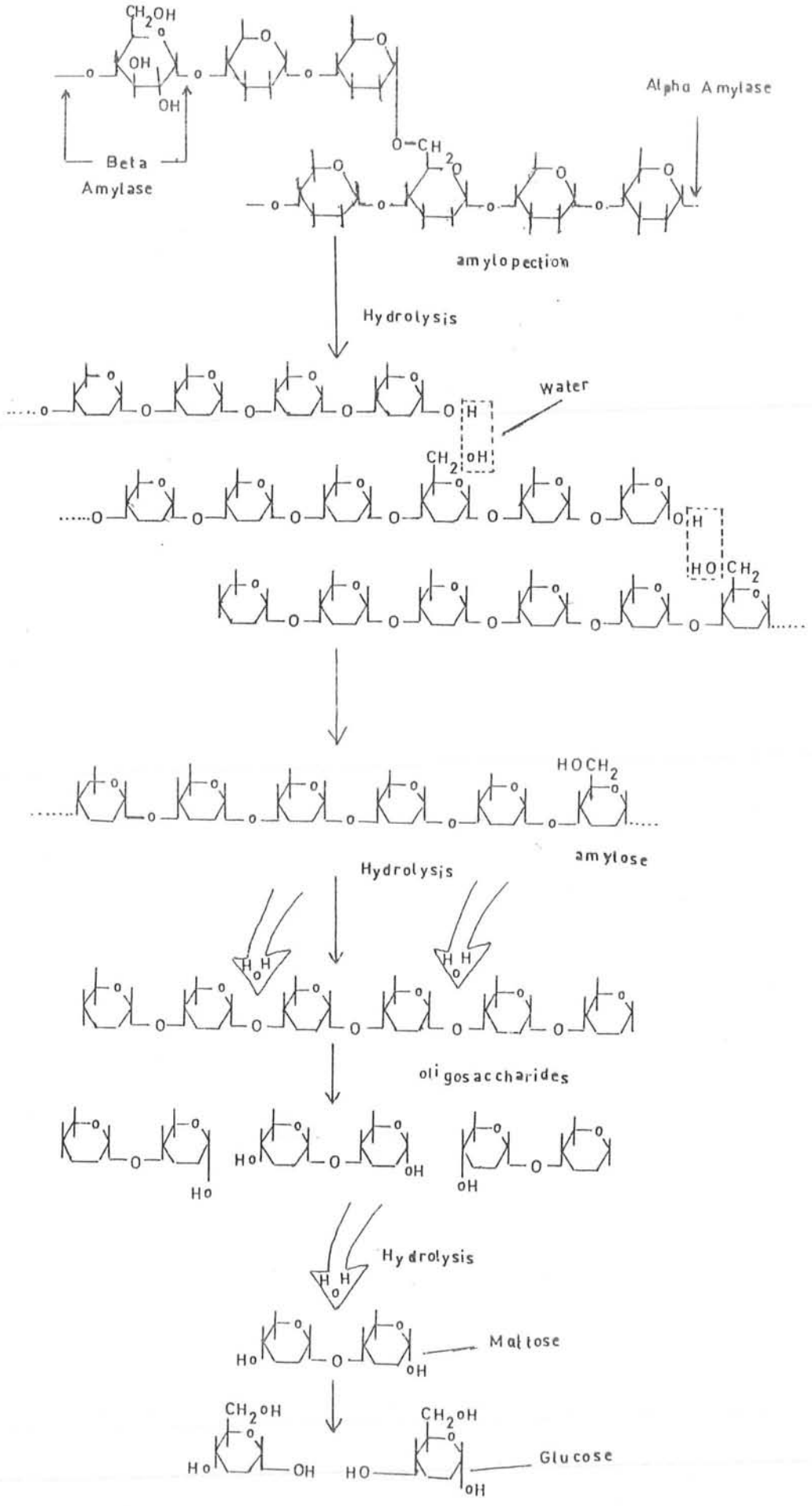

Alpha Amylase
Beta
Amylase
amylopection
Hydrolysis
water
amylose
Hydrolysis
oligosaccharides
Hydrolysis
Maltose
Glucose

Fig. 2

and coagulates a great deal of the protein material, which is known as the "hot break" or "trub". The boiling also sterilizes the wort, makes it more concentrated and provides an opportunity for the hops to be added. After boiling the wort is filtered quickely to remove the hops and also as much of the hot break as possible.

(c) Fermentation Process

The wort is run into the fermenting vessels, diluted and mixed with yeast and held at an appropriate temperature for 40-60 hrs. The living yeast cell secrete the enzyme maltase (converting maltose to glucose), invertase (converting sucrose to glucose) plus levulose and zymase (convert glucose to ethanal plus CO_2). The maltase causes the sugar, maltose, to hydrolyze into a simple sugar, glucose

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \xrightarrow{\text{maltase}} \underset{\text{Glucose}}{2C_6H_{12}O_6}$$

The glucose, in turn, is converted by yeast enzyme zymase mainly into ethanol and carbon dioxide.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \xrightarrow{\text{zymase}} 2CO_2 + \underset{\text{Ethanol}}{2C_2H_5OH}$$

Although small quantities of other substances, higher alcohols, succinic acid, glycerine, etc. are also produced[26-30].

1.1.5 Factors Influencing Fermentation

There are many factors that have a significant effect on alcohol yield and efficiency. These may be classified as: (a) the physiological condition of the inoculum, (b) the environmental factors present during fermentation, and (c) the quality of the raw materials.

(a) Physiological Condition of the Inoculum

The physiological condition of the inoculum, the quantity and the cell population are of considerable importance. Fortunately, most yeasts are resistant to adverse environmental conditions and are adaptable to a wide range of conditions[31]. The activity of the inoculum may be arrested by holding at low temperatures for use several hours later. it is not desirable to use a culture that is more than 24 to 26 hours old, since older cultures may result in a yield reduction of 2 to 4%[32].

(b) Environmental Factors

High yields can only be attained by maintaining such conditions that the residual amylases will continue to function until the conversion of residual dextrins is complete, and the fermentable sugar so produced is converted to alcohol. The critical environmental factors are: (a) pH (b) buffer capacity (c) initial load of contaminants (d) temperature (e) mash concentration (f) alcohol concentration (g) yeast nutrients.

(a) pH

Grain mashes, without pH adjustment with acid or stillage, will enter the fermentor at pH 5.4 to 5.6[33]. The principal microbial contaminants are latic acid formers. Above pH 5.0, their growth is rapid. Thus, it has been found most desirable to adjust the initial pH to 4.8 to 5.0 with either stillage or sulfuric acid. As soon as the pH of a fermentation falls below 4.1, the amylases are inactivated. The adjustment to as low as pH 4.5 gives successful results.

(b) Buffer capacity

The buffer capacity of mash is important. Grain mashes are generally well buffered from pH 5.0 to 6.0, poorly buffered from pH 4.4 to 5.0, and well buffered

from pH 3.5 to 4.4. Stillage is of added value, since it adds considerably to the buffer capacity of the mash between 3.5 and 4.4. This aids in stabilizing the pH above 4.1 for maximum period and is one reason why stillage mashes give higher average yields than mashes from the grains without stillage.

(c) Initial load of contaminants

Good process sanitation is most important in the control of contamination and the realization of maximum alcohol yields. The piping installation and equipment design should make provision for adequate steam sterilization[35], ease of cleaning, and the elimination of mash. It is desirable to operate the mashing and cooling and cooling systems for the maximum length of time between clean-ups.

(d) Temperature

The molasses fermentation may be conducted at higher temperature[36] than the grain fermentation, since the carbohydrate is present initially in a fermentable state. The optimum temperature for the growth of certain yeast is 84° to 90°F as compared with an optimum fermentation temperature of 100°F in the absence of alcohol, and 90°F at alcohol concentrations above 4.5% by volume.

Failure to control the temperature within the optimum-growth temperature range destroys yeast activity in a few hours and thus sharply reduces the alcohol yield. Furthermore, the high temperatures favor the growth of bacterial contaminants.

(e) Mash concentration

The initial concentration of the mash governs both the final alcohol concentration and the heat release per unit volume. It is, therefore, necessary to employ a concentration that will neither generate more heat than can be dissipated without exceeding $90^{\circ}F$ nor potentially result in an alcohol concentration in excess of the practical alcohol tolerance of the yeast strain.

(f) Alcohol concentration

The fermentation rate of yeast is greatly reduced in the presence of ethyl alcohol (above 12%). It is apparent that a high mash concentration and resultant high final alcohol concentration will protract the fermentation period, possibly to a dangerous degree, since the added time favors development of contaminants

(g) Yeast nutrients

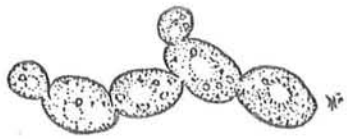
Stillage and urea has appreciable nutrient value

and is added for this reason and also for its buffering value and aid in adjusting the pH. Small grain mashes (wheat, rye, barley, malt)[34] are relatively rich in nutrients, while corn mash with 8 to 10% of barley malt is marginal in nutrient level[37]. None of these require supplementing with inorganic nitrogen, as this does not result in yield increases. The nutrient for yeast can be sometime overcome by addition of 0.5 to 1.0% of malt sprouts[38].

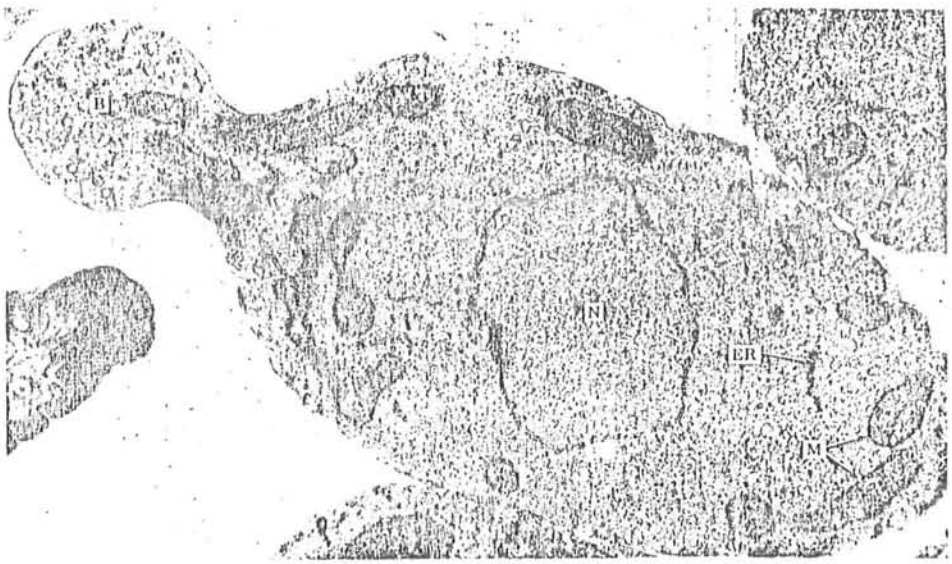
1.1.6 Yeast

Yeasts are the living entities (unicellular fungi) and as such they require food to maintain them in a healthy state so that reproduction (sexually or asexually) of their kind can continue; this food must include sources of carbon, nitrogen, sulfur, and certain minerals and many yeasts also required certain nutrilites[39,40]. Only three of the 349 known species of yeast are involved in alcoholic fermentation.

Yeasts used in brewing can be classified as two species of the genus Saccharomyces: Saccharomyces cerevisiae (Fig. J and K) and saccharomyces urarum.



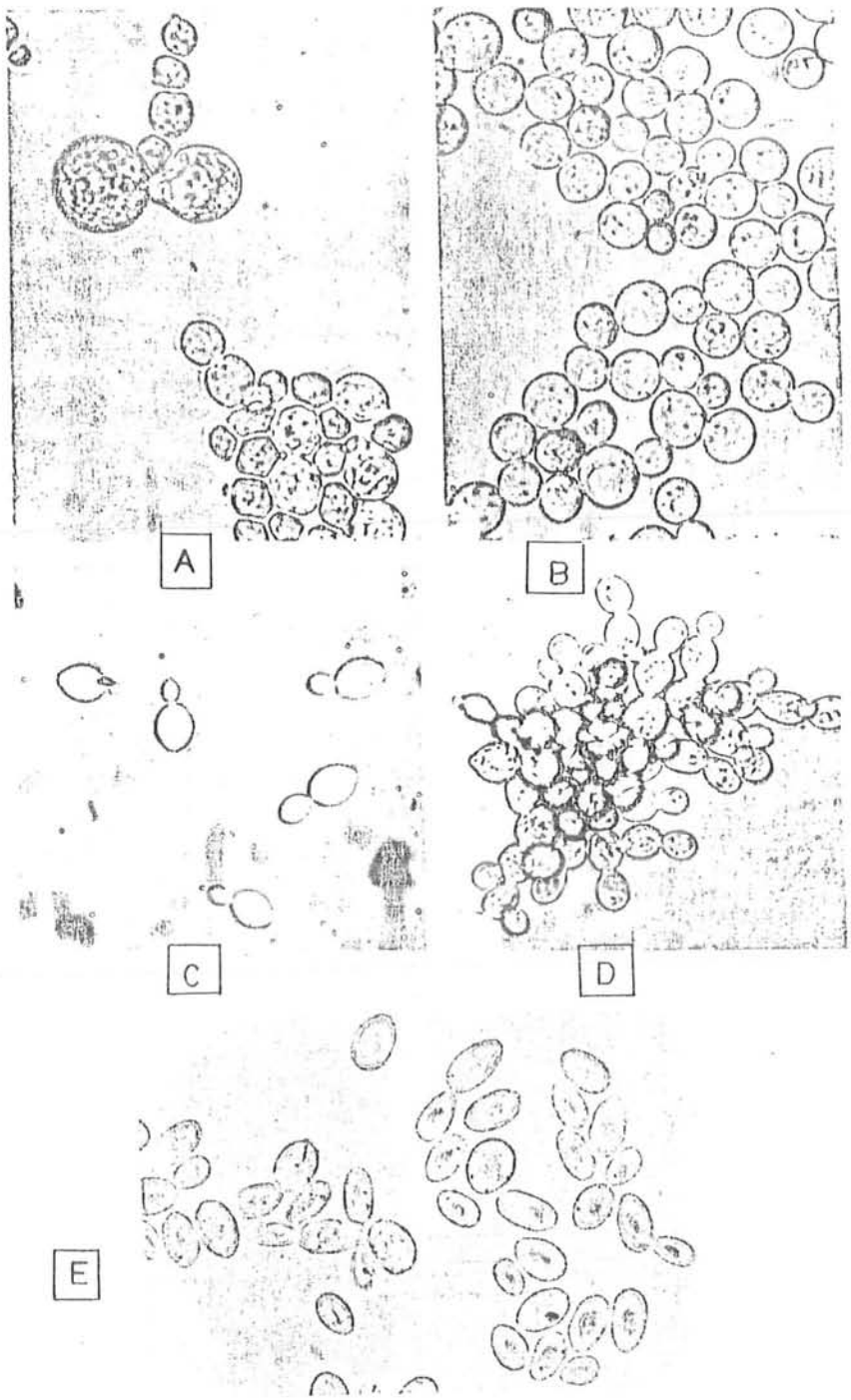
Chain of yeast cells.



A cell of Saccharomyces cerevisiae under high power microscope.

N = nucleus, M = mitochondria,
ER = endoplasmic reticulum, B = bud.

Fig. J



Reproduction Pattern of Saccharomyces cerevisiae.

A: Large cells in a culture of Saccharomyces cerevisiae.

B: Variation in the size of the cells in a culture of Saccharomyces cerevisiae.

C: Cells from a strain of Saccharomyces cerevisiae in which the daughter cells separate from the mother cells at maturity.

D: Cells from a strain of Saccharomyces cerevisiae in which the daughter cells do not separate from the mother cells.

E: Diploid cells.

Fig. K

Yeast preparation

Several ways are widely employed for the maintenance of pure cultures[41-44].

(a) A pure culture of yeast strain is maintained on a suitable medium, usually malt extract or glucose yeast extract agar. New stock cultures on agar slants are prepared every 30 to 60 days. Subcultures are incubated statically at 27°C for 3 days cultures are stored at 4°C for 6 months prior to resubculating.

(b) Agar-solidified MYPG medium

Loop-inoculated from a previous slant culture and incubated for 3 days at 28°C and then kept at 2°-4°C. They can be transferred to fresh slants every 3 months[45].

Yeast propagation and handling

When the laboratory culture (selected strain of S. cerevisiae) has fully developed, it is transferred to the vessel (100 ml) of sterile wort. After 2 days incubation at 26°C the cultures are microscopically examined. The vessel in which grwoth and normal cell development has taken place is transferred to large volume of sterile wort used for further propogation again incubation is carried out for 2 days at 25°C. These are then used for

inoculation. If not needed may be kept at $4^{o}C$ to $2^{o}C$ for upto 3 weeks as a stock culture, and ready to be used for another inoculation[46-50].

1.1.7 Starches

Starches are found as reserve materials in plants and occur as granules characteristic. The major portion of the grains is composed of starch. Complete hydrolysis of starch with strong acids gives glucose. Partial acid hydrolysis gives a complex mixture of dextrins, maltose, and glucose. Enzymatic hydrolysis with amylase gives maltose. About 20% of the starch granule is composed of amylose, which is soluble in water. The remainder is insoluble amylopectin which absorbs water and swells to form starch pastes[51].

Amylose

Amylose is a straight-chain polymer composed of glucose units bound by α-1,4-linkages. The compound gives a blue color with I_2 and is completely hydrolyzed by β-amylase to maltose[52] (Fig. D, E).

Amylopectin

Amylopectin has a molecular weight of 200,000 to

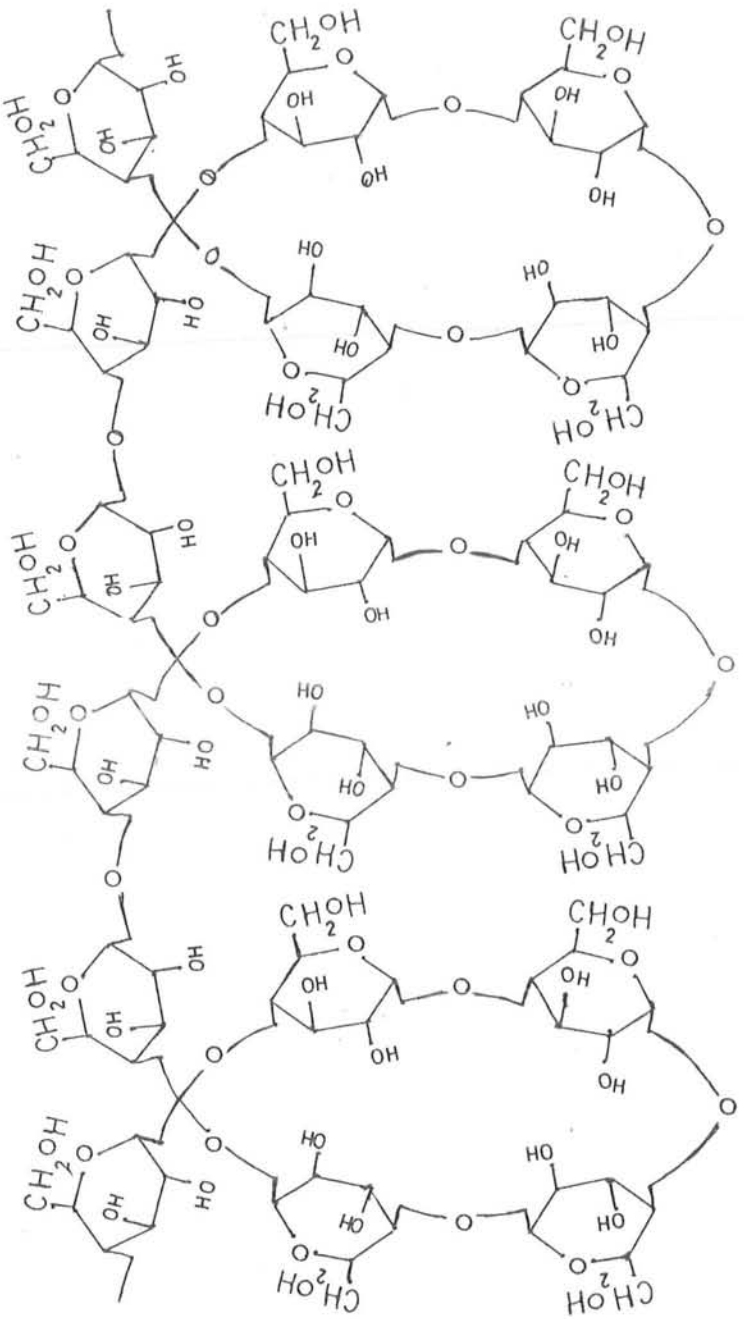


Diagram of Helical coil of glucose units that is formed when amylose is suspended in water. Iodine occupies a position in the interior of helical coil of glucose units

Fig.D

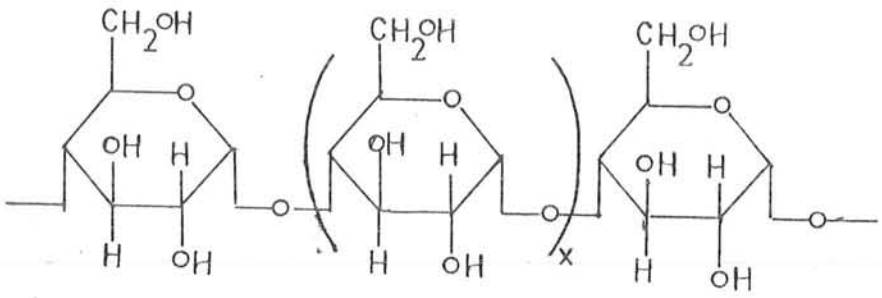


Diagram of amylose structure. X =100 to 400

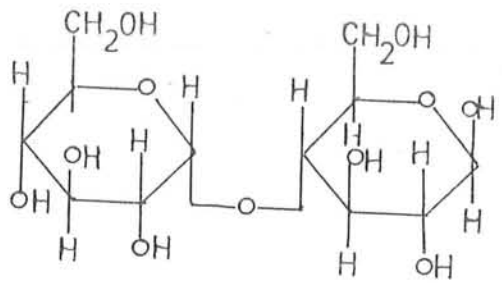


Diagram of maltose structure.

Fig.E

1,000,000 (Fig.F). It gives a red-violet colour with I_2 solution and is enzymatically hydrolyzed with β-amylase to give about a 60 per cent yield of maltose. A combination of β-amylase which catalyzes the splitting of α-1,4-linkages and α-amylase which catalyzes the splitting of α-1,6-linkages gives much higher yield of maltose[53]. On the basis of these enzymatic studies and chemical evidence the structure of amylopectin has been found to be that of a branched polymer containing α-1,4 and α-1,6-linkages.

1.1.8 By-products

The chief by-products are carbon dioxide, yeast and the spent grains from the mash. For more yeast is produced during fermentation than is needed for subsequent brews and it has a high nutritive value, containing 50% protein and 2% fat by dry weight, a good content of the B vitamins and minerals such as calcium and iron, although small quantities of other substances, higher alcohols (D-amyl alcohol, isoamyl alcohol), succinic acid, glycerine, etc, are also produced. The brewers spent grains are used for animal feeding since they contain about 20% protein and 8% fat by dry weight. The spent hops are dried and sold as hop manure.

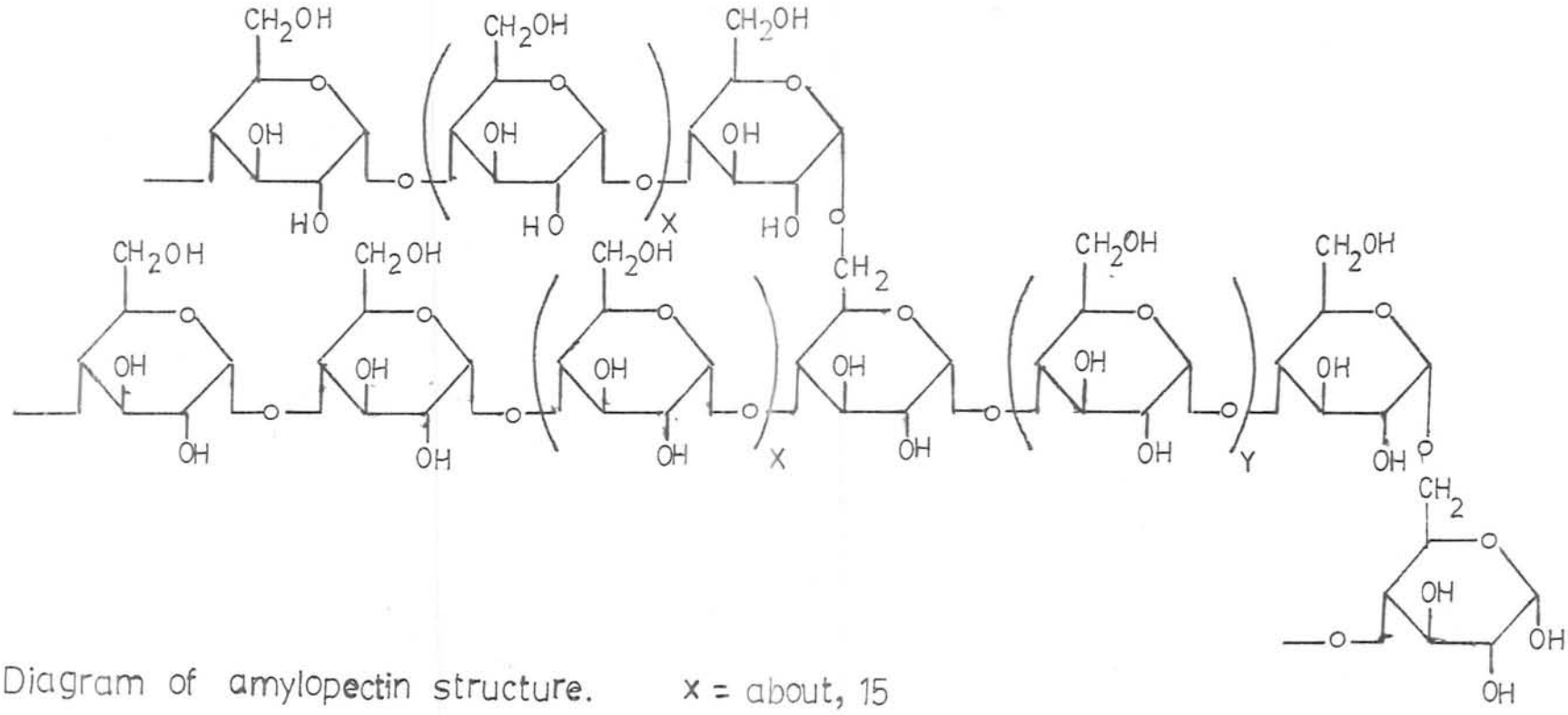


Diagram of amylopectin structure. x = about, 15
y = about 8.

Fig. F

1.1.9 Significance

The fermentations are of great economic importance in industries such as manufacture of certain beverages, bakery products, dairy products, souerkraut, pickles and other foods, frequently involve microbiological procedures. The best-known chemical product obtained industrially by fermentation, and the one of the largest volume, is ethyl alcohol, but other fermentation chemicals, including n-butyl alcohol, acetone, lactic acid, citric acid, sorbose, gluconic acid, itaconic acid, glycerol, 2,3-butanediol, riboflavin and other vitamins, pencillin and other antibiotics, various enzymes, and other substances, are or have been produced on commercial or pilot plant scale. Still other processes for producing chemicals by fermentation are continually under investigation in laboratory and pilot plant.

1.1.10 High Power Fuel

Fuel is a material whose combustion is used to supply heat for any purpose. Fuels are characterized by their physical form at normal temperatures, whether they are solids, liquids, or gases, by their heating value, that is, by the amount of heat given off when a unit weight or volume of the material is burned under

standard conditions, and by their combustion characteristics, which cover such points as ease of ignition, rate of combustion, flame temperature and flame puminusity[62].

Fuel having maximum purity (i.e., 99%) and allows higher combustion characteristics, maximum heat prodution is termed as high power fuel and most suitable for utilization as pure fuel[60].

(a) Power alcohol

Ethanol-water mixture possessing high percentage of water has no significance value for burning purpose. The most energy-intensive step involves the recovery of 95% alcohol from fermented mash (ethanol; 4-5%) and subsequently dehydration through azeotropic distillation to anhydrous product[64].

The fermented mesh (ethanol-water mixture) is distilled to increase the strength of alcohol component by virtue of the composition of the vapors being stronger in the more volatile constituent than liquid from which these vapors arise and obtain ethanol upto 95 by conventional distillation (fractional distillation).

The ethanol 95% is of above 190-proof. It is further distilled with quick lime to obtain 99.5% alcohol called absolute alcohol. Hundred per cent alcohol (power ethanol) is obtained by treatment with magnesium turning and subsequent redistillation[63].

Power alcohol has the composition carbon, 52.2; Hydrocarbon, 13.1; oxygen, 34.7; octone number, 0.5 and with a calorific value of 5048 Kcal/litre[60,61]. When ignited burns in air with a pale blue, transparent flame producing water and carbon dioxide[62].

(b) Gellified fuel

Pure alcohol (power alcohol; 99.95%) produced is converted into a liquid system of high viscosity that contains structures that are derivable from colloidal particles (salts of carbohydrate derivatives), which exhibits elastic properties in resisting deformation and it frequently possesses a high degree of order (gel)[54,57] The said composition may be used in a proper way for bruning purposes (burns with pale blue, transparent flame), without sacrificing any of the advantages inherent to the physical condition of a solid body and which chiefly reside in the reduction of shipping, transport, packing and storage costs[58,59].

The feature possessed by gel which is responsible for their distinctive properties is a three-dimensional network. This network may be temporary by virtue of secondary bonds, as in case with gelatin[57]. Gels are frequently described as concentrated solutions of a liquid in a solid. The presence of macro-molecules is essential for the formation of network. The rigidity and strength of the network structure endows the gel with its ability to undergo elastic deformation and to imbibe large quantities of liquid[55,56].

1.2 PROTEINS

Proteins are compounds of higher molecular weight from a few thousand to a few million, which vary in appearence from white amorphous powders to brilliantly coloured crystals[65,69]. They have indeterminate melting points and are subject to denaturation by fluctuations of temperature and pH. The average composition of protein is C, 50; H, 7; O, 23; N, 16; S, 0-3; P, 0-3%. Elements such as Fe, Cu, Mn, Zn are found in specific proteins[66,67].

The complete hydrolysis of proteins yields a mixture of about 20 amino acids[67], with two exceptions (proline and hydroxyproline, are α-imino acids). These substances have a primary amino function and a carboxyl function joined to the same carbon atom; hence they are termed α-amino acids and are derivatives of the general structure[68-72] NH_2-CHR-CooH. The amino acids are classified into seven groups[73-75].

Fibrous Proteins

These proteins are associated with cellular elements and serve function of supporting specific structure of cell. Examples are wool, silk fibroin, collagen (connec-

tive tissue), myosin (muscle), keratin (hair) and fibrin (blood clot).

Globular Proteins

These consist of polypeptides (chains of amino acid residues) that are held together by cross-linked groups or in an aggregated state.

Glutelins

These are insoluble in neutral aqueous solutions but soluble in dilute acid or alkali. Examples are glutenin (wheat) and oryzenin (rice).

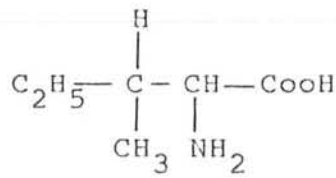
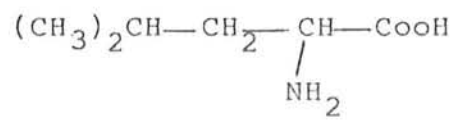
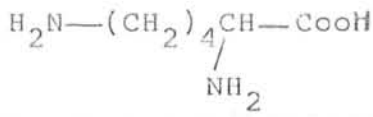
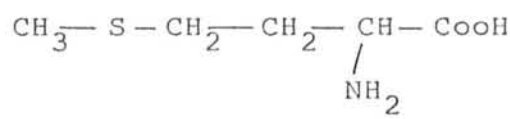
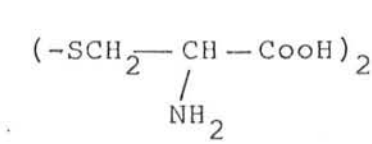
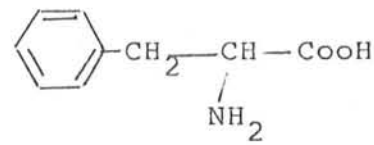
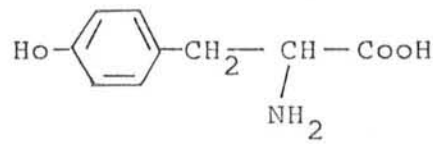
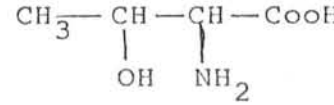
Gliadins (Prolamins)

These are soluble in 70 to 80% ethanol and insoluble in water or absolute ethanol. Examples are gliadin (wheat), hordein (barley), and Zein (corn). The proteins of barley, wheat, rye, rice, oats and corn contain the same types of amino acid (Table 2.1) but in different proportions and combinations[88]. Fig. G

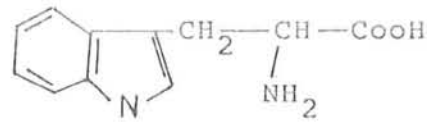
Albumins (egg, serum) are water soluble proteins, Globulins are insoluble in salt free water and are soluble in dilute salt solution. Histones, protamines are also water soluble proteins[65-69].

TABLE 2.1

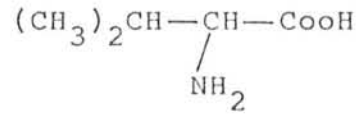
AMINO ACIDS ENCOUNTERED IN CEREAL PROTEINS

Amino acid	Abbreviation	Structure
1- Isoleucine	Ile	
2- Leucine	Leu	
3- Lysine	Lys	
4- Methionine	Met	
5- Cysteine	Cys Cys	
6- Phenylalanine	Phe	
7- Tyrosine	Tyr	
8- Threonine	Thr	

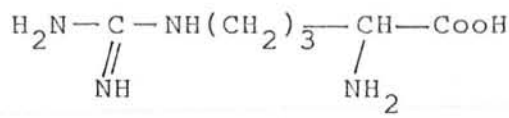
9- Tryptophan Trp



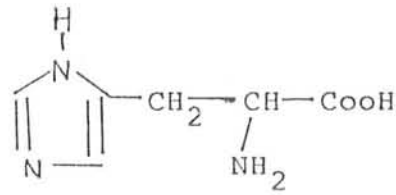
10- Valine Val



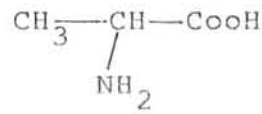
11- Argenine Arg



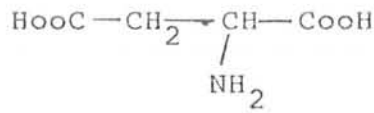
12- Histidine His



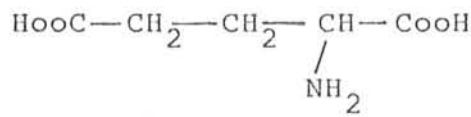
13- Alanine Ala



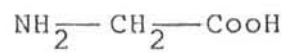
14- Aspartic acid Asp



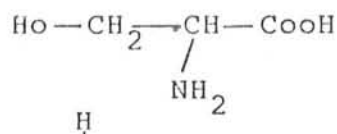
15- Glutamic acid Glu



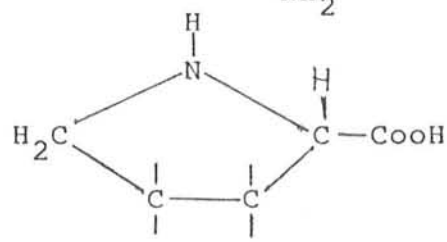
16- Glycine Gly



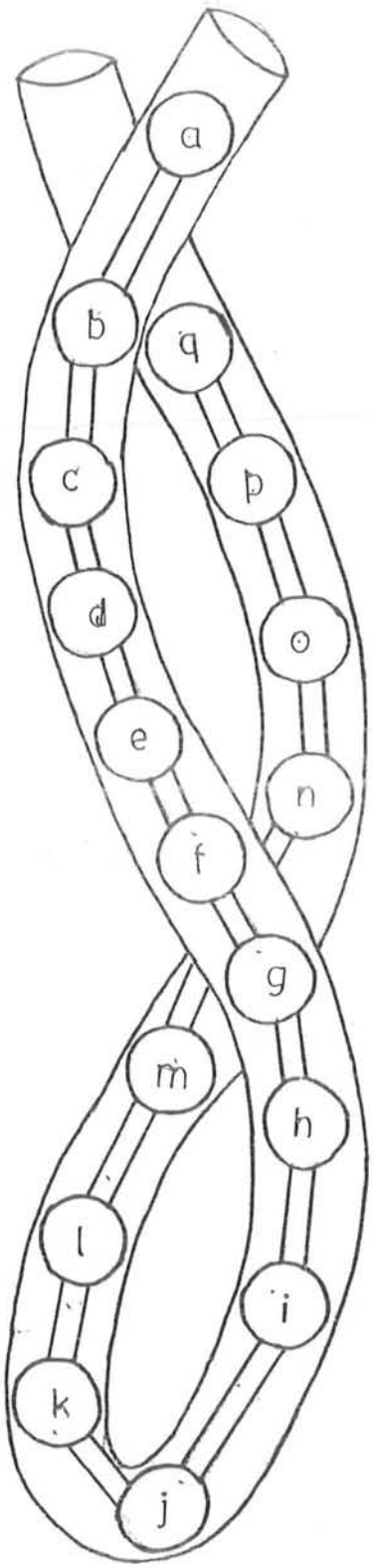
17- Serine Ser



18- Proline Pro



(a) Ile
(b) Leu
(c) Lys
(d) Met
(e) Phe
(f) Cys — cys
(g) Thr
(h) Trp
(i) Val
(j) Arg
(k) His
(l) Ala
(m) Asp
(n) Glu
(o) Gly
(p) Ser
(q) Pro
Tyr



Polypeptide chain of cereal protein (each circle represents one amino acid)

Fig. G

1.2.1 Chemistry of Proteins in the Fermentation Process

(a) Role of Proteases

The protein in the fermented material is derived from malt and usually provides some indication of the amount of malt that was used in the original brew. The proteases are proteolytic enzymes, they break down the large protein molecules into smaller sub-units. The malt proteases of the mash are a series of enzymes each acting on a specific protein substrate. Much of the malt protein is permanently insoluble, only about 40% of the total malt protein is solubilized in the mash and thus available for the reaction[81].

(b) Proteolytic Activity

Quantitatively, the ratio of proteolytic activity during malting and during mashing is about one to one.

During mashing three kinds of proteolytic activity take place under an optimum temperature range 50-60^{o}C and optimum pH range of 4.2-5.3.

(i) Solubilization of Insoluble Proteins

A small portion of insoluble proteins is reversibly soluble and is ultimately coagulated by heat and precipitated during the boiling of the wort in the vessel. The remainder of this fraction is permanently soluble.

(ii) Breakdown of Souble Protein

The permanently soluble protein (left after precipitation of protein during the boiling of wort) along with the previously soluble high molecular weight, high complex protein molecules are enzymatically broken down into simpler colloidal molecules of albumoses, peptones and polypeptides. These substances gives flavour, tactile impression (mouth feel), to fermented syrup.

(iii) Peptone Group Degradation

Further degradation of peptone group is accomplished mainly by the enzyme peptidase. This process occurs in an optimum temperature range of 45.5^{o}C and pH range of 4.2 to 5.3. The medium size peptones and polypeptides are broken down into simpler protein residues, i.e. peptides and amino acids. These comprise the yeast

assimilable nitrogen fraction of the wort, along with ammonia and, as such are extremely important to the welfare of the yeast and the quality of the finished product[80-82].

1.2.2 Recovery of Insoluble and Soluble Proteins

(a) Insoluble Protein

The insoluble protein can be recovered from fermentable material (barley, wheat, rye, oat, corn) during fermentation process on two stages:

(i) During mashing a small portion of insoluble protein is reversibly soluble and is ultimately coagulated by heat and precipitated during the boiling of wort.

(ii) The residue (protein from malt, fibrous material, yeast) which is recovered after fermentation contains 50% protein on dry bases. It is recovered first by dissolving in suitable solvent (70% ethanol) and filtering off undissolved fibrous and residual material. The filterate is diluted with excess of distilled water until precipitate appear. After filteration the precipitation is dried in oven.

(b) Soluble Protein

During fermentation the permanently soluble protein (in the form of haze) further broken down into simpler molecules. Coagulation of these molecules is done first by cooling the fermented liquid and then centrifugation. The recovered protein is dried in oven at 35 $\pm$ 1^{o}C. Further purification of protein is done in water and ethanol (70%).

1.2.3 Purification of Proteins

The purification of proteins from natural sources is usually a formidable task. Many of the usual methods of purification of organic materials, such as distillation and solvent extraction, are not suitable for the purification of proteins owing to their size and instability. Proteins are fragile molecule. In general, exposure to even moderate temperature, for example 37^{o}C elicits their slow denaturation. Consequently, most protein purification procedures should be carried out near the freezing point of the solvent in use. On the other hand, if the protein of interest happens to be unusually stable to heat, a brief heat treatment may serve to precipitate the bulk of the contaminating material, thus effecting a substantial purification[69,72,73,79].

Many fractionation methods are available for the purification of proteins.

(a) Salt Precipitation

Depend upon the proteins, which are very markedly in their solubilities in concentrated salt solutions. Consequently, purification may be achieved by addition, for example, of sufficient salt to precipitate some of the contaminating proteins, which may then be collected by centrifugation and discarded, a further addition precipitating the protein of interest but not all the remaining contaminating proteins. The protein of interest together with other proteins that may have precipitated over the same concentration range, may then be collected. The salts most frequently employed for this purpose are ammonium sulfate and sodium sulfate; the former is usually preferable.

(b) Isoelectric Precipitation

Most proteins have a minimum solubility near their isoelectric point, suitable adjustment of the pH may result in the precipitation of the protein of interest, most of the contaminating proteins remaining in the solution.

(c) Precipitation with Organic Solvents

A variant of methods involves the use of organic solvents, particularly acetone, methanol, and ethanol, in attempts to precipitate certain proteins selectively from a mixture. These solvents often easily denature proteins, and the use of very low temperatures is advisable.

(d) Ion-Exchange Chromatography

Proteins, like amino acid, may frequently be purified by column chromatography on ion-exchange resins. Resins with hydrophilic backbones, usually cellulose, are the most satisfactory for this purpose. Two commonly used column materials for protein purification include DEAE-cellulose, an anion-exchange resin formed by linking diethyl-aminoethyl functions to a cellulose backbone, and CM-cellulose, a cation exchange resin formed by linking carboxymethyl functions to a cellulose backbone. Such columns are developed by washing with buffers of increasing ionic strength or increasing or decreasing values of pH, as appropriate.

(e) Adsorption Chromatography

The selective adsorption of proteins onto certain materials and the selective elution of proteins following

adsorption may often be used as a purification procedure. This chromatography may be performed either on a column or in slurries of the adsorbant. Frequently employed materials for the adsorption chromatography of proteins include calcium phosphate gel, alumina gel, diatomaceous earth (celite), starch and hydroxylapatite.

(f) Electrophoresis

Electrophoresis of protein mixtures by zone method employing a high capacity support, usually starch blocks, may effect excellent separations and permits near quantitative recovery of the protein of interest. Thus, zone electrophoresis is a good purification procedure.

(g) Molecular Sieves

The use of molecular sieves, which sort out molecules according to size, may be adopted to protein purification. Diethyl aminoethyl and carboxymethyl function have been linked to sephadex, so that a single run through a column may fractionate both on the basis of size and charge.

(h) Crystallization

Conditions for the crystallization of a given

protein must be of careful control of pH, ionic strength protein concentration, and temperature is essential.

(i) Lyophilization (Freeze Drying)

Freeze drying is an important method of concentrating protein solutions or reducing them to dryness. In this procedure, the protein solution is frozen in an acetone-dry ice bath and connected to a vacuum line. The rate of cooling due to evaporation of solvent from the frozen solution is sufficient to maintain the solution in the frozen state throughout the course of the concentration. Most proteins are not denatured by this procedure and may be stored in the dry state, in the cold, for long periods of time without deterioration of the sample. Frozen solutions are, in general, less stable. Owing to the low surface tension of protein solutions, they may not be concentrated by evaporation under vacuum in the liquid state. Pumping on a protein solution results in extensive foaming, so that most of the protein ends up in the pump oil; furthermore, proteins are denatured on surfaces, and hence foaming must be avoided.

1.3 CARBON DIOXIDE

Corbon dioxide is obtained in large quantities as a by product during the alcoholic fermentation. For each molecule of glucose fermented, two molecules of ethyl alcohol and two molecules of carbon dioxide are produced. The chemical reaction is exothermic and heat is liberated.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + \text{Heat}$$

From every 100 g of glucose fermented approximately 46 g of alcohol and 44 g of carbon dioxide are produced[83-84].

1.3.1 Recovery of Carbon Dioxide

Carbon dioxide is collected from closed fermentors after a vigorous fermentation has set in and the dissolved gases and air have been purged from the fermentor. The gas at this stage while relatively pure contains water vapor and traces of ethyl alcohol and other organic chemicals including aldehydes, esters, acetates and ketones. These are responsible for an odor and impurities, must be removed prior to utilization of CO_2 to carbonate beverages or to be processed into

solid by compression. Two general purification methods are used. The Backus process[85,86] and the Reich process[87].

The Backus process depends primarily on a direct adsorption of the impurities, while in the Reich process the impurities are first oxidized and then removed by adsorption or absorption.

Carbon dioxide is purified as following:

(a) The gas is washed in a water serubber which removes entrained solids and all but traces of aldehydes and alcohols. Some 0.5 to 1% of the total alcohol produced in the fermentation is recovered by distillation of the carbon dioxide wash water.

(b) Water vapour is removed from gas by an acid scrubber containing sulfuric acid and this is followed by (3) passage through an activated-carbon for removal of odors.

Gaseous carbon dioxide can be compressed to form liquid carbon dioxide in two- or three-stage compressors, depending on the refrigerant used. By suitably rapid evaporation of liquid carbon dioxide, the solid is

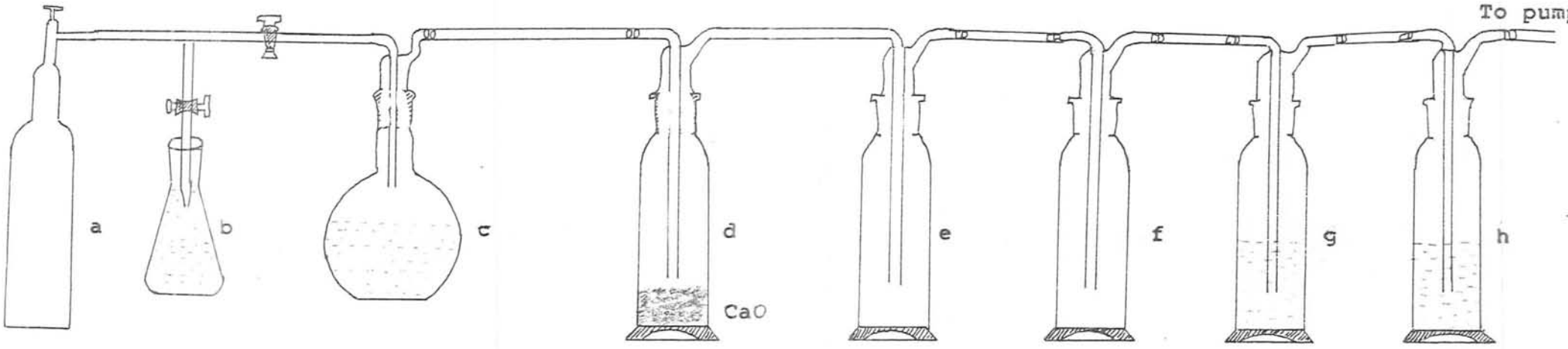


Fig H Schematic diagram for the determination of carbon dioxide (a) cylinder (Helium gas), (b) water flask, (c) fermented vessel (d) gas washing bottle, (e), (f), (g) gas absorption bottle ($Ba(OH)_2$ solution), (h) test bottle ($Ca(oH)_2$).

formed as a snow which may be compressed into cakes for use as 'dry ice'.

1.3.2 Determination of carbon dioxide

The carbon dioxide to be determined from fermentation process can be absorbed in known concentration of potassium hydroxide solution. The oxygen and nitrogen coming along with carbon dioxide is not determined because of minor importance. The solution of potassium hydroxide used for absorbing carbon dioxide had the concentration recommended by Clemens[160], namely, one part potassium hydroxide to four parts of water. The absorption of carbon dioxide in (1:1) potassium hydroxide by Engelder method[161-163], by replacement of petroleum using an apparatus[166], by using Hg manometer[164,165] and the method devised by Cain and Maxwell by absorption in $Ba(oH)_2$ solution[167] has been reported. The form of apparatus used for carbon dioxide determination is shown in Fig.H

1.3.3 Uses of carbon dioxide

Solid carbon dioxide can be used primarily as a

refrigerant for cooling and shrinking machine parts and for reliquefaction after transport to make liquid carbon dioxide. Liquid carbon dioxide can be used extensively for carbonating beverages, in fire extinguishers, in food preservatives, in refractories, and in the chemical industry.

1.4 RECOVERY OF NON-ALCOHOLIC DRINK

The fermented liquid from starch containing material (wheat, barley, rye, oats and corn) consists of nearly 92.1% water, 3.4 to 3.6% alcohol by weight (4.3 to 4.8% alcohol by volume), calcium, iron, sodium, potassium, vitamin A, thiamine, riboflavin, niacin and ascarbic acid (vitamin C)[158,159]. The ethanol is removed by distillation and non-alcoholic syrup can be treated with flavour, and preservatives. It may be preserved for human consumption.

1.5 SENSIBLE SOURCES

The sensible sources consist of sugar bearing materials, starches and sucrose. Barley, wheat, rice, rye, oats and maize contain starches, while sucrose (non crystalline sugar) is found in blackstrap molasses (a by-product from cane). The production data of sensible sources in Pakistan[99] are presented in Tables (5.1-5.5).

1.5.1 Barley (Hordeum vulgare)

Barley apparently originated by domestication of wild two-rowed from, H. vulgare spontaneum, in or near the area bordering Syria and Iraq with Iran and Turkey. Six-row barley was being cultivated by about 6000 B.C. Barley culture spread to India, Europe and North Africa, during the Stone Age.

Barley belongs to the grass tribe Hordeae, in which the spikes have a zig zag rachis. The vegetative portion of the barley plant is similar to that of the other cereal grasses except that the auricles on the leaf are conspicuous. The grains are about 8 to 12 millimeters long, 3 to 4 millimeters wide, and 2 to 3 millimeters thick. Five colour conditions are recognized in the

barley grain[89]: white, black, red, purple and blue.

About 85% of the malt is used in making beer, 10% for making industrial alcohol and whisky and the remainder for malt sirups in the world. Barley requires grinding or rolling for satisfactory feeding to animals other than sheep.

1.5.2 Wheat (Triticum aestivum)

The origin of cultivated wheat is still speculative. Wheat was cultivated throughout Europe in Prehistoric times, and was one of the most valuable cereals of ancient Persia, Greece, and Egypt. Archaeologists have come upon carbonized grains of wheat in Pakistan and Turkey, the tombs of Egypt, and in storage vessels found in many other countries.

Wheat is an annual or winter-annual grass with a spikelet inflorescence which consists of a sessile spikelet placed at each notch of the zig zag rachis. It is classified in the grass tribe Hordeae and in the genus Triticum. This genus is characterized by two- to five flowered spikelats placed flat at each rachis point of the spike. The average spike (head) of common

wheat contains 25 to 30 grains in 14 to 17 spikelets.

Wheat is a more efficient feed than other grains in weight grain per feed unit. The chief food use of wheat is in the form of flour for baked products other food uses include prepared breakfast foods, bread flour, cake, bread, cracker, pastry and family flours[90].

1.5.3 Rice (Oryza sativa)

Rice, probably originated in India or Southeastern Asia, where several wild species are found, possibly evolving from a wild form, which no longer exists. Rice culture is believed to have spread into China by 3000 B.C. and westward into Europe by 700 B.C. The first commercial rice planting in the United States was at Charleston, S.C., about 1685.

It belongs to the grass tribe Oryzeae characterized by one flowered spikelets, laterally compressed, and two short glumes. It is an annual grass with erect culms 60 to 180 cm tall. The rice grain is enclosed by the lemma and Palea (called hull). The hulled kernels vary from 3.5 to 8 millimeters in length, 1.7 to 3 millimeters in breadth, and about 1.3 to 2.3 millimeters in

thickness . The colour of unmilled kernel may be white, brown, amber red, or purple. An average rice panicle contains 100 to 150 seeds[91].

Rice is used as food in many countries. The raw rice is used, for satisfactory feeding to animals.

1.5.4 Oat (Avena sativa)

Cultivated oats formerly were believed to have been derived chiefly from two species, the common wild oat (A. fatua) and wild red oat (A. sterilis)[92]. Apparently cultivated oats were unknown to the ancient Egyptians, Chinese, Hebrews, and Hindus. The common oat, first found growing in Western Europe, spread to other parts of the world It was believed to have first cultivated by the ancient Slavonic peoples who inhabited this region during the Iron and Bronze Ages.

The oat plant is an annual grass classified in the genus Avena. Under average conditions the plant produces three to five hallow culms from 1/8 to 1/4 inch in diameter and from 2 to 5 feet in height. The average leaves are about 10 inches long and 5/8 inch wide. Oats are harvested in summer and can be safely

stored when their moisture content is less than 14 per cent. The oat caryopsis is narrowly oblong or spindle-shaped, deeply furrowed, and usually covered with fine hairs, especially at the upper end.

Oats often fed whole to horses and sheep. Ground or chopped oats are fed extensively to dairy cattle, breeding stock, and young stock. Oats are fed extensively to poultry.. Oats flour is used in breakfast food and other foods. Oat product is used as an anti-oxidant and stabilizer in ice cream and other dairy products[93].

1.5.5 Rye (Secale cereale)

Rye was unknown to the ancient Egyptians and Greeks, but is supposed to have come into cultivation in Asia Minor more than 4,000 years ago[94].

Secale cereale is an annual or winter annual grass classified in the tribe Hordeae to which wheat and barley also belong[95]. The stems of rye are larger and larger than those of wheat. The leaves of the two plants are similar except that those of rye are coarser and more bluish in colour.

Rye are used for making whisky and alcohol and also for food. Rye usually is mixed with 25 to 50% wheat flour for bread making. Distillers prefer a plump light-coloured grain when using rye for whisky. Rye straw formerly was used for packing nursery stock, crockery, and other materials and for stuffing horse collars.

1.5.6 Corn (Maize; Zea mays)

Maize is perhaps the most completely demesticated of all field crops. It cannot exist as a wild plant. Corn was seen by Columbus in Cuba on November 5, 1492, on his first voyage to America. The most likely center of origin of corn is Mexico or Central America, with a possible secondary origin in South America.

Maize is a coarse annual grass, classified in the tribe May deae, which has monoecious (separate staminate and pistillate) inflorescences[96].

Corn is used to feed hogs, cattle, poultry, horses, and sheep in that order. It is also used for feed, alcohol, breakfast foods and flour.

1.5.7 Blackstrap Molasses

Blackstrap molasses (Saccharine Materials) used in ethyl alcohol fermentation, is a by-product of the sugar industry, obtained after the sucrose has been crystallized and centrifuged from defecated, evaporated cane juice. The process of evaporation and crystallization is usually repeated three times until the invert sugar, non-sugar organic constituents and high viscosity of the molasses will permit no further crystallization of the sucrose. The residue is known as final or blackstrap molasses. Blackstrap molasses is, therefore, a rather crude complex mixture, containing sucrose, invert sugar, salts, and all of the alkali-soluble non-sugar ingredients normally present in the defecated cane juice, as well as those formed during the process of sugar manufacture[97].

In addition to sucrose, glucose, fructose and raffinose, which are fermentable, molasses also contains reducing substances not fermentable by yeast. These copper-reducing, nonfermentable compounds are mainly caramels, free from nitrogen, produced by the heating necessary during sugar manufacture and melanoidins, containing nitrogen and derived from condensation products of sugars and amino compounds[98].

TABLE 5.1

PRODUCTION OF BARLEY IN PAKISTAN ('000' TONNES)

Year	Punjab	Sind	N.W.F.P.	BALUCHISTAN	TOTAL
1975-76	68.8	8.8	48.3	4.2	130.1
1976-77	64.5	10.1	47.4	1.6	123.6
1977-78	55.4	11.2	51.0	3.0	120.6
1978-79	65.1	10.8	49.7	3.7	129.3
1979-80	59.8	9.7	43.6	5.0	118.1
1980-81	112.5	7.0	52.2	3.8	175.5
1981-82	85.7	9.9	56.9	5.0	157.5
1982-83	90.0	10.9	62.8	21.6	185.3
1983-84	55.0	13.7	62.1	8.7	139.5
1984-85	45.7	13.0	62.2	10.7	131.6
1985-86	49.8	13.0	60.1	10.8	133.7
1986-87	43.9	12.6	64.3	13.4	134.2
1987-88	35.1	12.9	52.0	11.8	111.8
1988-89	39.7	12.9	52.1	17.8	122.5

TABLE 5.2

PRODUCTION OF WHEAT IN PAKISTAN ('000' TONNES)

Year	Punjab	Sind	N.W.F.P.	BALUCHISTAN	TOTAL
1975-76	6571.6	1320.9	660.4	137.8	8690.7
1976-77	6807.7	1478.6	711.6	146.0	9143.9
1977-78	6090.2	1427.0	688.6	161.4	8367.2
1978-79	7323.6	1680.1	737.5	208.8	9950.0
1979-80	7913.5	1849.4	862.5	231.1	10856.5
1980-81	8350.0	1945.8	940.8	238.0	11474.6
1981-82	7962.1	2061.7	962.2	318.2	11304.2
1982-83	8935.1	2066.7	998.4	414.2	12414.4
1983-84	7622.8	1945.8	859.8	453.5	10881.9
1984-85	8315.1	2078.7	872.1	437.1	11703.0
1985-86	10431.6	2172.2	906.5	412.7	13923.0
1986-87	8334.3	2211.5	959.4	510.7	12015.9
1987-88	9203.8	2180.4	899.2	391.7	12675.1
1988-89	10517.0	2360.6	1003.7	537.9	14419.2

TABLE 5.3

PRODUCTION OF RICE IN PAKISTAN ('000' TONNES)

Year	Punjab	Sind	N.W.F.P.	BALUCHISTAN	TOTAL
1975-76	1207.2	1286.1	84.6	39.6	2617.5
1976-77	1332.0	1292.0	85.4	28.0	2737.4
1977-78	1507.8	1315.3	87.6	38.9	2949.6
1978-79	1765.9	1340.9	104.0	61.2	3272.0
1979-80	1518.4	1499.1	104.7	93.6	3215.8
1980-81	1361.7	1549.9	105.1	106.5	3123.2
1981-82	1450.9	1584.2	110.7	283.9	3429.7
1982-83	1407.0	1560.1	112.7	364.9	3444.7
1983-84	1409.4	1478.8	115.8	335.5	3339.5
1984-85	1534.9	1345.0	115.5	319.8	3315.2
1985-86	1478.2	1071.7	113.8	255.2	2918.9
1986-87	1534.8	1548.5	118.3	284.7	3486.3
1987-88	1352.3	1537.5	107.5	243.6	3240.9
1988-89	1367.3	1439.9	117.8	279.2	3200.0

TABLE 5.4

PRODUCTION OF MAIZE IN PAKISTAN ('000' TONNES)

Year	Punjab	Sind	N.W.F.P.	BALUCHISTAN	TOTAL
1975-76	386.3	12.8	401.5	1.9	802.5
1976-77	361.7	13.2	386.1	2.8	763.8
1977-78	391.7	12.2	414.5	2.5	820.9
1978-79	384.5	12.5	398.9	2.7	798.6
1979-80	420.6	13.6	438.4	2.6	875.2
1980-81	444.7	12.3	510.9	2.5	970.4
1981-82	405.3	10.9	512.2	2.0	930.4
1982-83	450.1	9.8	542.9	2.6	1005.4
1983-84	450.0	9.8	550.9	2.8	1013.5
1984-85	460.0	10.4	554.0	3.2	1027.6
1985-86	415.0	10.1	580.6	3.7	1009.4
1986-87	453.3	10.4	643.5	4.0	1111.2
1987-88	404.8	8.7	709.3	4.1	1126.9
1988-89	455.3	9.7	735.1	4.0	1204.1

TABLE 5.5

PRODUCTION OF MOLASSES IN PAKISTAN ('000' TONNES)

Year	Cane crushed	Molasses produced
1982-83	404415.965	14560.0
1983-84	330998.480	14110.0
1984-85	339491.805	11828.0
1985-86	141702.197	5226,0
1986-87	81209.040	2769.4
1987-88	480995.683	18107.4
1988-89	522214.791	18700.16
1989-90	263578.982	9056.0

CHAPTER 2

TECHNIQUES

ANALYTICAL TECHNIQUES

2.1 Conventional Techniques

2.1.1 Moisture (% dry matter)

The moisture content of a sample can be determined by drying the sample in an oven at 105^{o}C. The loss in weight after drying is moisture.

However, this method does not apply to those samples with high volatile compounds. There are several methods to determine the moisture content of samples in this category; drying in vacuum ovens, freeze drying and distillation with toluene by using dean and stark receiver.

2.1.2 Crude protein

The nitrogen of protein and other compounds are transformed into ammonium sulphate by sulfuric acid digestion. The digest is cooled, diluted with water and alkalified with sodium hydroxide. The released ammonia is distilled into a boric acid solution, or a known volume (always in excess) of standardized acid. If the

distillate is in boric acid, then it is titrated with standardized acid to quantify the ammonia evolved. For the latter case, a standardized alkali is used to back titrate the excess acid so that a quantity of acid neutralized by the ammonia can be found. That is equal to the quantity of ammonia evolved. The following analytical method is a combination of macro Kjeldahl digestion with semi-micro distillation by using Markam still apparatus.

2.1.3 Ether extract (fat)

Diethyl ether or petroleum ether is continuously evaporated, then condensed and it passes through sample in thimble placed in extractor. When the extract in the extractor reaches its maximum height it is siphoned into a receiving flask. Then the solvent is again evaporated and passes through the sample for second extraction. This is a continuous cyclic process until all the ether soluble materials have been extracted.

2.1.4 Crude fibre

Crude fibre is defined as an organic residue that

remains after being digested first with a weak acid solution, and then a weak alkaline solution. Therefore, a moisture free and ether-extracted sample is first digested with a dilute H_2So_4 and then a dilute NaoH solution. The residue collected after digestion is ignited. The loss in weight on ignition is registered as crude fibre.

2.1.5 Ash

The sample is ignited at 600°C to burn off all organic matters. The inorganic carbon-free substance which remains at that temperature is called ash .

2.1.6 Carbohydrates

Starch is converted by acid hydrolysis into reducing sugars which are determined by volume using Fehling's solution[100-103].

2.1.7 Energy values of food

When organic substances are oxidized, they liberate a certain amount of heat. This calorific energy varies according to the type of organic matter undergoing oxi-

dation. In arriving at the total energy value of any given diet it is possible to burn weighed samples of the various food in an oxygen atmosphere in an apparatus called a bomb calorimeter. Thus, while proteins yield about 5.7 calories per gram when burned in a calorineter, correction for incompletely oxidized urea and other N compounds reduces this value in the body to 4.1. Further correction must be made for indigestible carbohydrates usually grouped together under designation 'crude fiber' which resists hot acid and alkaline digestion is an accurate measure of the polysaccharides which in vivo resist enzymatic digestion[103].

The energy values of food protein, fat, and carbohydrates are as follows:

Protein = 4.1 calories per g.

Fat = 9.3 calories per g.

Carbohydrates = 4.1 calories per g.

To allow for the incomplete digestion of these nutrients these caloric conversion factors were multiplied by the coefficients of digestibility and thus rounded off to 4, 9 and 4, respectively. The calorific value of foods may be estimated by applying these figures to the percentage composition as determined by

chemical analysis. For this purpose, the 'proximate' analysis includes moisture, ash, protein, fat, crude fiber, and carbohydrates[104,105].

2.2 INSTRUMENTAL TECHNIQUES

2.2.1 CHN Analyzer

Carbon, Hydrogen and Nitrogen was estimated by CHN Elemental analyzer, Model 240 B, Perkin-Elmer Norwalk Connecticut, U.S.A.

The Elemental analyzer estimate the carbon, hydrogen and nitrogen content in organic compounds accurately. Combustion occurs in pure oxygen under static conditions. The combustion products are then analyzed automatically in self-integrating, steady-state thermal conductivity analyzer which eliminates the tedious weighing of absorption traps associated with classical gravimetric analysis. Results are recorded with the programmable calculator system. The complete sample run is stored into memory for automatic operation. Blanks, sensitivity factors and analytical results are computed directly in weight percent with calculator system. Results of the analysis are given in Table 3.1-3.2. The specification of the instrument are: furance; combustion induction; detector; thermal conductivity; carrier gas; helium; atmosphere; oxygen. Diagram of the instrument is shown in Fig. 1.

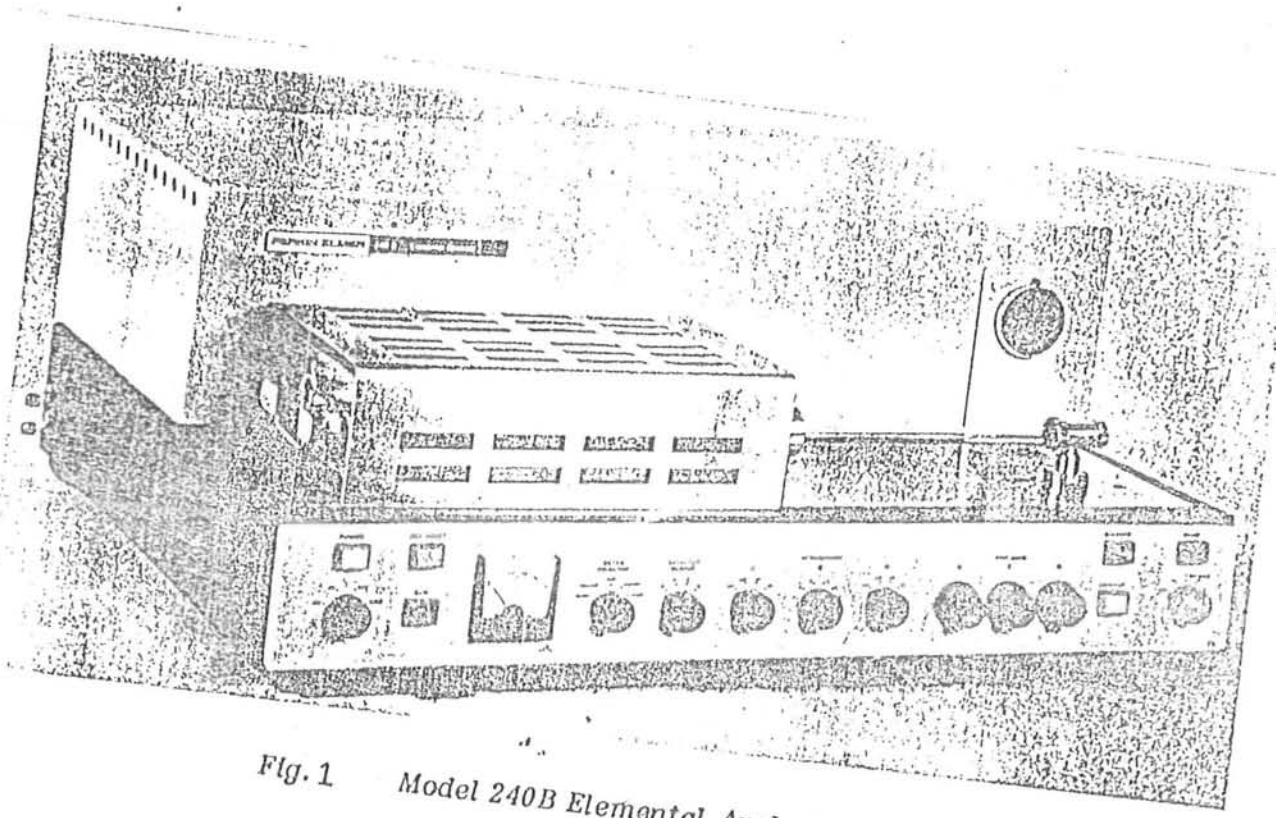


Fig. 1 *Model 240B Elemental Analyzer*

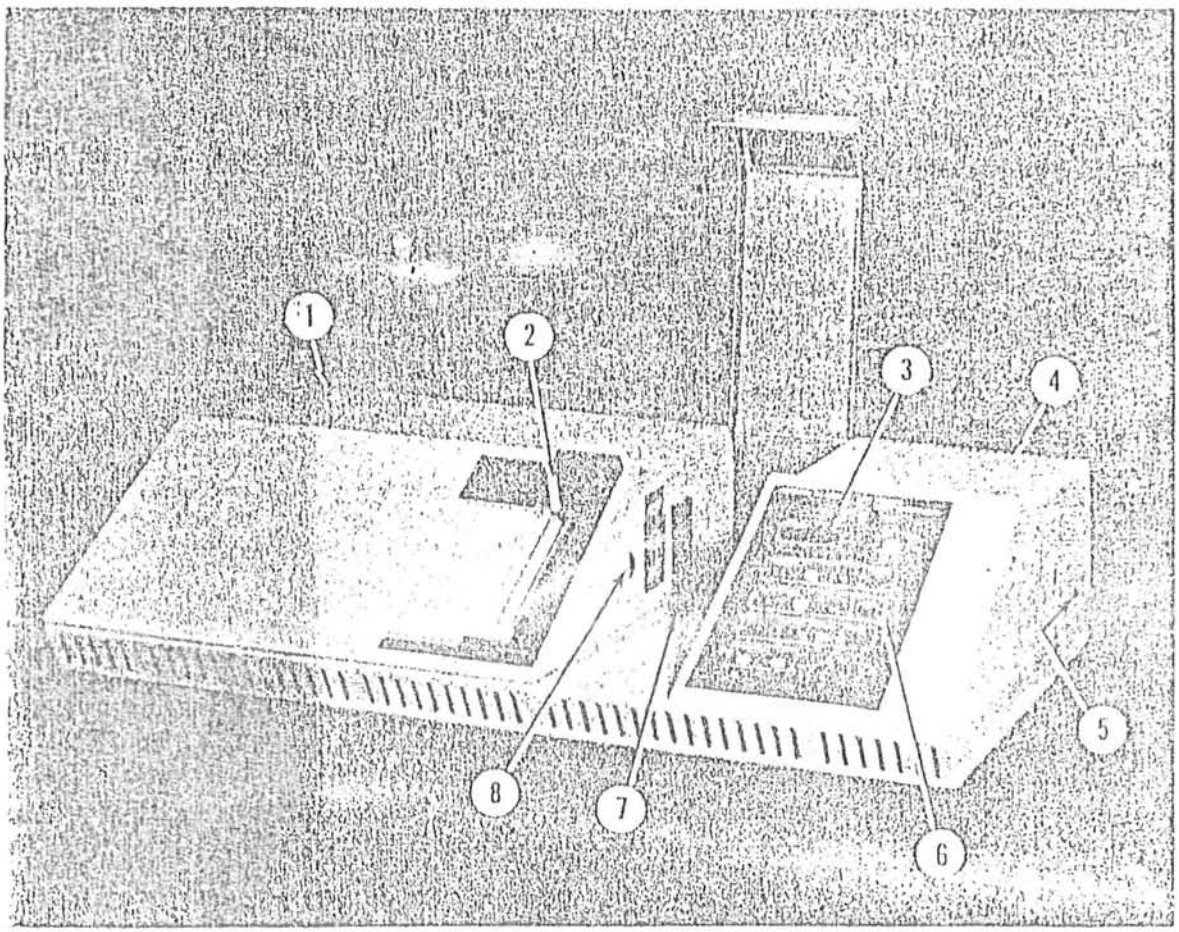
2.2.2 Infrared Spectroscopy

The infra-red region refers to that part of the electromagnetic spectrum between the visible and micro-wave regions, extending from 0.75 μm to about 1000 um. Infrared spectroscopy provides a powerful tool for qualitative analysis of organic compound on the basis of streching or bending vibration relative to other bonds in molecules. Inorganic compounds containing poly-atomic cations or anions also give useful I.R. spectra[107]. The common sub-divisions of I.R. are:

Near infra-red: where majority of the absorption bands are due to overtones of the hydrogen stretching vibrations, which are useful for the quantitative analysis of various functional groups.

Fundamental infra-red region: where a vast amount of quantitative information of about functional groups and molecular structure are given

For infra-red region: which gives information mainly about rotational, transitions, vibrational modes of crystal lattices and skeletal vibrations of large molecules.



1. *Monochromator/Photometer and Electronics Compartment*
2. *Recorder Pen*
3. *Frequency (Wavenumber) Display*
4. *Source Compartment*
5. *Power ON-OFF Switch*
6. *Control Panel*
7. *Sample Area*
8. *Baseline Control*

Figure 2 = Model 1330 IR Spectrophotometer

The marked contrast to the visible and near ultra-violet region, almost all substances show absorption in the infra-red region, the only exception being monoatomic or homopolar molecules such as Ne, He, O_2 and H_2. Further no two compounds with different structures have the same infra-red spectrum except for optical isomers and some high molecular weight polymers, differentiating only slightly in molecular weight[108].

Infra-red studies of prepared compounds were carried out on infra-red spectrophotometer, Hitachi Model 270-50, Japan, with data processor. Spectra were scanned in full wavelength range of the instrument i.e. 4000-250 cm^{-1}. Spectra of solid compound were recorded as transparent KBr disc, while spectra of liquid compounds as thin film using Nacl windows while gel between pressed KBr disc. Transparent disc was made by mixing and grinding appropriate amount of sample and KBr. Fine grind powder was pressed by hydraulic press at high pressure to get transparent disc[109].

Band positions of different compounds are tabulated in Tables 4.1-4.7. Schematic diagram of instrument is shown in Fig. 2.

2.2.3 Thermal Analysis

Thermal analysis may be defined as a 'group of techniques in which change in properties (physical or chemical) are studied as a function of temperature when subjected to controlled temperature program'.

Quite a number of methods of thermal analysis have been developed in the course of time to investigate thermal processes occurring when a solid product is heated or cooled.

Methods associated with a change in mass

(a) Thermogravimetry (TG)

(b) Derivative Thermogravimetry (DTG)

Methods associated with change in energy

(a) Differential Thermal Analysis (DTA)

(b) Differential Scanning Colarimetry (DSC)

Methods associated with the evolution of volatile product

(a) Evolved Gas Oetection (EGD)

(b) Evolved Gas Analysis (EGA)

Methods associated with dimensional changes

Thermomechanical Analysis (TMA)
or
(Dilatometry)

As we see it can be divided into three groups of techniques in which we study change in mass, change in energy and change in dimension.

The instrumental techniques associated with change in mass are TG; EGA and EGD. Among these three techniques TG is most important technique and is also used in our present work.

TC (thermogravimetry) may be defined as "change in mass of sample as a function of temperature when subjected to controlled temperature program".

The resulting mass change versus temperature curves (thermograms) provide information concerning the thermal stability and composition of the initial sample, intermediate and the composition of residue, if any[110]. The method of TG is basically quantitative in nature and mass change can be accurately measured. TG is widely used in almost all of the area of chemistry and allied fields[111-119].

The techniques associated with change in energy are DSC (Differential Scanning Calorimetry) and DTA (Differential Thermal Analysis). Both techniques are very important and are used for determination of enthalpic changes in organic and inorganic compounds.

DSC may be defined as 'measurement of change in energy of sample with respect to reference as a function of temperature when subjected to controlled temperature program'.

While DTA which is also used in our work may be defined as "measurement of change in temperature of sample with respect to reference as a function of temperature when subjected to controlled temperature program".

The application of these techniques (DSC and DTA) to organic compounds have been reviewed[120-125]. Specifically DTA applications to melting point determination[126,127], fusion characteristics[128,129], phase transitions and heat of vaporization[130] of organic compounds are given. DTA has also been used for studying aromatic compounds and for number of polymers[131-143].

2.2.3.1 Thermogravimetry

Thermogravimetric analysis was carried by NETZSCH Simultaneous thermal analyzer STA 429. This is an instrument which detects and records the changes in mass as a linear function of temperature or time. The resulting mass change versus temperature curve (thermogram) provides information concerning the thermal stability and composition of the initial sample.

Sample was contained in Platinium Crucible (8 mm Diam. x 10 mm depth) with a central base recess. The cruible was then adjusted on a palladium Ruthenium Crucible support plateform, which was connected to the micro balance. Such that slight change in weight of sample was indicated by Magnetic Compensation System (MCS), which gave a proportional signal to the recorder and computer interface to plot the weight loss of sample against temperature

The schematic diagram of thermobalance is shown in Fig. 3.

2.2.3.2 Differential Thermal Analysis (DTA)

DTA analysis was carried out by the NETZSCH TG, DTA SIMULTANEOUS THERMAL ANALYZER STA-429. Small sample

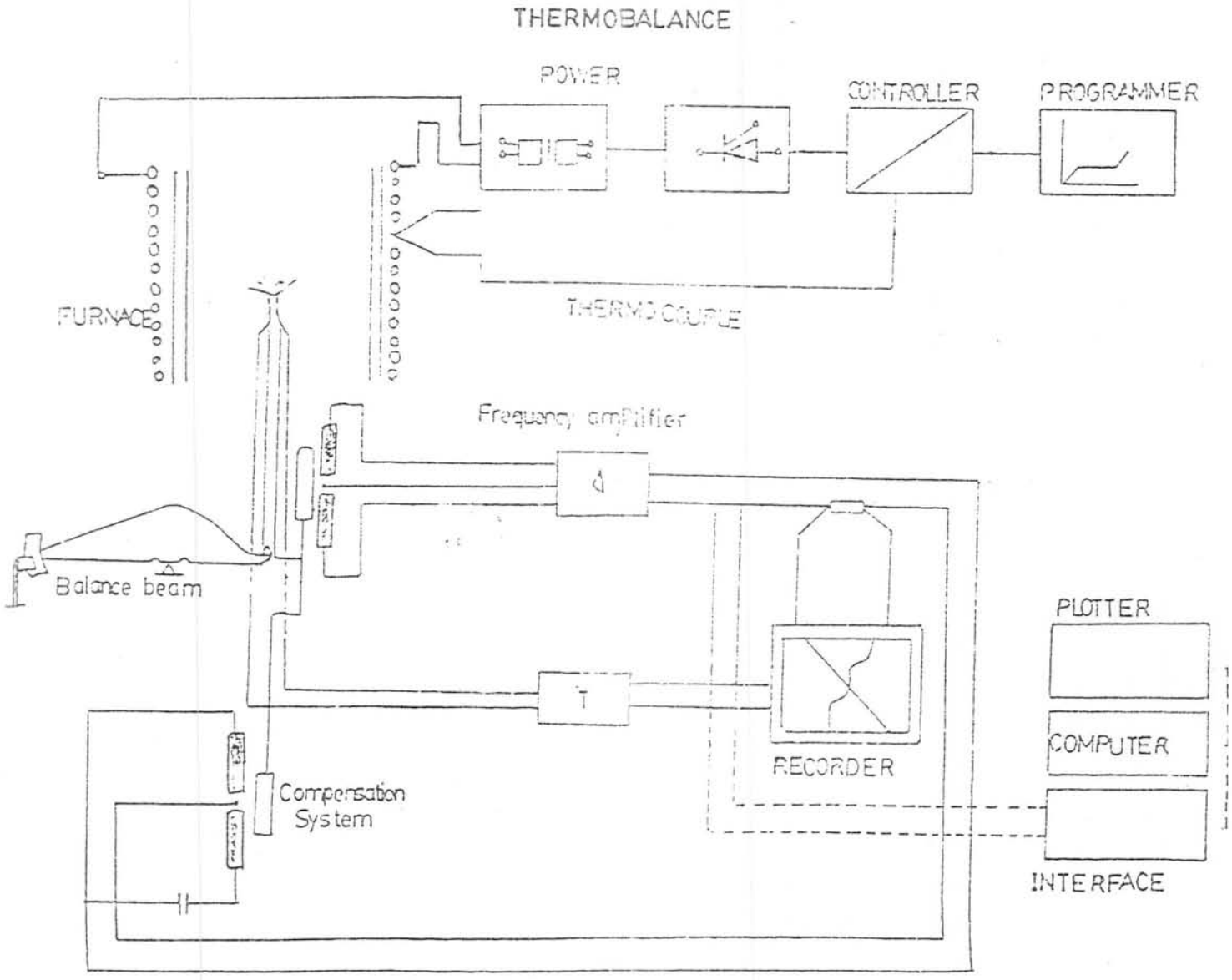

THERMOBALANCE
POWER
CONTROLLER
PROGRAMMER
FURNACE
THERMOCOUPLE
Frequency amplifier
Balance beam
PLOTTER
COMPUTER
RECORDER
Compensation System
INTERFACE

Fig. 3

quantities can be tested on this instrument in the temperature range of -160 to +2400^{o}C under vacuum, in static or dynamic atmosphere using inert, reactive or corrosive gases.

Thermaly inert kaoline or alumina was used as a reference material. The sample and reference are contained in platinum crucibles. The crucibles are mounted. So that beeds of two pt/pt 13% Rh thermocouples come within the base recess of each crucible. The sample temperature (T) is taken from the sample thermocouple, the differential temperature ($\triangle$T) is amplified and then 'T' and '$\triangle$T' are recorded on a leeds Northrop X_1-X_2 10 in recorder.

Instrument was run with samples of 10-30 mg and heating rate of 10^{o}C per minute

Schematic diagram of DTA instrument is illustrated in Fig. 4 and 5. **Results of analysis are given in Table 5.1 and thermograms 5.2-5.4.**

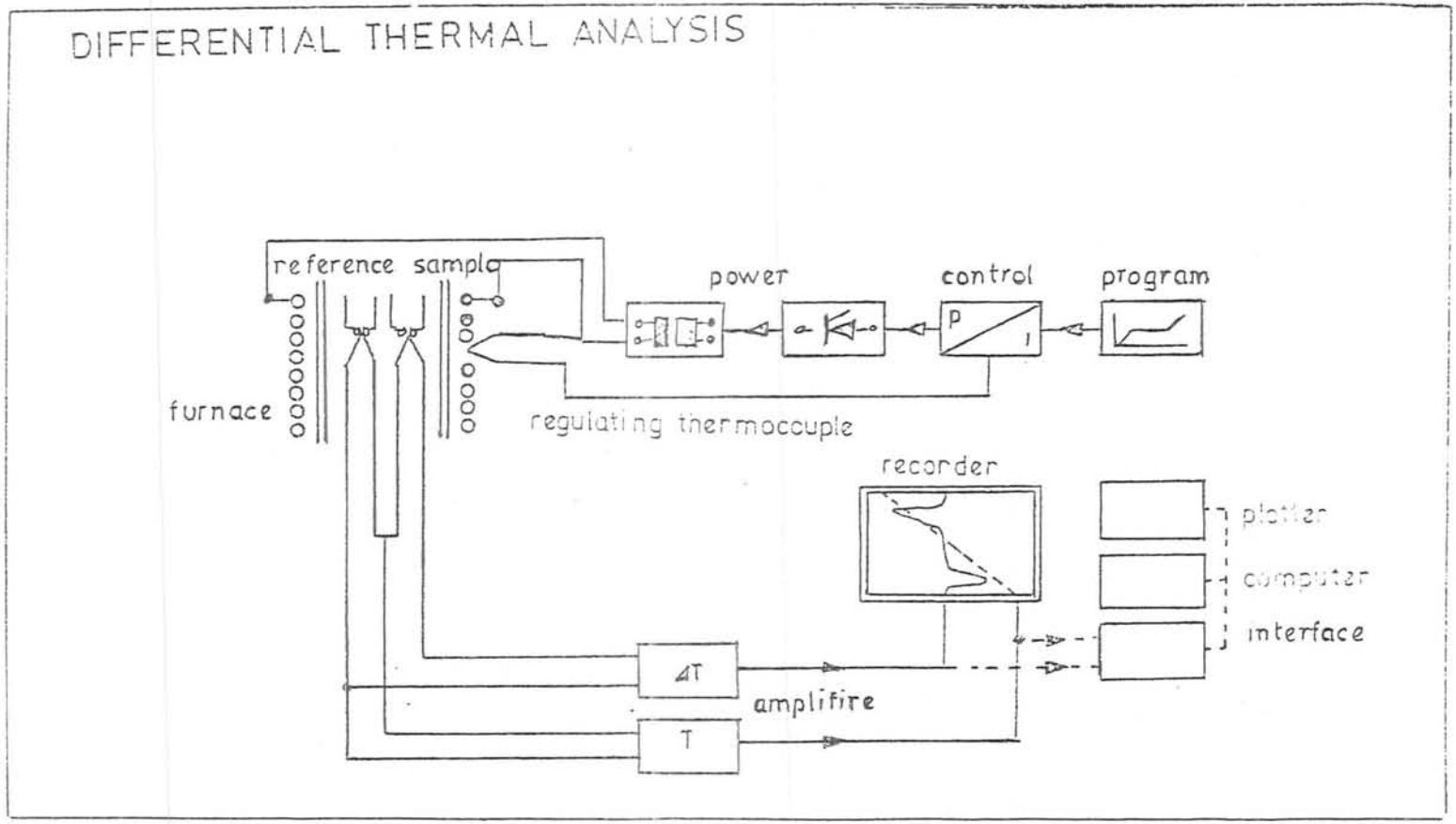

DIFFERENTIAL THERMAL ANALYSIS
reference
sample
power
control
program
P
I
furnace
regulating thermocouple
recorder
plotter
computer
interface
ΔT
amplifire
T

Fig. 4

Simultaneous Thermal Analysis STA 429

The Thermal Analyzer which also solves your measuring problems.
T – TG – DTG – DTA – DDTA – P – EGA – MS
Simultaneously on the same sample.

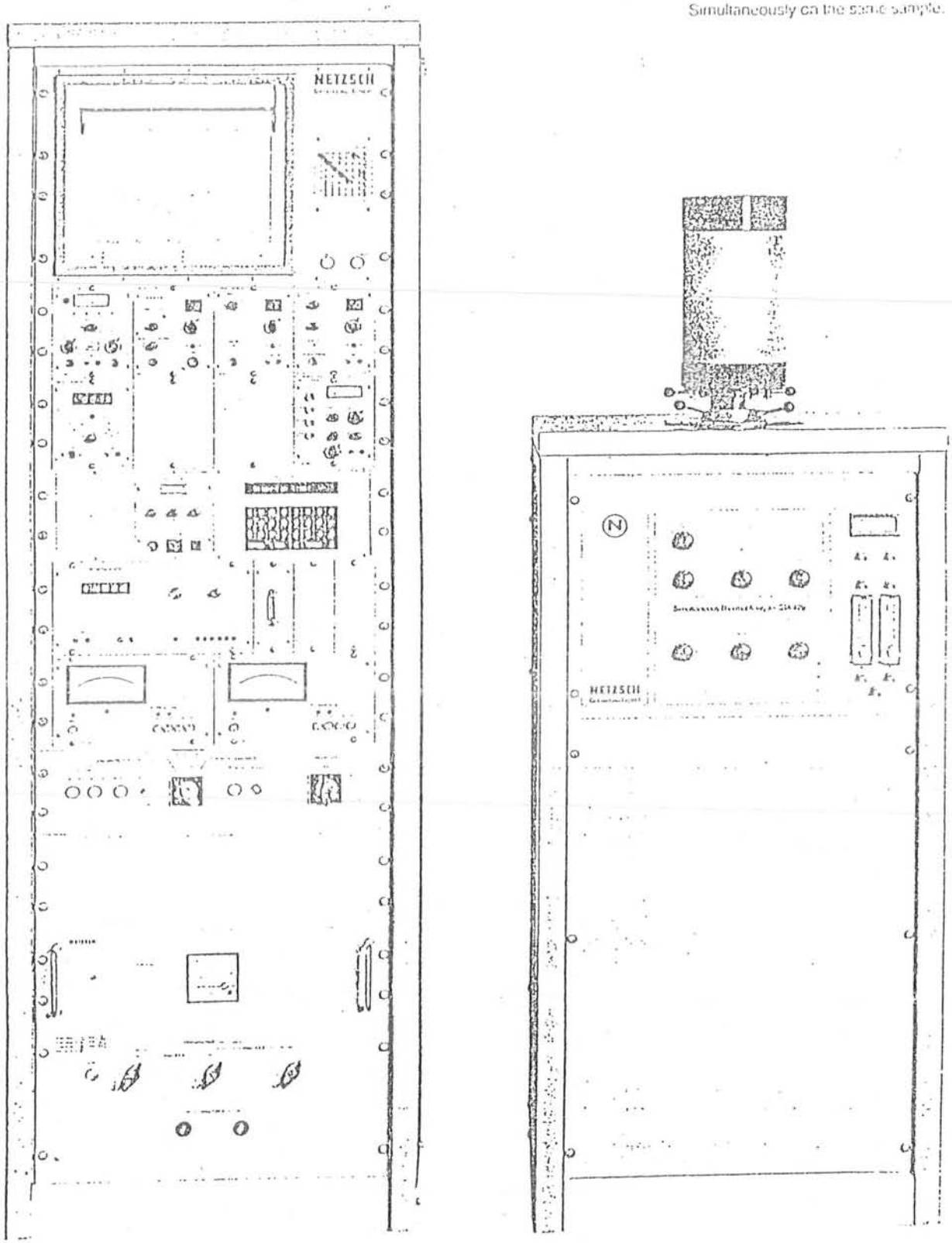


Fig. 5

2.2.4 Atomic Absorption Spectroscopic Method

Atomic absorption spectroscopy may be defined as a method for determining the concentration of an element in a sample by measuring the absorption of radiation in atomic vapour produced from the sample at a wavelength (λ) [144] that is specific and characteristic of the element under consideration. When radiation of a selected frequency passes through an enclosure containing free atoms which can emit that frequency when excited, resonance effects are accompanied by an absorption of incident radiation and so the intensity of the transmitted radiation is decreased[145-147].

For the determination of major and trace elements, Hitachi Zeman Atomic Absorption Spectrophotometer Model Z-8000 made of Japan was used. The standard analytical conditions established for the quantification of major and trace metals are listed in Table 2.4.

The Hitachi Zeman Atomic Absorption spectrophotometer is a high speed, dual frequency, simultaneous photometric background correction. Other salient features of the equipment are automatic selection of

TABLE 2.4

INSTRUMENTAL CONDITIONS USED FOR THE DETERMINATION OF MACRONUTRIENTS AND MICRONUTRIENTS FOR A.A.S.

Conditions / Elements	Detection limit µg/ml	Wave length (nm)	Slit width (nm)	Fuel flow rate (L/min)	Type of flame
Na	0.0003	589.0	0.5	1.6	Air-C_2H_5
K	0.003	766.5	0.5	1.9	"
Ca	0.001	442.7	0.5	2.0	"
Mg	0.0003	285.2	0.5	1.6	"
Fe	0.01	248.3	0.2	1.9	"
Cu	0.003	324.8	0.5	1.7	"
Mn	0.003	279.5	0.4	1.8	"
Zn	0.001	213.9	0.5	1.9	"
Cd	0.001	228.8	0.4	1.7	"
Cr	0.003	357.9	0.5	2.0	"
Pb	0.01	217.0	0.4	1.8	"
Hg	0.1 $(0.00003)^n$	253.7	0.5	1.6	"
Ni	0.01	232.0	0.5	1.7	"
As	0.1 $(0.003)^n$	193.7	0.5	1.9	"

^{n} = vapour generation, as hydrides for As and Se.

optimum operational conditions, automatic recording of the data, including the working curve and top level data-processing function to ensure high precision and high accuracy[148-150].

EXPERIMENTAL

EXPERIMENTAL

2.3 REAGENTS

All reagents were purchased from Merch Schuchardt (West Germany) and Fluka (U.S.A.), are listed below:

(a) Anhydrous α-D glucose (V)

(b) Anhydrous α-D fructose

(c) Anhydrous α-D galactose

(d) Acetone

(e) n-hexane

(f) Pyridene

(g) Sulfuric acid

(h) Chlorosulfunic acid

(i) Anhydrous cupric sulfate

(j) Sodium hydroxide

(k) Calcium hydroxide

(l) Potassium sulfate

(m) Potassium carbonate

(n) Calcium oxide

(o) Boric acid

(p) Magnesium turnings

(q) Methyl red

(r) Methylene blue

(s) Iodine

(t) Ether

(u) Absestos

(v) Chloroform

2.3.1 Purification of the Reagents

(a) Acetone

In acetone (1000 ml), successive quantities of potassium permanganate was added until the violet colour persisted. The solution was refluxed for 4 hrs. Then added anhydrous potassium carbonate (100 g), filtered the solution and distilled, dry acetone obtained 99.95% and refractive index 1.3554 [106].

(b) Ethanol

In final fermented solution (1 L), distilled water (1 L) was added. Distilled the solution exactly upto 1 L contain ethanol (4.8%). Again distilled it with the help of fractional distillation to obtaine ethanol (95%).

Poured the content (500 ml; 95% ethanol) into 1 L round-bottomed flask, calcium oxide (125 g; 2.23 mol.) was added. Refluxed the mixture gently for 6 hrs. and allowed it to stand overnight. Distilled the ethanol (discarded first 20 ml of distillate). The distillate contained pure ethanol of 99.5%, refractive index 1.3499.

In ethanol (50 ml, 99.5%), magnesium turnings (3 g)

was added, followed by iodine (0.4 g). Warmed the mixture until the iodine disappeared. Continued the heating until all the magnesium was converted into ethanolate, then added 450 ml of absolute ethanol (99.5%). Refluxed the mixture for 1 hr. Distilled off the ethanol into the vessel in which it was stored. The purity of ethanol was 99.95%, refractive index 1.3596.

$$Mg + 2C_2H_5OH \longrightarrow H_2 + Mg(OC_2H_5)_2$$

$$Mg(OC_2H_5)_2 + 2H_2O \longrightarrow Mg(OH)_2 + 2C_2H_5OH$$

2.4 COMPOSITION AND FERMENTATION OF SENSIBLE SOURCES

2.4.1 Composition

The composition of sensible sources were determined by the standard methods described in the literature, and results are reproduced in Tables (2.1 - 2.4).

2.4.1.1 Moisture (% dry matter)

Sample (2 g) was placed in a crucible with lid and heated the contents in an oven at 105^{o}C for 12 hrs. The contents were cooled in a desiccator and weighed. The percentage of moisture contents were calculated.

2.4.1.2 Crude protein

Sample (2 g) was transferred to Kjeldahl flask. Added catalyst (5 g) $CuSO_4.5H_2O$ and K_2SO_4 in the ratio of (1:13:3), concentrated H_2SO_4 (30 ml). The mixture was digested for 30 min. Transferred the contents into a 100 ml volumetric flask. Alocate the 5 ml of the contents and added 5 ml of NaoH (40% W/W). Distilled for 5 minutes and collected in a flask containing 5 ml of 2% boric acid. Distilled it for 5 min. and titrated against N/5 H_2SO_4. The percentage of protein contents were calculated.

2.4.1.3 Ether extract (fat)

Moisture free sample (2 g) was placed in dried extraction thimble. Plugged the thimble with absorbent cotten wool placed the thimble in an extractor. The extract was evaporated to dryness on water bath for 2 hrs. The percentage of fat contents were calculated.

2.4.1.4 Crude fibre

To the moisture free sample (2 g), dil H_2SO_4 was added to digest for 30 min. The extracted fibre was neutralized with N/10 NaOH solution, and transferred the content in beaker and added 200 ml of boiling dil NaOH and digested the content for 30 min. Neutralized the contents with 10 ml hot dil H_2SO_4, then with 10 ml hot water. Dried the content at 135^{o}C for 2 hrs. Ignited the content at 600^{o}C in furnace for 30 min., cooled and weighed.

2.4.1.5 Ash

Dried sample (2 g) was placed in cruible and ignited it at 600^{o}C for 4 hrs. Cooled the content and weighed.

2.4.1.6 Carbohydrates (suger)

Powdered sample (2 g) was dissolved in distilled water (60 ml), then added hydrochloric acid (5 ml). The

contents were kept at 60°C for 2 hrs. The pH of the solution was maintained at 7 by addition of NaOH solution (40%). Made the volume 100 ml and transferred it to burette. From burette, added the solution to the boiling liquid (Fehling's solution (5 ml of solution A and 5 ml of solution B)), until the blue colour of solution became hardly discernible then added methylene blue solution (indicator 1%) and titrated until the blue colour of indicator disappeared. The percentage of sugar contents were calculated.

2.4.1.7 Carbon dioxide determination

5 g of fermentable material (either wheat, corn, barley, rye and oat including hop (rice; 40%) was dissolved in appropriate water and made the volume 50 ml. Three traps were connected with the fermentation flask (Fig. H) each containing 0.1 N barium hydroxide solution. After completion of fermentation helium gas was flashed through the apparatus and solutions from three traps were taken, mixed and titrated against 0.1 N hydrochloric acid using phenalphthalein as an indicator. The difference between the volume of the base would give the volume of carbon dioxide absorbed according to the following equation:

1 ml 0.1 N $Ba(OH)_2$ = 1.119 ml of CO_2 at N.T.P.

At the beginning of the experiment 0.1 N $Ba(OH)_2$ was titrated against 0.1 N Hcl to have the first reading for later calculations. From the volume of CO_2 and known volume of fermentable liquid the percentage of carbon dioxide was calculated.

2.4.2 FERMENTATION

2.4.2.1 Barley (A)

Barley (150 g) was converted into malt by steeping for 24 hrs. (including shaking after every six hrs.), then filtered. The filtrate was rejected. The residue was stored for germination for 8 days at 18-25°C (humidity, 40-65%) and was allowed to shake after every 12 hrs. The germinated product (green malt) contain roots, which were rejected, the rest of the product was heated at 66°C for 18 hrs. and the malt (140 g) contain 5% moisture. The malt was then converted into fine powder and mixed with rice powder (60.0g). The mixture was changed into slurry by adding 1 L of water.

The slurry was heated at 45°C by stirring for ½ an hr. Then added distilled water (1 L), and further heated at 70°C for 1 hr. The product was recovered as light brown colour syrup (saccharified mash), cooled at 20-30°C (iodine test indicted the complete conversion of starch into sugar). The saccharified mash was then filtered. The residue (malt husks; 5 g) was analysed (dry matter, 25.42; protein 7.96; fat, 1.20; crude fiber, 15.94; ash, 1.37; carbohydrate, 15%).

To the filtrate (wort; pH 5.4) further added yeast starter* (10.0 g). The mixture temperature (9-12°C) was then maintained for fermentation. The fermentation was completed within 7-8 days. Fermented liquid was filtered. The filtrate (50 ml) was analyzed (protein, 0.3; C_2H_5OH; 4.7; fat, traces; carbohydrates, nil; ash, nil; crude fibre, nil%, pH, 4.5).

The residue (50 g) after filteration was analysed (dry matter, 35.90; protein, 16.21; ash, 1.59; carbohydrates, nil%).

2.4.2.2 Wheat (B)

Wheat (100 g) was converted into malt by steeping for 24 hrs. (including shaking after every six hrs.), then filtered. The filtrate was rejected. The residue was stored for germination for 8 days at 18-25°C (humidity, 40-65%) and was allowed to shake after every 12 hrs. The germinated product (green malt) contain roots, which were rejected. The rest of the product was heated at 65°C for 18 hrs. and the malt (95.0 g; contain 4.5% moisture). The malt was then converted into fine powder and mixed with rice powder (40 g). The mixture was changed into slurry by adding 1 L of water. The slurry was heated at 45°C by stirring for ½ an hr. Then added distilled water (600 ml) and further heated at 70°C for

*swelled yeast.

1 hr. The product was recovered as light brown colour syrup (saccharified mesh), cooled at 20-30°C (iodine test indicted the complete conversion of starch into sugar). The saccharified mesh was then filtered. The residue (malt husks; 5 g) was analyzed (dry matter, 19.42; protein, 7.93; fat, 1.1; crude fibre, 10.91; ash, 0.8; carbohydrate, 17%). To the filtrate wort (pH, 5.3) further added yeast starter (10 g). The mixture temperature (9-13°C) was then maintained for fermentation. The fermentation was completed within 7-8 days. The liquid was filtered. The filtrate (50 ml) was analysed (protein, 0.3; C_2H_5OH, 4.5; fat, traces; carbohydrates, nil; ash, nil; crude fibre, nil%; pH 4.7). The residue (50 g) after filtration was analysed (dry matter, 35.90; crude protein, 16.21; ash, 1.59; carbohydrates, nil %).

2.4.2.3 Oat (D)

Sample (150 g) was converted into malt by steeping for 24 hrs. (including shaking after every six hrs.), then filtered. The filtrate was rejected. The residue was stored for germination for 8 days at 18-25°C, (humidity 50-70%) and was allowed to shake after every 12 hrs. The germinated product (green malt) contain

roots, which were rejected and the rest of the product was heated at 65°C for 20 hrs. and the malt (144 g) contain 5.2% moisture. The malt was then converted into fine powder and mixed with rice powder (60.0 g). The mixture was changed into slurry by adding 1.0 L water. The slurry was heated at 45°C by stirring for ½ an hr. Then added distilled water (1.0 L), and further heated at 70°C for 1 hr. The product was recovered as light brown colour syrup, cooled at 20-30°C (iodine test indicated the complete conversion of starch into sugar). The saccharified mesh was then filtered. The residue (malt husk; 5 g) was analyzed (dry matter, 20.41; protein, 7.33; fat, 1.0; crude fibre, 9.81; ash, 0.9; carbohydrate, 9%). To the filtrate wort (pH 5.3) further added yeast starter (10.0 g). The mixture temperature (9-12°C) was then maintained for fermentation. The fermentation was completed within 7-8 days. Fermentated liquid was filtered. The filtrate (50 ml) was analysed (protein, 0.26; C_2H_5OH, 4.4; fat, traces; carbohydrates, nil; ash, nil; crude fibre, nil %; pH 4.8). The residue (5 g) after filtration was analysed (dry matter, 32.31; crude protein, 12.31; ash, 1.01; carbohydrates, nil%).

2.4.2.4 Rye (E)

Rye (200 g) was converted into malt by steeping for

24 hrs. (including shaking after every 6 hrs.), then filtered. The filtrate was rejected. The residue was stored for germination for 8 days at 20-30°C (humidity 30-60%), and was allowed to shake after every 12 hrs. The germinated product (green malt) contain roots, which were rejected, the rest of the product was heated at 65°C for 18 hrs. and the malt (180 g) contain 5% moisture. The malt was then converted into fine powder and mixed with rice powder (60.0 g). The mixture was changed into slurry by adding 1.0 L of distilled water. The slurry was heated at 45°C by stirring for ½ an hr. Then added distilled water (1.0 L) and further heated at 70°C for 1 hr. The product was recovered a light brown colour syrup (saccharified mesh), cooled at 20-30°C (iodine test indicated the complete conversion of starch into sugar). The saccharified mesh was then filtered. The residue (malt husks, 5 g) was analyzed (dry matter, 30.91; protein, 7.11; fat, 0.81; crude fibre, 8.99; ash, 0.9; carbohydrate, 13%). To the filterate wort (pH 5.4) further added yeast starter (10.0g). The mixture temperature (9-12°C) was then maintained for fermentation. The fermentation was completed within 7-8 days.

Fermented liquid was filtered and filtrate (50 ml)

was analysed (protein, 0.25; C_2H_5OH, 4.4; fat, traces; carbohydrates, nil; ash, nil; crude fibre, nil%; pH 4.7). The residue (50 g) after filtration was analysed (dry matter, 34.71; crude protein, 11.35; ash, 1.61; carbohydrates, nil%).

2.4.2.5 Corn (F)

Corn (150 g) was converted into malt by steeping for 24 hrs. (including shaking after every 6 hrs.) then filtered. The filterate was rejected. The residue was stored for germination for 8 days at 22-25°C (humidity, 50-60%) and was allowed to shake after every 12 hrs. The germinated product (green malt) contain roots which were rejected, the rest of the product was heated at 65°C for 18 hrs. and the malt (140 g) contain 5% moisture. The malt was then converted into fine powder and mixed with rice powder (60 g). The mixture was changed into slurry by adding 1 L of water. The slurry was heated at 45°C by stirring for ½ an hr. then added distilled water (1.0 L), and further heated at 70°C for 1 hr. The product was recovered as light brown colour syrup (saccharified mesh), cooled at 20-30°C (iodine test indicated the complete conversion of starch into sugar). The saccharified mesh was then filtered. The residue

(malt husks; 5 g) was analyzed (dry matter, 21.31; crude protein, 6.31; fat, 1.23; crude fibre, 9.92; ash, 1.1; carbohydrate, 14%.

To the filtrate wort (pH, 5.5) further added yeast starter (10 g).The mixture temperature (9-12°C) was then maintained for fermentation. The fermentation was completed within 7-8 days. Fermented liquid was filtered. The filterate (50 ml) was analysed (protein, 0.26; C_2H_5OH, 4.2; fat, traces; carbohydrates, nil; ash, nil; crude fibre, nil%; pH, 4.9).

The residue (50 g) after filteration was analysed (dry matter, 34.0; crude protein, 10.95; ash, 1.71; carbohydrates, nil%).

2.4.2.6 Molasses (G)

Molasses (sugar 54%) was diluted in distilled water (1 L). The solution was kept at density between 1.06 and 1.07 (contain molasses 195 g; sugar, 10.8%). The diluted material (solution) was called mesh. Mesh was adjusted to the optimal temperature (25-30°C) and yeast (10. g) was mixed (prepared by cultivation of an appropriate organism such as saccharomyes cerevesiae). The

fermentation process was completed in 52 hrs. The resulting solution (200 ml) was mixed with distilled water (200 ml) and distilled at temperature (100°C) until 200 ml was distilled. The solution was analyzed (protein, nil; fat, nil;; carbohydrate, nil; C_2H_5OH, 4.3; pH, 4.7).

2.5 RECOVERY OF THE PRODUCTS

2.5.1 Malt Husks

The spent grains (5 g) recovered during mesh filtration were dried in oven by maintaining temperature (65^{o}C) for 6 hrs. The dried malt husks was obtained (A, 25.42%; B, 19.42; O, 20.4%; E, 20.10%, F, 21.31%).

2.5.2 Solid Protein

Solid protein was recovered from fermented material at two different stages.

In Stage-1 when wort was boiled and then centrifuged, the protein was coagulated. After filteration it was dried at 35 $\pm$ 1^{o}C. the protein was recovered as solid product (A, 1.2; B, 1.1; D, 1.3; E, 1.0; F, 0.9%).

In State-2 fermented mixture was filtered. The residue was dried at 40 ± 1°C for 4 hrs. Solid crude protein was isolated (A, 35.90; B, 32.88; D, 32.31; E, 34.71; F, 34.0%).

It was further purified in 70% ethanol undissolved fibrous material was filtered off. Filterate was diluted with water and the mixture was cooled to isolate 90% solid protein at 35 ± 1°C.

2.5.3 Liquid Protein

After the recovery of the solid protein the filterate contained the liquid protein (A, 0.3; B, 0.28; D, 0.26; E, 0.25; F, 0.26%) which was recovered by cooling then centrifugation.

2.5.4 Carbon dioxide

Carbon dioxide evolved during fermentation (contains traces of ethanol vapour, organic chemicals, including aldehydes, esters, acetates and air) was passed through the traper 1 containing distilled water, then passed through trapers 2 and 3 containing activated carbon and calcium oxide respective by the purified gas was collected in cylinder by pumping.

2.5.5 Ethanol

Ethanol was recovered after the completion of fermentation process. It was isolated by converting it to an azeotropic mixture and then distilled. The distillate contain ethanol (A, 4.8; B, 4.7; D, 4.6; E, 4.2; F, 4.1; G, 4.3%).

2.5.6 Non-alcoholic Soft Drink

The alcohol free mixture was pasteurized at 60°C for half an hr. It was stored for drinking.

2.6 ESTIMATION OF MACRONUTRIENTS AND MICRONUTRIENTS IN SENSIBLE SOURCES BY A.A. SPECTROSCOPY

2.6.1 Experimental Procedure

The cereal samples were first dried in electric oven maintained at 40 $\pm$ 1^{o}C for 24 hrs. for the purpose of weight normaliation. After complete removal of moisture, the sample was grinded in a mortor until fine powder was obtained. An exactly weighed of 1 g of each sample was transferred to a China dish and 10 ml of 65% nitric acid was added to it. The dish was subsequently placed for about 30 min. in oven maintained at 40 $\pm$ 1^{o}C. The digested sample was diluted with distilled water and transferred, with constant and careful washings, to a measuring flask (50 ml) by suitably making up the valume. This solution was aspirated directly on to the A.A. spectrophotometer for the estimation of trace metal content (Tables 6.1-6.2).

2.7 PREPARATION OF THE RAW MATERIALS IN THE SYNTHESIS OF HIGH POWER FUEL

2.7.1 Ethanol (99.95%) .. .. (98)

2.7.2 Preparation of Isopropylidene Derivatives

2.7.2.1 1,2:5,6-Di-O-isopropylidene-∝-D-glucofuranose(IV)

Anhydrous ∝-D glucose(V) (37.5 g; 0.20 mol.) was dissolved in acetone (250 ml). Then pulverized anhydrous zinc chloride (30 g; 0.21 mol.) followed by phosphoric acid (1.87 ml; 85%) was added respectively. The mixture was stirred for 30 hrs. at ambient. the undissolved D-glucose (15.45 g) was filtered off. Filtrate was cooled and made alkaline with NaoH solution (21.25 g, 40%). The insoluble inorganic salts were removed and washed with acetone. The concentrated filtrate was diluted with 37.5 ml of distilled water and extracted with chloroform (3 x 37.5 ml). The extract was washed with water and finally concentrated to form a white crystalline compound of 1,2;5,6 di-o-isopropylidene∝-D-glucose(VI); recrys-

tallized the product in chloroform and n-hexane (1:2 V/V)[151-153].

(Found C = 54.93, H = 7.21, O = 37.86.

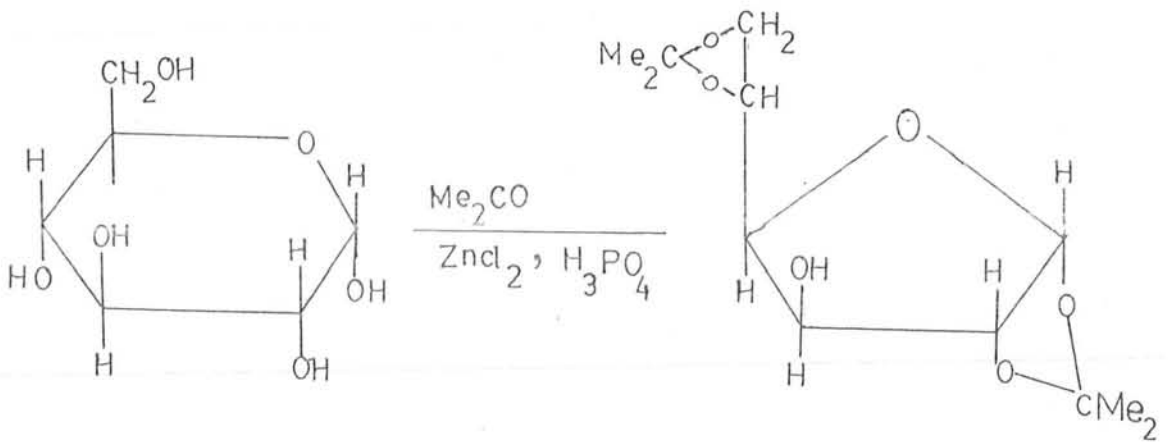
Calculated C = 55.38, H = 7.69, O = 36.92%)

Yield = 65%

m.p. = 109°C

Rf = 0.76 cm.

It is partially soluble in acetone, completely soluble in chloroform while insoluble in n-hexane.



2.7.2.2 1,2:4,6-Di-O-isopropylicdene-∝-D-Sorbofuranose

Anhydrous ∝ -D-fructose (75 g; 0.41 mol.) was dissolved in acetone (500 ml). The pulverized zinc chloride (60 g; 0.44 mol.) and phosphoric acid (3.74 g; 85%) was added respectively. The mixture was stirred for 30 hrs. at ambient. The mixture was filtered off. The filtrate was cooled and the solution was made alkaline with sodium hydroxide (42.5 g, 40%). The mixture was filtered. Filtrate was concentrated. The residue

was diluted first with distilled water (75 ml) and then extracted with chloroform (3 x 75 ml). The extract compound was washed and concentrated to form white crystalline compound of 1,2:4,6-Di-O-isopropylidene-α-D sorbofuranose. Recrystallized the product in chloroform and n-hexane (1:2 V/V).

Found C = 55.02 H = 7.23, O = 37.75

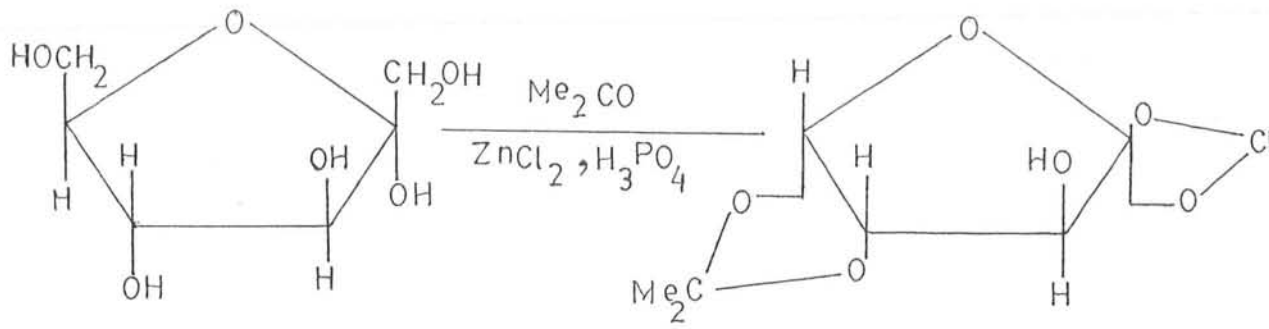
Calculated C = 55.38, H = 7.67, O = 36.92%)

Yield = 55%

m.p. = 71.47°C

Rf = 0.77 cm.

It is partially soluble in acetone, completely in chloroform while insoluble in n-hexane.



2.7.2.3 1,2:3,4-Di-O-isopropylidene-α-D galactopyranose(X)

Powdered of anhydrous D-galactose, (45 g; 0.25 mol) powdered anhydrous cupric sulfate (100 g; 0.625 mol.) and conc. sulfuric acid (5 ml; 1.84 d) was mixed together

and finally acetone 1 L (13.7 mol.) was added to the mixture and stirred for 24 hrs. at ambient. Cupric sulfate was filtered and washed with little anhydrous acetone. Filtrate was neutralized by powdered calcium hydroxide (4.7 g) until the solution is neutralized to give congo red colouration. The insoluble inorganic materials ($Ca(OH)_2$ and $CaSO_4$) was filtered off, and washed with dry acetone. The filtrate was concentrated by distillation to form a light yellow oil of 1,2:3,4, Di-O-isopropylidene-α-D-galactopyranose.

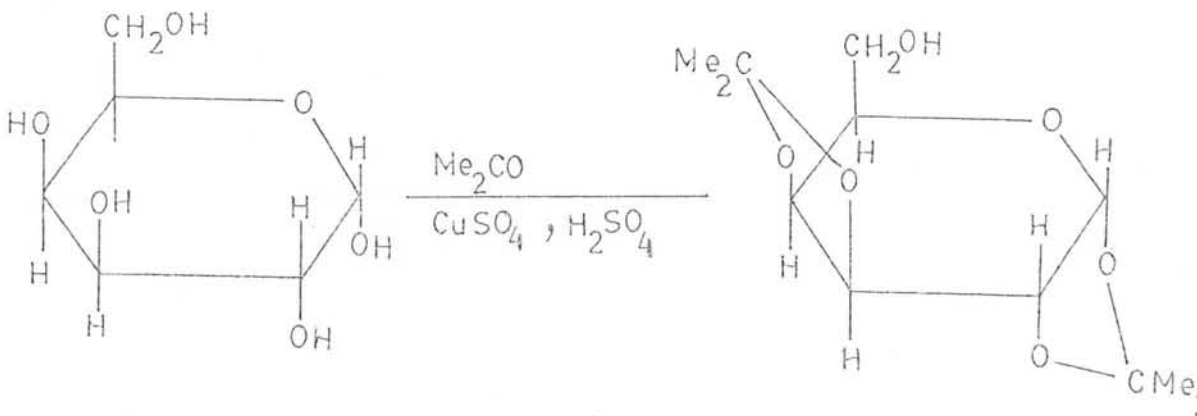
(Found C = 55.79, H = 7.21 , = 37.00

Calculated C = 55.38, H = 7.69, O = 36.92).

Yield = 66.65%

Rf = 0.72

It is partially soluable in acetone, completely in chloroform, while insoluble in n-hexane[154-157].



2.7.3 Preparation of Appropriate Salts of Isopropylidene Derivatives

2.7.3.1 Potassium salt of acid sulfuric ester of 1,2:4,6 -Di-O-isopropylidene-α-D-Sorbofuranose

Anhydrous 1,2:4,6-Di-O-isopropylidene-α-D-sorbofuranose (10 g; 0.038 mol.) was mixed with well-cooled mixture ($-5^{o}C$) of pyridene (15 ml) and chlorosulfonic acid (2.560 ml)(mixture was prepared slowly by addition of $HclSo_3$ dropwise into pyridene). The mixture was stirred for 4 hrs, then allowed the mixture to stand for 3 hrs. Excess pyridene was removed by distillation and added K_2CO_3 (4.04 g) stirred the mixture for 8 hrs. allowed it to stand for over night. Colourless crystalline compound so produced was washed with acetone.

Yield 42 %

m.p. = $113^{o}C$

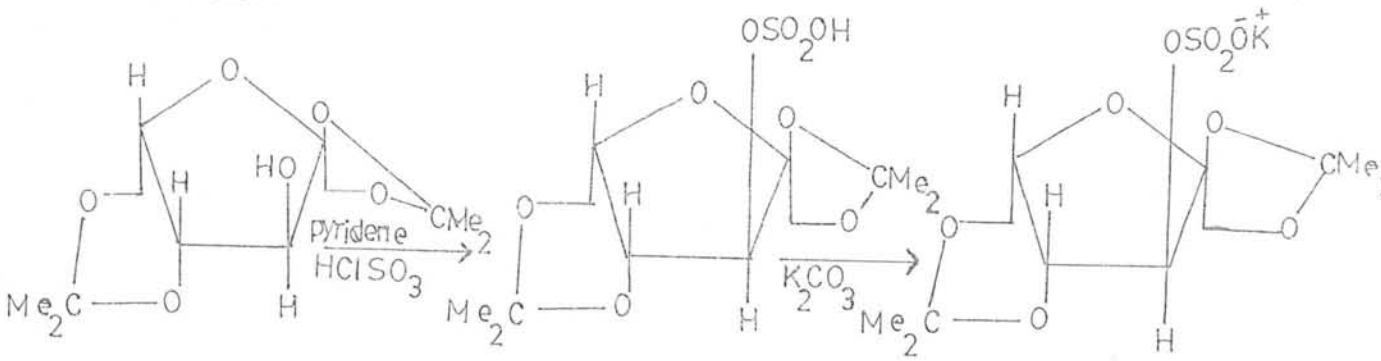
Rf = 1.2 cm

(Found C = 37.81, H = 5.38, O = 37.82, K = 9.93%

Calculated C = 38.09, H = 5.02, O = 38.09, S = 8.46,

K = 10.31%).

It is insoluble in acetone, n-hexane while soluble in water.



The appropriate salts of 1,2:5,6-Di-O-isopropylidene- ∝ -D-glucofuranose(VI) and 1,2:3,4-Di-O-isopropylidene- ∝ -D-galactopyranose(X) were prepared by the same procedure as described in 2.7.3.1.

2.7.3.2 Potassium salt of acid sulfuric ester of 1,2:5,6-Di-O-isopropylidene- ∝ -D-glucofuranose

Yield = 3.58 g

m.p. = 108°C

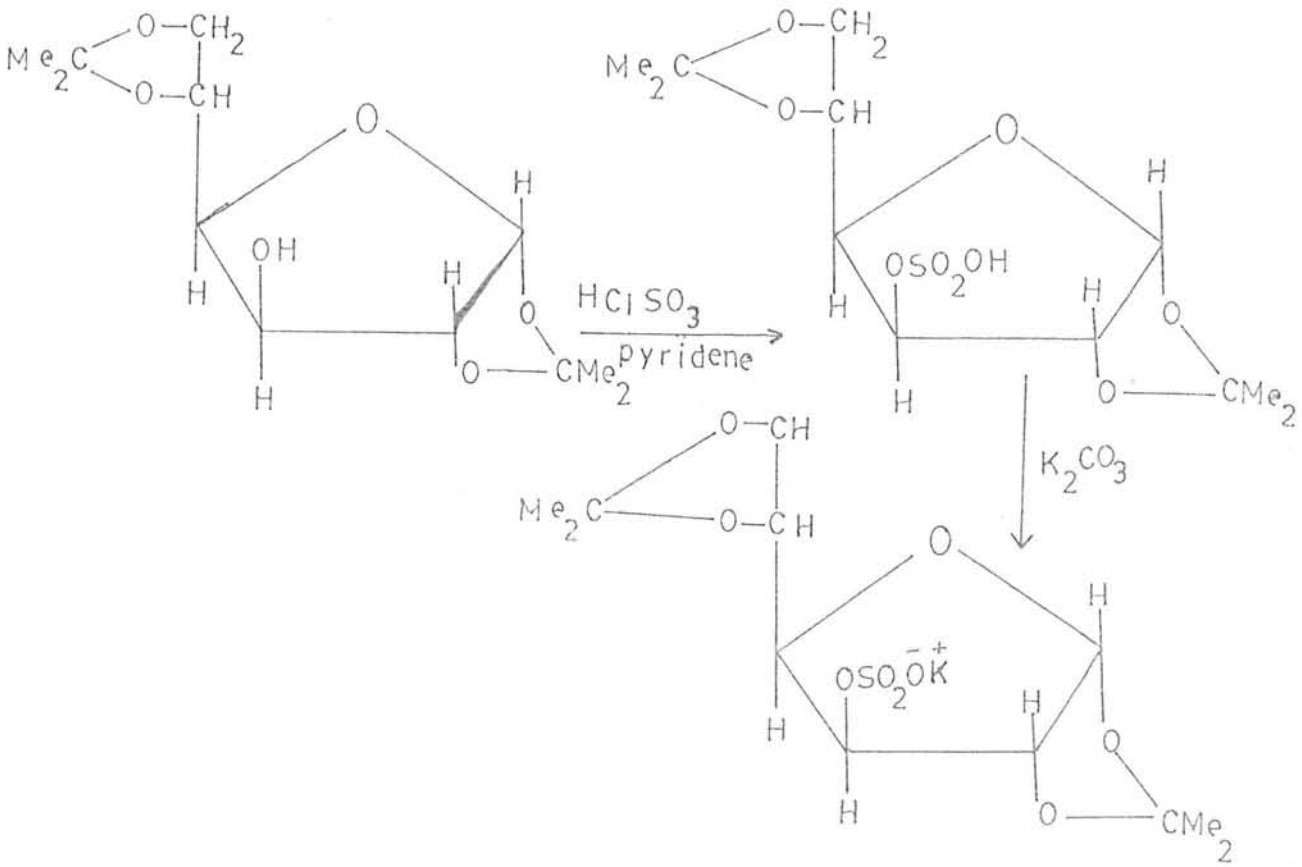
Rf = 1.4 cm

(Found C = 38.0, H = 4.95, O = 37.99, K = 9.98%,

Calculated C = 38.09, H = 5.02, O = 38.09, S = 8.46,

K = 10.31%)

It is insoluble in acetone, n-hexane while soluble in water.



2.7.3.3 Potassium salt of acid sulfuric ester of 1,2:3,4-Di-O-isopropylidene- -D-galactopyranose

Yield = 3.3 g

m.p. 60°C

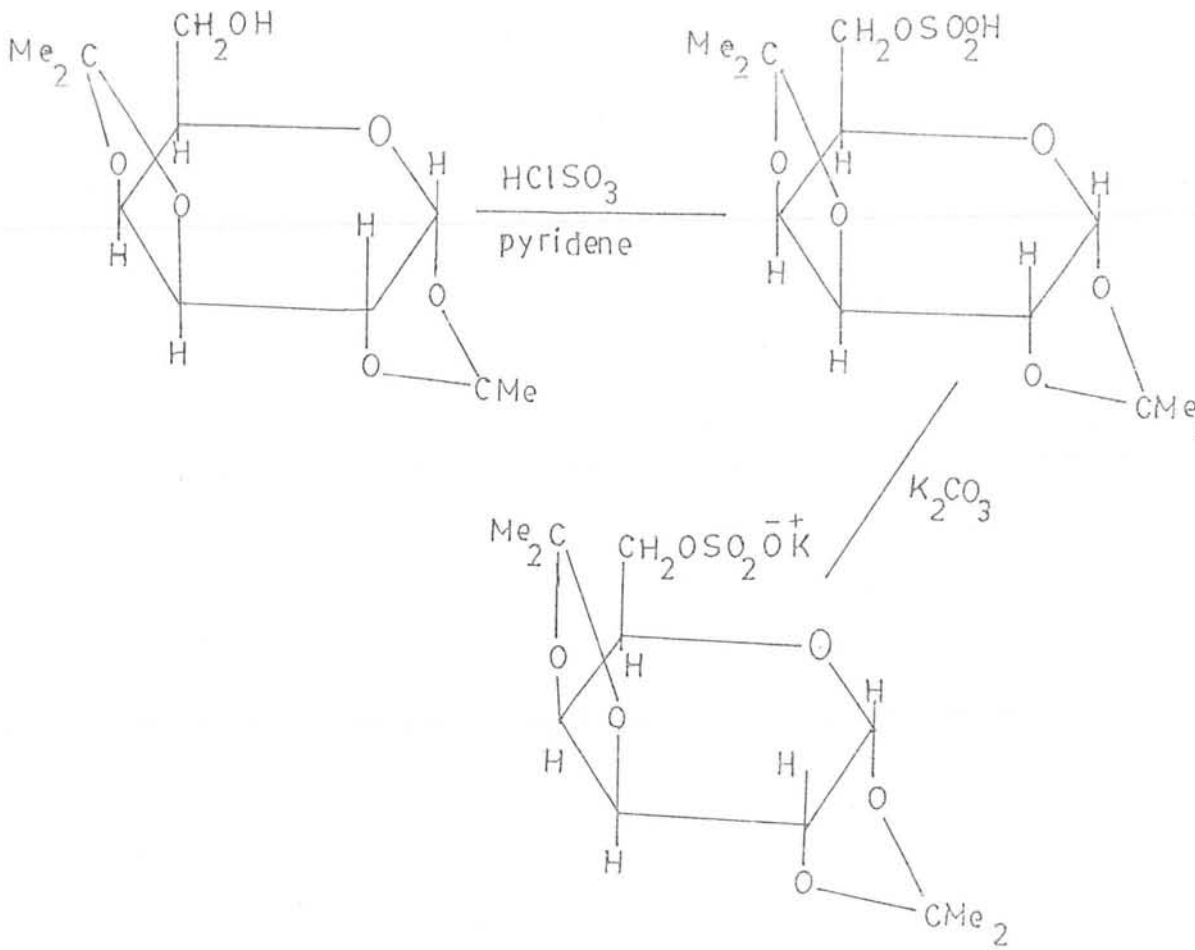
Rf = 1.1 cm

(Found C = 37.32 H = 4.89 = 38.91,K = 10,55%

Calculated C = 38.09, H = 5.02, O = 38.09, S = 8.46,

K = 10.31%

It is soluble in acetone, n-hexane while soluble in water.



2.8 PREPARATION OF HIGH POWER FUEL

Anhydrous potassium salt of acid sulfuric ester of 1,2:4,6-Di-O-isopropylidene-α-D-sorbofuranose (4 g; 0.01 mol.) was dissolved in distilled water (4 ml) heat the content at 100^{o}C for 10 min. To the solution, added ethyl alcohol (50 ml, 99.95%), by constant stirring until a clear solution was obtained. Allowed it to stand and cooled for half an hr. The product was light yellow thick colloidal gel (60 g).

(Found C = 41.21 H = 10.92, O = 42.60, K = 1.92

Rf = 1.2 cm.

It is soluble in methanol, ethanol and dimethyl sulfoxide, while insoluble in acetone, chloroform and ether. It burns with presistant blue flame.

TABLE 2.

SENSIBLE SOURCES

Sample Number	Common Name	Biological Name	Colour
A	Barley	Hordevm vulgare	Brown
B	Wheat	Tritium aestivum	Brown
C	Rice	Oryza sativa	White
D	Oats	Avena sativa	Light cream
E	Rye	Secale cereale	Brown
F	Corn (maize)	Zea mays	Light yellow
G	Molasses (non-crystalline sugar)		Dark Brown

TABLE 2.1

COMPOSITION OF SENSIBLE SOURCES (per 2 gm)

Sample Number	Dry matter %	Protein %	Fats %	Crude fiber %	Ash %	Carbo-hydrate %
A	92.44	12.00	1.30	4.50	1.81	72.75
B	90.06	12.08	3.25	3.41	1.72	69.82
C	87.71	8.31	2.13	1.40	0.68	74.93
D	86.91	12.85	6.90	1.30	2.90	63.61
E	85.30	10.91	1.00	2.20	1.70	69.11
F	88.30	8.91	3.20	2.30	1.30	72.23
G	83.74	3.55	-	-	1.37	54.00

TABLE 2.2

COMPOSITION OF MALT HUSKS (Per 5 g)

Sample Number	Dry matter %	Protein (solid) %	Fat %	Crude fibre %	Ash %	Carbo-hydrate %
A	25.42	7.96	1.20	15.94	1.37	15
B	19.42	7.93	1.10	10.91	0.8	17
D	20.41	7.33	1.0	9.81	0.9	9
E	20.10	7.11	0.81	8.99	0.9	13
F	21.31	6.31	1.23	9.92	1.1	14

TABLE 2.3

COMPOSITION OF ALCOHOLIC PRODUCT (Per 50 ml)

Sample Number	Protein (liquid) %	Fat %	Alcohol %	pH
A	0.3	Trace	4.7	4.5
B	0.28	Trace	4.5	4.7
D	0.26	Trace	4.4	4.8
E	0.25	Trace	4.4	4.7
F	0.26	Trace	4.2	4.9
G	-	-	4.3	4.7

TABLE 2.4

COMPOSITION OF SOLID ALCOHOLIC PRODUCT

REMAINED AFTER FERMENTATION (Per 50 gm)

Sample Number	Dry matter %	Crude protein (solid) %	Ash %
A	35.90	16.21	1.59
B	32.88	15.21	1.41
D	32.31	12.31	1.01
E	34.71	11.35	1.61
F	34.00	10.95	1.71

TABLE 3.1

ELEMENTAL ANALYSIS OF ISOPROPYLIDENE DERIVATIVES

S. No.	Compound	Carbon %		Hydrogen %		Oxygen %	
		Found	Calc.	Found	Calc.	Found	Calc.
1.	1,2:5,6-Di-O-isopropylidene -α-D-glucofuranose	54.93	55.38	7.21	7.69	37.86	36.92
2.	1,2:4,6-Di-O-isopropylidene -α-D-sorbofuranose	55.02	55.38	7.23	7.69	37.75	36.92
3.	1,2:3,4-Di-O-isopropylidene -α-D-galactopyranose	55.79	55.38	7.21	7.69	37.00	36.92

TABLE 3.2

ELEMENTAL ANALYSIS OF THE SALT OF ACID SULFURIC ESTER OF ISOPROPYLIDENE DERIVATIVES

S. No.	Compound	Carbon %		Hydrogen %		Oxygen %	
		Found	Calc.	Found	Calc.	Found	Calc.
1.	Potassium salt of acid sulfuric ester of 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose	38.00	38.09	4.95	5.02	37.99	38.09
2.	Potassium salt of acid sulfuric ester of 1,2:4,6-Di-O-isopropylidene-α-D-sarbofuranose	37.81	38.09	5.38	5.02	37.82	38.09
3.	Potassium salt of acid sulfuric ester of 1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose	37.32	38.09	4.89	5.02	38.91	38.09

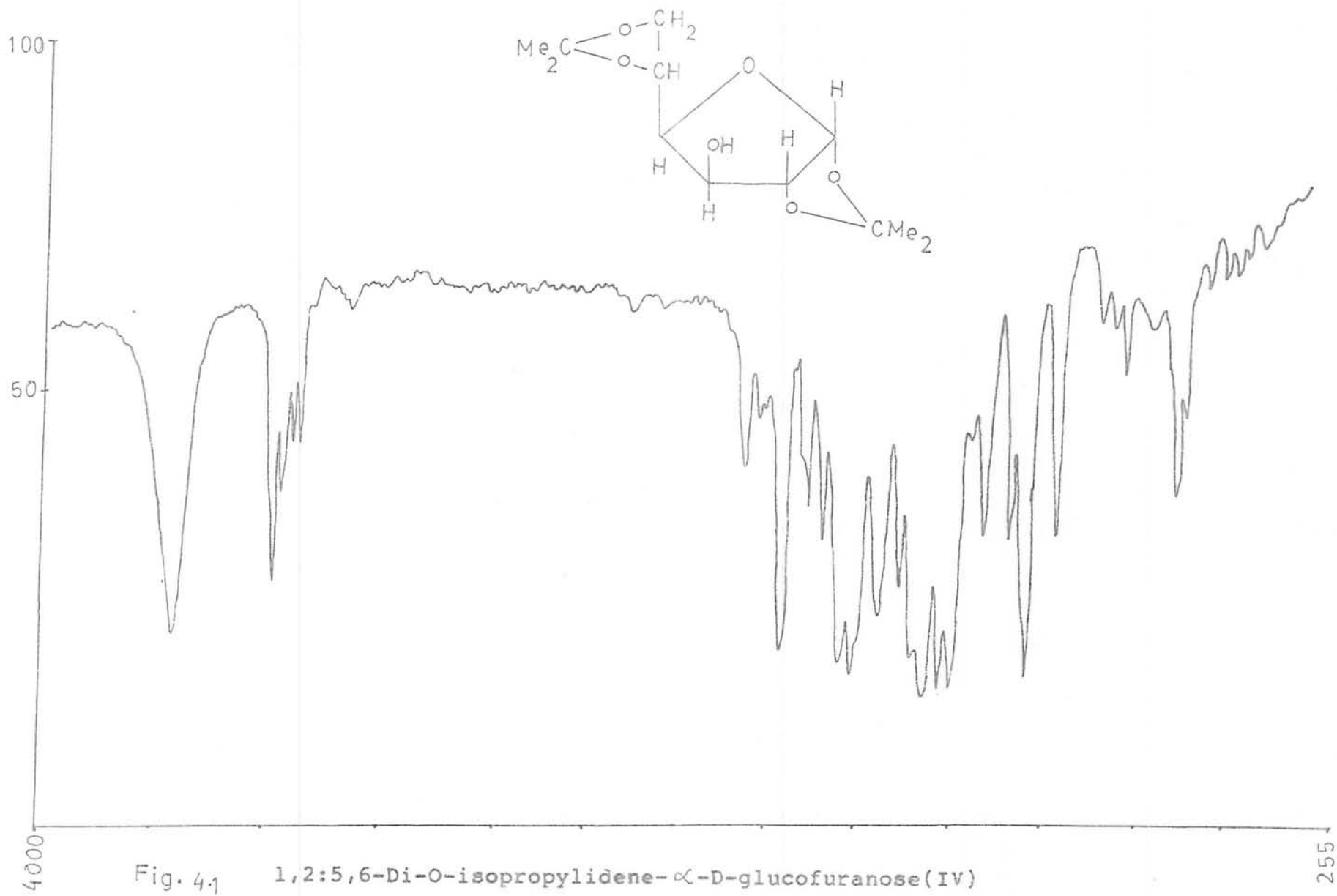


Fig. 4.1 1,2:5,6-Di-O-isopropylidene-∝-D-glucofuranose(IV)

TABLE 4.1

ASSIGNMENT OF INFRARED SPECTRAL DATA OF

1,2:5,6-Di-O-isopropylidene-∝D-Glucofuranose

Observed Frequencies (cm^{-1})	Band Assignment
3424	H-bonded-OH (S)
2986	$-CH_3$ (S)
2896	C—H (M)
2872	C—O (M)
1713	C=O (V)
1458	C—CH_2 (S)
1431	C—O (M)
1380	C—$(CH_3)_2$ doublet (S)
1320	O—H bending (M)
1287	
1245	C—oH with five member ring (M)
1221	C—O (S)
1161	C—$(CH_3)_2$
1116	C—O stretching (S)
1062	
1029	

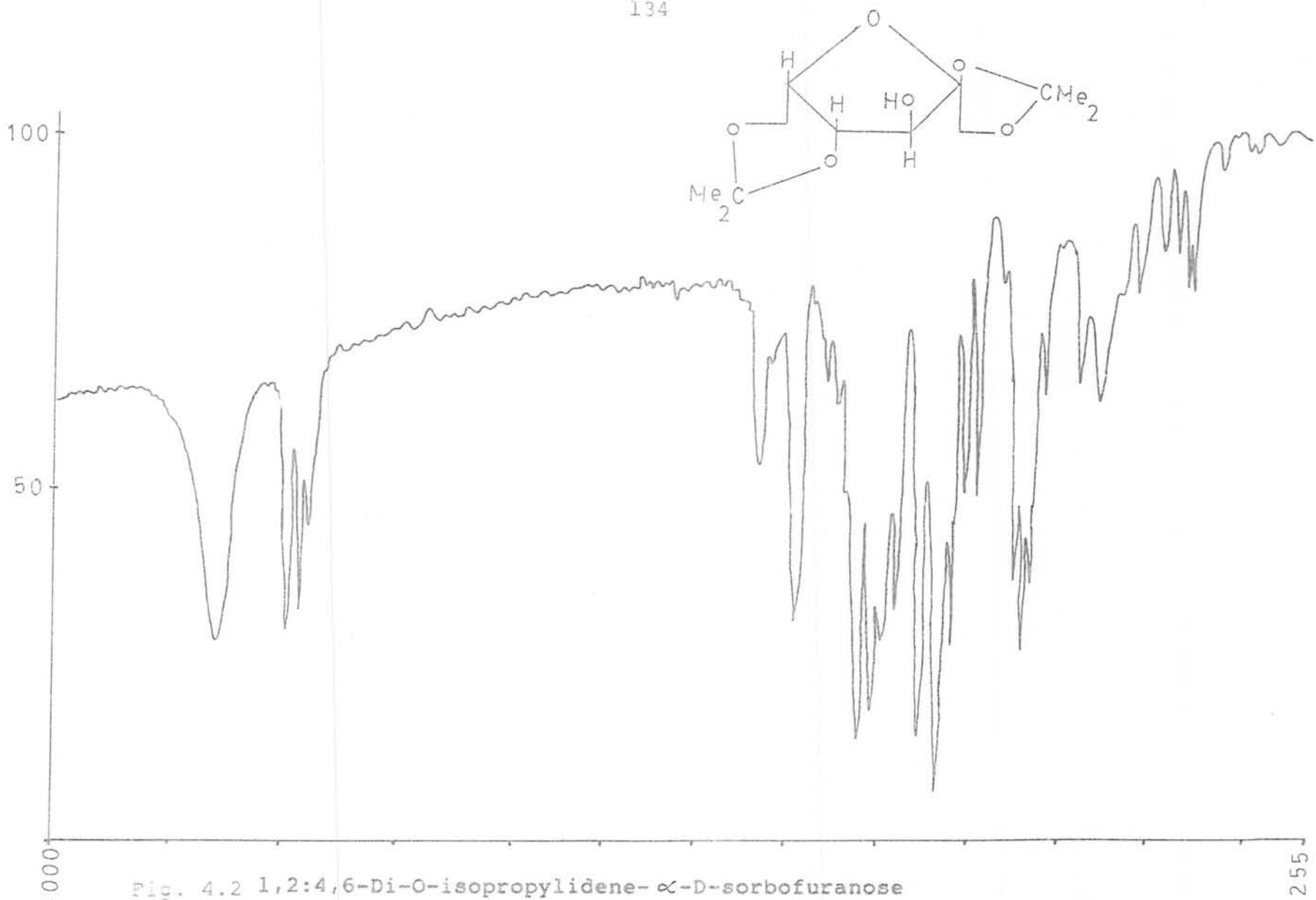


Fig. 4.2 1,2:4,6-Di-O-isopropylidene-∝-D-sorbofuranose

TABLE 4.2

ASSIGNMENT OF INFRARED SPECTRAL DATA OF

1,2:4,6-Di-O-isopropylidene-α-O-sorbofuranose

Observed Frequencies (cm^{-1})	Band Assignment
3292	H-bonded -oH (b)
2986	CH_3 (S)
2932	CH_2 (S)
2896	C—H stretching (M)
1455	C—CH_3 (M)
1377	C—$(CH_3)_2$ bending vib. (V)
1242	C—oH with five member ring (S)
1212	C—O stretching (S)
1194	C—$(CH_3)_2$
1158	sketal vib. (M)
1107	oH deformation vib.(S)
1068	C—O stretching (S)
1038	

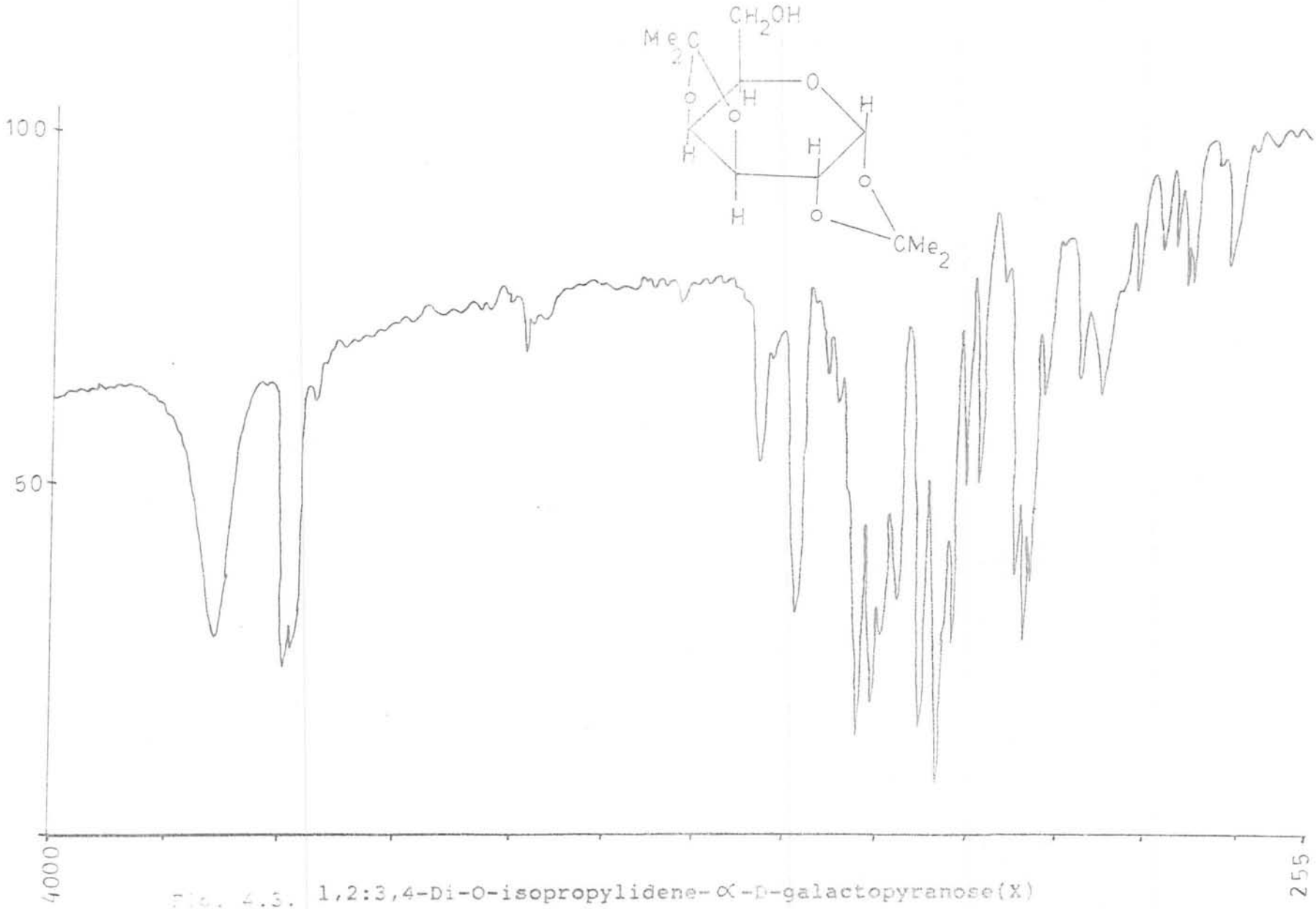


Fig. 4.3. 1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose(X)

TABLE 4.3

ASSIGNMENT OF INFRARED SPECTRAL DATA OF 1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose

Observed Frequencies (cm^{-1})	Band Assignment
3472	H-bonded—oH (S)
2986	$-CH_3$ (S)
2932	CH_2 (S)
2734	C—H stretching (V)
1458	C—CH_3 (S)
1386	C—$(CH_3)_2$ bending vib(S)
1254	C—oH (S)
1212	C—O (S)
1164	C—$(CH_3)_2$ sketal vib. (b)
1068	C-o stretching (b)

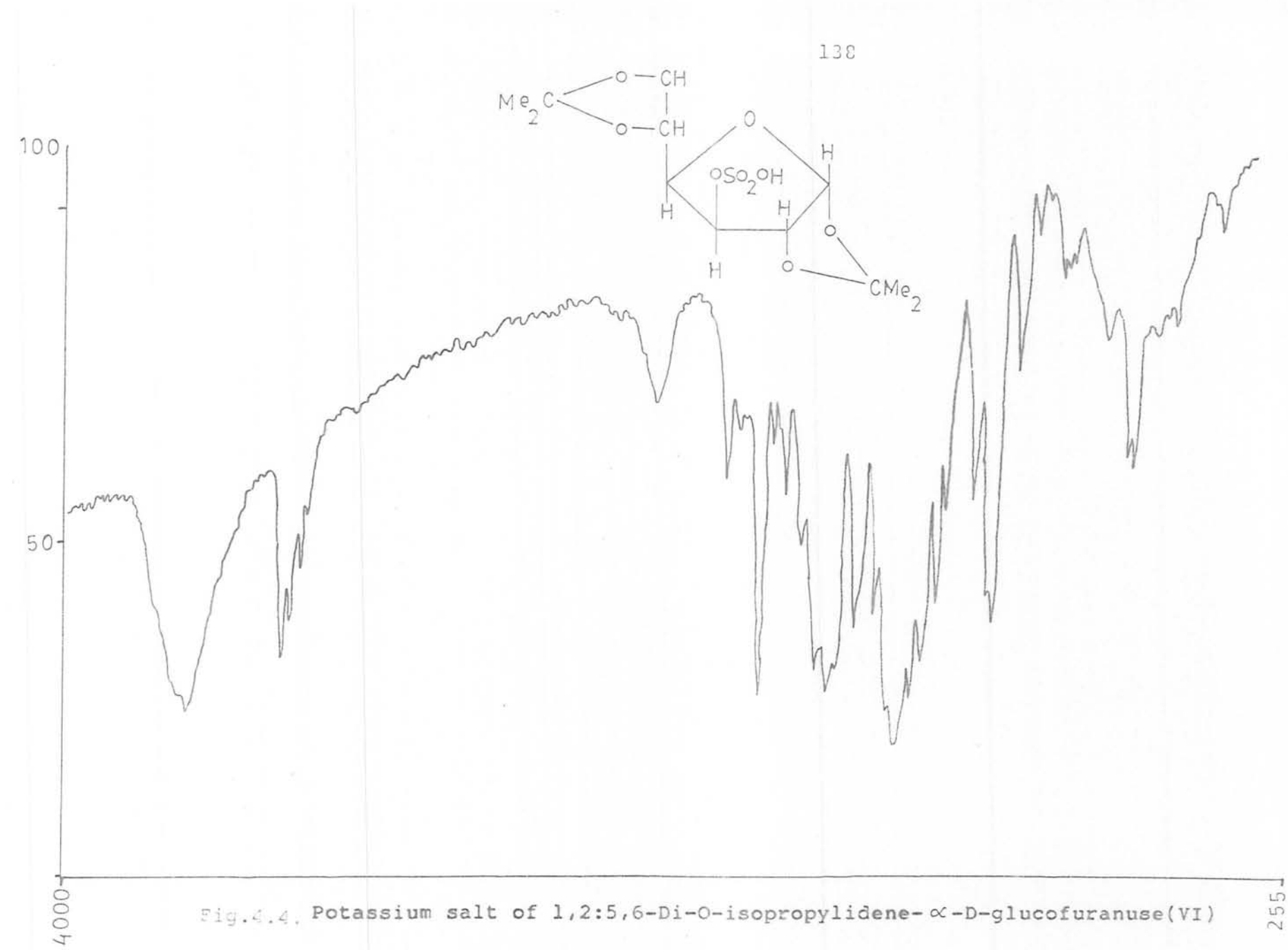


Fig.4.4. **Potassium salt of 1,2:5,6-Di-O-isopropylidene-∝-D-glucofuranuse(VI)**

TABLE 4.4

ASSIGNMENT OF INFRARED SPECTRAL DATA OF
potassium salt of acid sulfuric ester of
1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose

Observed Frequencies (cm^{-1})	Band Assignment
3424	H-banded —oH (S)
2980	CH_3 (S)
2932	CH_2 (S)
2855	C—H stretching (V)
1629	Ring
1416	C—CH_3 (M)
1353	C—$(CH_3)_2$ bending vib (S)
1320	$-So_2-$ O— (S)
1278	C—oH with five member
1251	ring (b)
1221	$-SO_2-$ O-(S)
1161	C—$(CH_3)_2$ sketal vib. (b)
1116	
1028	S=O stretching (V)
1005	

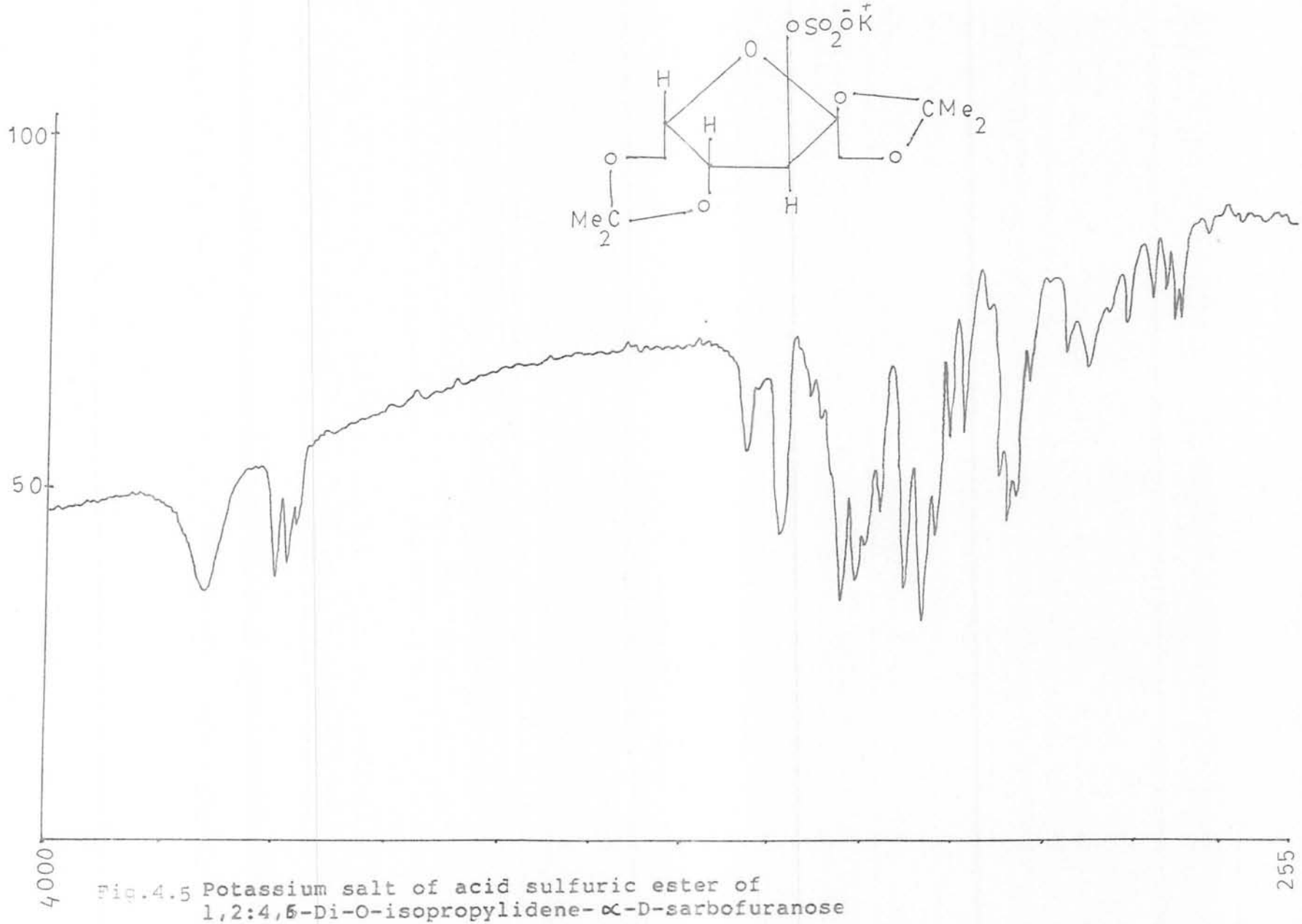


Fig.4.5 Potassium salt of acid sulfuric ester of 1,2:4,5-Di-O-isopropylidene-∝-D-sarbofuranose

TABLE 4.5

ASSIGNMENT OF INFRARED SPECTRAL DATA OF
potassium salt of acid sulfuric ester of
1,2:4,6-Di-O-isopropylidene-α-D-sarbofuranose

Observed Frequencies (cm^{-1})	Band Assignment
3292	H-bonded-oH (b)
2980	CH_3 (S)
2932	CH_2 (S)
2890	C—H stretching (V)
1455	C—CH_3 (M)
1377	C—$(CH_3)_2$ bending vib.(V)
1314	$-SO_2-$ O— (V)
1242	C—oH with five member ring (S)
1212	$-SO_2-$ O— (S)
1158	C—$(CH_3)_2$ sketal vib. (S)
1104	o—H bending (S)
1065, 1038, 1008	S=o stretching (S)

b = board; M = medium; V = very weak; S = sharp.

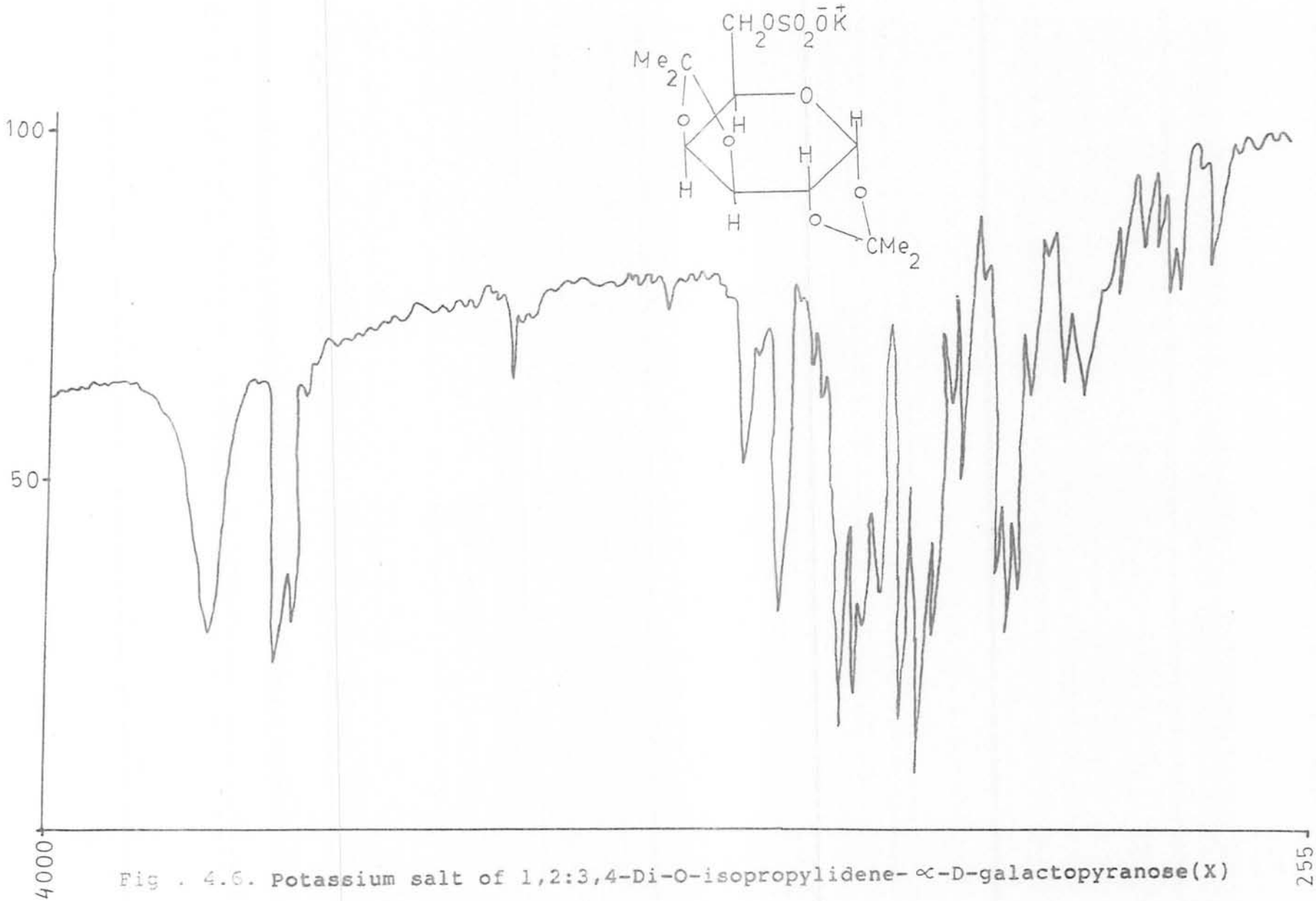


Fig . 4.6. **Potassium salt of 1,2:3,4-Di-O-isopropylidene-∝-D-galactopyranose(X)**

TABLE 4.6

ASSIGNMENT OF INFRARED SPECTRAL DATA OF
potassium salt of acid sulfuric ester of
1.2:3,4-Di-O-isopropylidene-α-D-galactopyranose

Observed Frequencies (cm^{-1})	Band Assignment
3286	H-bonded-oH (b)
2980	CH_3 (S)
2932	CH_2 (S)
2896	C—H stretching (V)
1455	C—CH_3 (S)
1380	C—$(CH_3)_2$ beding vib.(S)
1314, 1242	SO_2— O— (M)
1212, 1194	-SO_2— O— (S)
1158, 1124	C—$(CH_3)_2$ sketal vib. (S)
1065	C—$(CH_3)_2$ (S)
1038, 1008	C—o stretching (M)

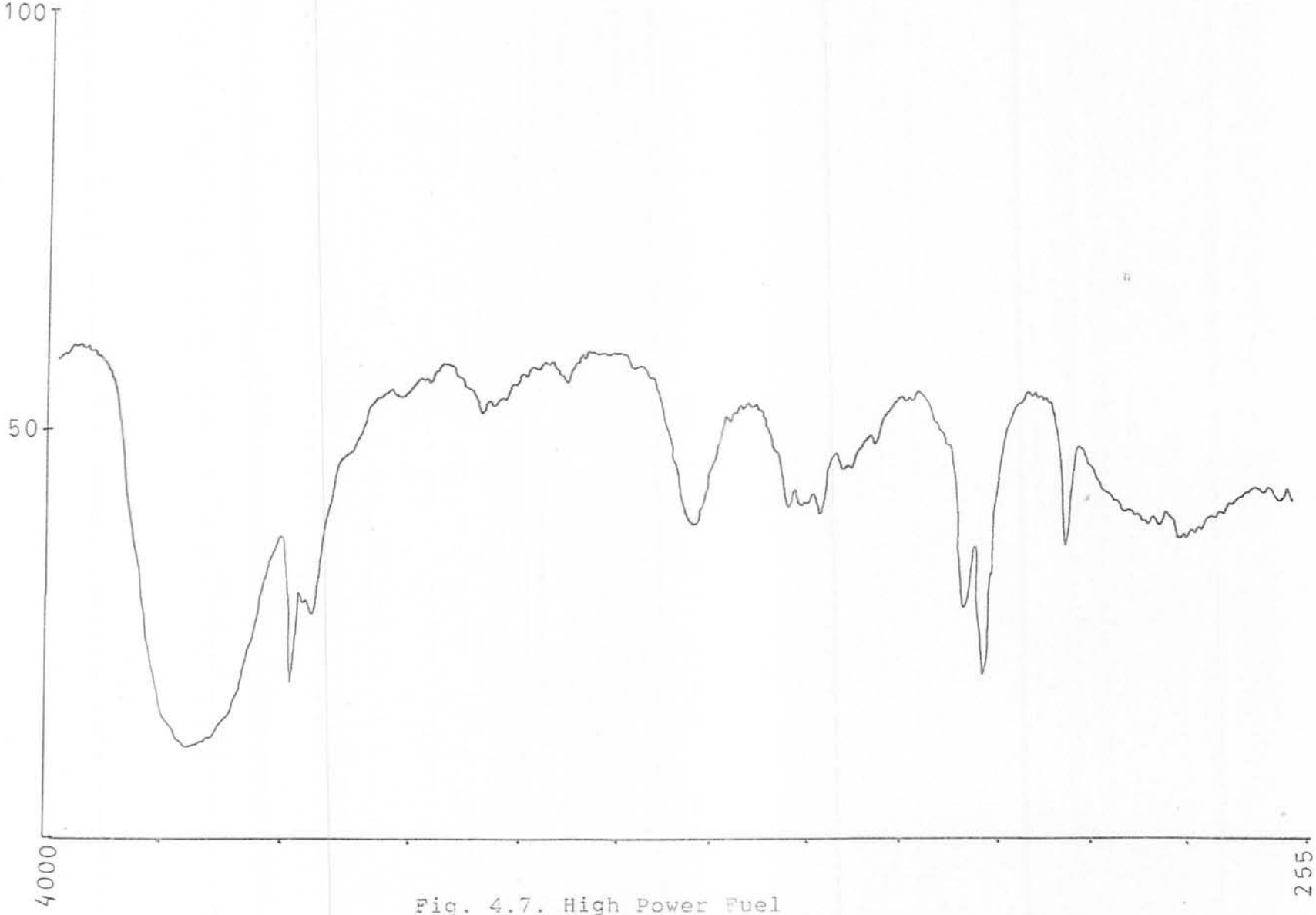


Fig. 4.7. High Power Fuel

TABLE 4.7

ASSIGNMENT OF INFRARED SPECTRAL DATA OF

HIGH POWER FUEL

Observed Frequencies (cm^{-1})	Band Assignment
3406	Free o — H stretching (b)
2974	$-CH_3$ sym. deformation doublet (S)
2890	$\geq CH_2$ (b)
1650	C — o — C (b)
1452	$-CH_3$ sym. deformation (M)
1425	SO_2 — o — (V)
1386	$\geq C(CH_3)_2$ sym. doublet (V)
1275	O — H bending
1083	C — O stretching (Alcohol)
1044	C — o primary alcohol (S)
876	CH_3 — CH_2 — (S)

TABLE 5.1

DTA AND DSC RESULT

Sample	Temperature °C	Loss in weight(%) Found	Calculated	DTA	DSC	Volatile matter	Intermediate
Gellified matter	91	80	81	End.	-	$C_2H_5OH+H_2O$	$C_{12}H_{19}O_9SK$
	301	-	-	Exo.	-	CO	K_2SO_4
	357	-	-	-	Exo.	-	-
	358	-	-	Exo.	-	Crystalline transition	K_2SO_4
	388	-	-	-	Exo.	-do-	K_2SO_4

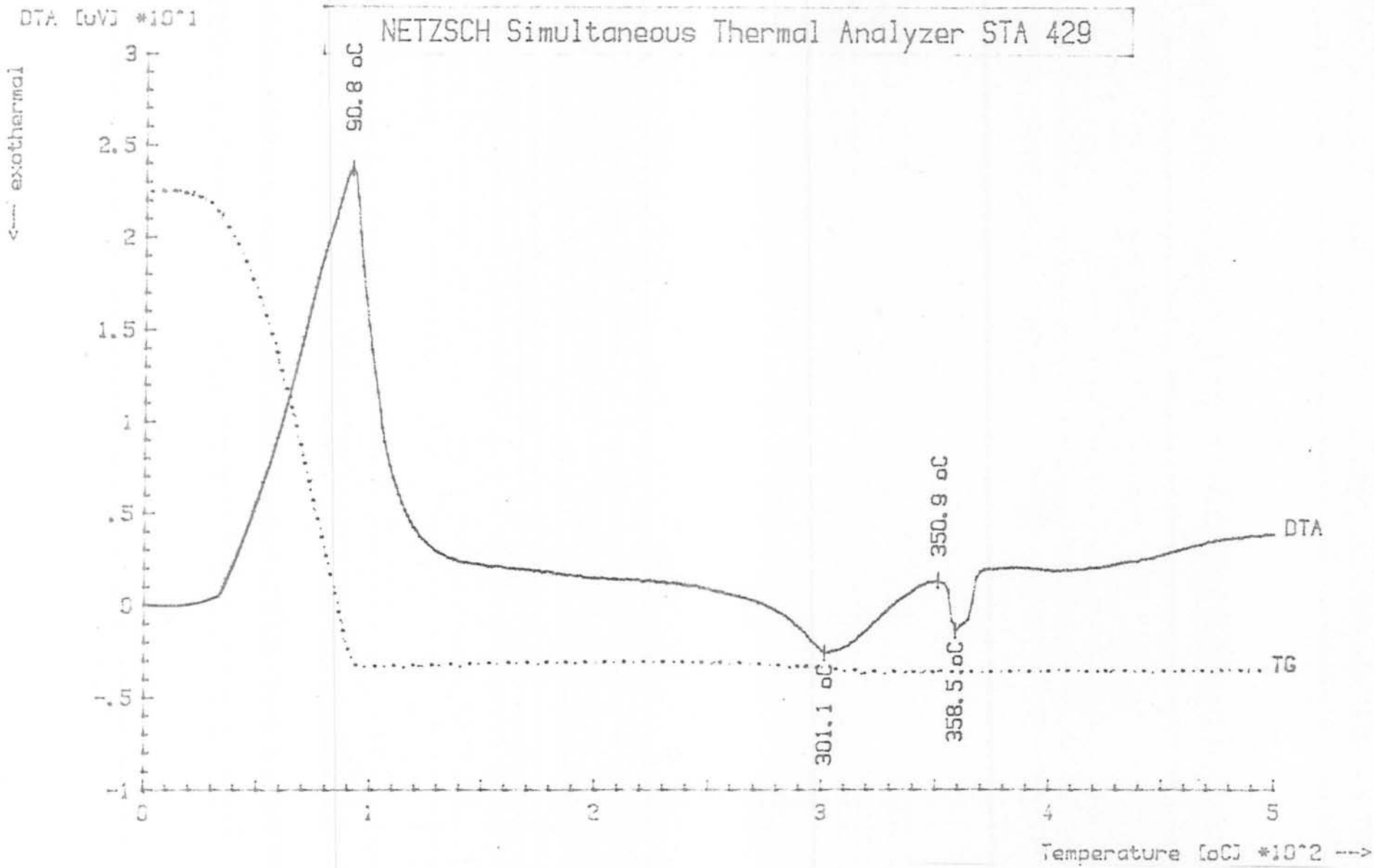


Fig. 5.2. Combined DTA and TG of High Power Fuel

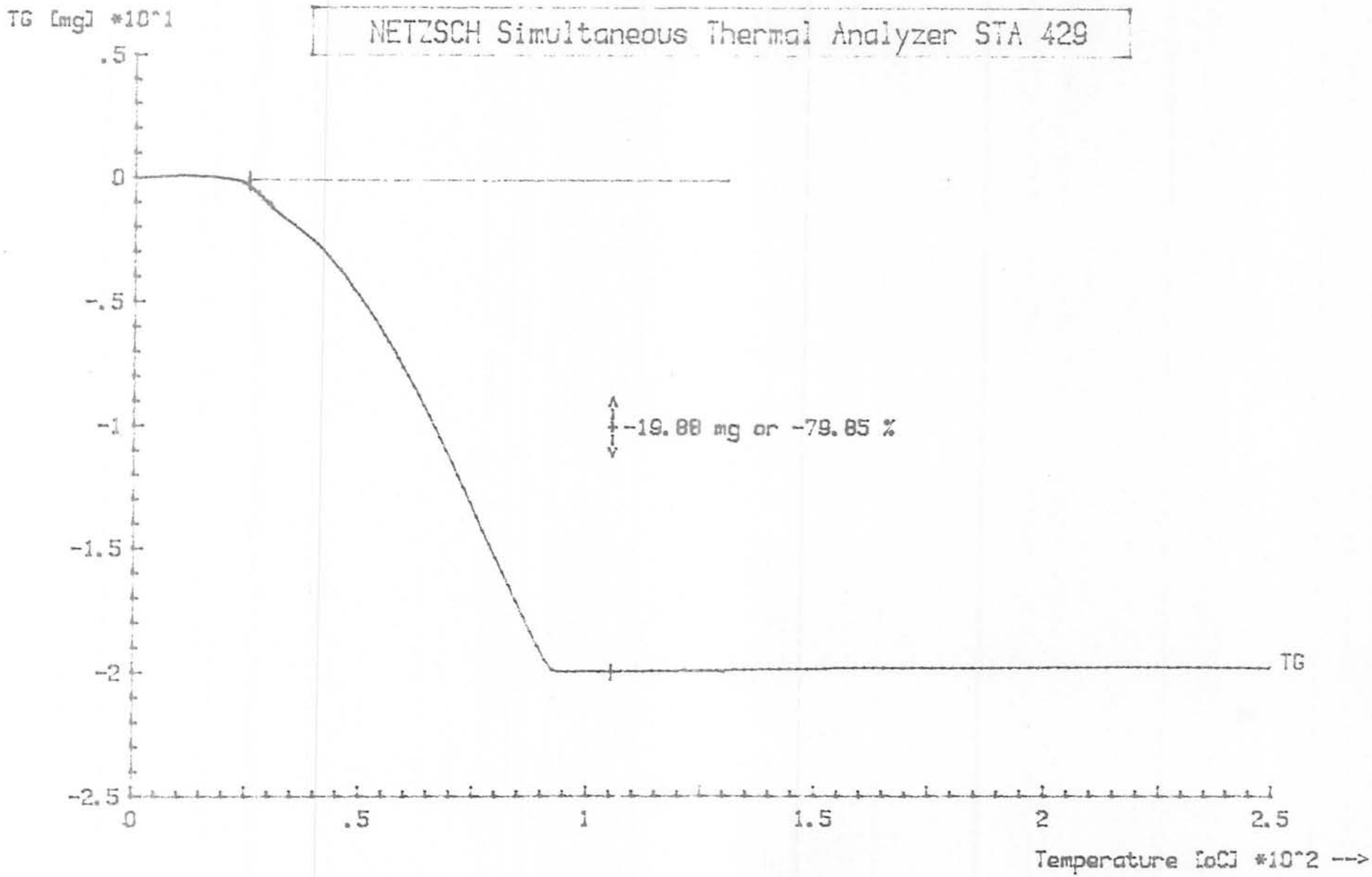


Fig. 5.3. TG of High Power Fuel

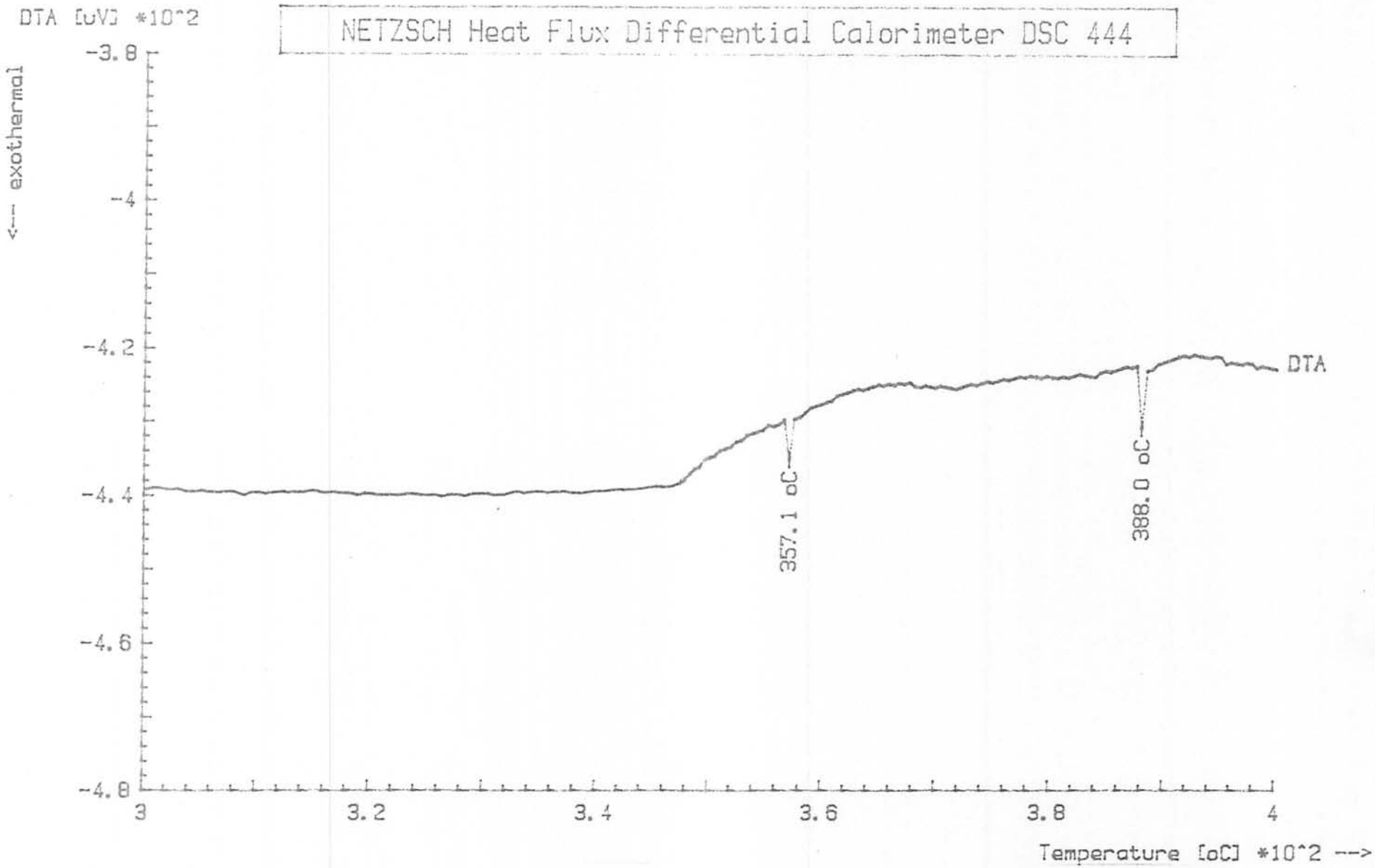


Fig. 5.4. DSC of High Power Fuel

TABLE 6.1

CONCENTRATION OF MACRONUTRIENTS IN SENSIBLE SOURCES
(mg%, dry weight)

Sample Number	A	B	C	D	E	F	G
Metals							
Sodium (Na)	0.43 ± 0.004	0.063 ± 0.001	0.031 ± 0.001	0.041 ± 0.002	0.32 ± 0.003	0.074 ± 0.001	66.51 ± 0.01
Potassium (K)	6.797 ± 0.061	5.193 ± 0.047	1.752 ± 0.011	1.631 ± 0.018	4.312 ± 0.032	3.613 ± 0.022	1383.22 ± 0.018
Calcium (Ca)	0.456 ± 0.003	0.868 ± 0.007	0.0817 ± 0.001	0.613 ± 0.006	0.723 ± 0.006	0.031 ± 0.003	18.98 ± 0.02
Magnesium (Mg)	2.785 ± 0.012	1.812 ± 0.023	0.353 ± 0.003	1.732 ± 0.022	1.413 ± 0.02	2.021 ± 0.014	23.84 ± 0.03

TABLE 6.2

CONCENTRATION OF MICRONUTRIENTS IN SENSIBLE SOURCES
(mg/kg, dry weight)

Sample Number	A	B	C	D	E	F	G
Metals							
Manganese (Mn)	22.460 ± 0.200	31.480 ± 0.32	8.952 ± 0.081	18.441 ± 0.192	17.312 ± 0.30	7.651 ± 0.068	2.1 ± 0.02
Iron (Fe)	22.343 ± 0.189	16.281 ± 0.162	4.401 ± 0.038	17.941 ± 0.178	15.345 ± 0.143	12.691 ± 0.0923	7.7 ± 0.05
Zinc (Zn)	29.061 ± 0.188	30.897 ± 0.281	17.503 ± 0.147	22.4421 ± 0.192	21.385 ± 0.200	22.131 ± 0.214	7.24 ± 0.24
Copper (Cu)	3.261 ± 0.030	2.580 ± 0.023	0.818 ± 0.006	2.213 ± 0.029	2.100 ± 0.021	1.710 ± 0.009	1.46 ± 0.02
Chromium (Cr)	1.254 ± 0.011	1.280 ± 0.013	1.151 ± 0.011	0.934 ± 0.011	0.953 ± 0.012	*	0.57 ± 0.012
Cadmium (Cd)	0.253 ± 0.002	0.069 ± 0.001	0.303 ± 0.002	0.153 ± 0.003	0.092 ± 0.002	0.298 ± 0.003	1.29 ± 0.02
Mercury (Mg)	0.0213 ± 0.001	0.098 ± 0.001	0.003 ± 0.001	0.010 ± 0.001	0.0092 ± 0.0012	0.115 ± 0.001	*

Continued Table 6.2

Lead (Pb)	0.2010 ± 0.0023	0.439 ± 0.0039	0.503 ± 0.002	0.1701 ± 0.0021	0.1231 ± 0.001	1.300 ± 0.009	*
Arsenic (As)	0.108 ± 0.001	0.119 ± 0.001	*	*	*	0.91 ± 0.001	*
Nickle (Ni)	1.460 ± 0.013	0.048 ± 0.001	1.16 ± 0.011	1.021 ± 0.021	0.910 ± 0.02	1.790 ± 0.0092	*

* = below detection limit.

TABLE 6.3

CONCENTRATION OF TRACE ELEMENTS IN HIGH POWER FUEL

Trace Element	Concentration (mg%)
Fe	0.13
Cr	*
Ca	*
Na	0.10
Mg	0.3
K	31.72
Zn	*
Pb	0.2
Cu	*
Cd	*
As	*
Hg	*
Ni	*
Mn	*

* Below detection limit.

CHAPTER 3

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

3.1 The initial analysis results of sensible source of wheat, corn, barley, rye, oat and molasses are reproduced in Table-1.

3.2 Fermentation of these sensible sources exhibit the production of high fibrous protein, HFP; liquid protein, LP; carbon dioxide, ethanol and soft drink. Comparison of the results of sensible sources after fermentation are recorded.

WHEAT: HFP, 3.85; LP, 0.3; CO_2, 44; EtoH, 4.5; soft drink, 92%.

BARLEY: HFP, 3.94; LP, 0.3; CO_2, 43; EtoH, 4.7; soft drink, 91%.

OAT : HFP, 4.87; LP, 0.26; EtoH, 4.4; CO_2, 43.5; soft drink, 92%.

RYE:, HFP, 2.54; LP, 0.25; EtoH, 4.4; CO_2, 43; soft drink, 90%.

CORN: HFP, 2.34; LP, 0.26; EtoH, 4.2; CO_2, 42; soft drink, 92%.

MOLASSES: HFP, nil; LP, nil; CO_2, 43; EtoH, 4.3; soft drink, nil%.

TABLE 1

ANALYSIS OF THE PRODUCTS

	Corn	Wheat	Rye	Barley	Oats	Molasses
Crude fiber (%)	2.3	3.41	2.2	4.5	1.3	-
Total dietry fiber (%)	84.34	85.15	81.2	86.05	83.36	54
Soluble (%)	81.7	81.74	79.0	81.55	82.06	-
Insoluble (%)	2.3	3.41	2.2	4.5	1.3	-
Protein(%)	8.91	12.08	10.91	12.00	12.85	-
Total Carbo-hydrates(%)	74.54	73.23	71.31	77.25	64.91	54
Avail, Carbo-hydrtes(%)	72.23	69.82	69.11	72.75	63.61	54
Calories (per 100 g)	353.36	356.77	329.08	350.70	367.94	216
Fat(%)	3.20	3.25	1.00	1.30	6.90	-
Ash(%)	1.3	1.72	1.70	1.81	2.90	1.37
Moisture(%)	11.70	9.94	14.70	7.56	13.09	16.26
Calcium(mg%)	0.131	0.868	0.723	0.456	0.613	18.98
Sodium(mg%)	0.074	0.06	0.32	0.43	0.041	66.51
Potassium(mg%)	3.61	5.19	4.31	6.79	1.63	138.3
Iron (mg/kg)	12.69	16.28	15.34	22.34	17.94	7.7
Copper(mg/kg)	1.71	2.58	2.10	3.26	2.21	1.46
Zinc (mg/kg)	22.10	30.89	21.38	29.06	22.44	7.24
Manganese((mg/kg)	7.65	31.48	17.31	22.46	18.44	2.1

3.3 The exploted sensible sources show the following recoveries are made during the fermentation. High Fibrous Protein 2-4%, which can be used for human consumption in the bakeries etc. Liquid Protein 0.2-0.3% can be used in lieu of or in addition to fresh water for cattle. Recovery of carbon dioxide 43% from fermentation vapour. This can then be sold as a gas or further processed into dry ice. Non-alcoholic drink 93% is treated with flavour and can be used for export.

3.4 Down stream processing of 5% ethanol into high power fuel (gellified fuel) for cooking and defence purposses. Ethanol is reproduced as a power fuel. The characteristics properties are given in Table 2 and compared to commonly known fuels. Ethanol[168,169] is completely soluble in gasolin, diesel or fuel oil, provided no water is present in the system. The combustion properties of the power alcohol falls very close to that of gasoline. It mixes and burns well with gasoline in internal combustion engines. The power fuel such as cannot be used as diesel substitute, because of difference in their combustion properties.

3.5 The power fuel is further converted to high power

TABLE 2

MAIN PHYSICAL/CHEMICAL PROPERTIES OF
POWER ALCOHOL AND HYDRO CARBON FUELS

Property	Ethanol/ power alcohol	Gasoline	Diesel	Fuel Oil
Formula	C_2H_5OH	C_4- C_{12} Hydro carbon	$C_{14}C_{19}$ Hydro carbon	C_{20} Hydro carbon
Molecular weight	46.1	100-105 avg.	240 avg.	-
Composition (Wt.%)				
Carbon	52.2	85-88	85-88	85-87
Hydrocarbon	13.1	12-15	12-15	10-11
Oxygen	34.7	Negative	Negative	Negative
Specific gravity	0.79	0.72-0.78	0.83-0.88	0.88-0.98
Octane Number				
Research	106-111	79-88	-	-
Motor	89-100	71-90	-	-
Octane Number	0.5	5-10	45-55	NA
Solubility in water	Infinite	0	0	0
Calorific value Kcal/mole	5048	7700	8738	8795

fuel (ix-xii) by treating it with derivative of carbohydrates (glucose, fructose galactose) prepared by the interaction of these carbohydrate (0.20 mol.) with acetone (250 ml) in the presence of $ZnCl_2$, H_3Po_4 and maintain the experimental conditions. The derivatives so produced are further converted into salts by treating it with chlorosulphonic acid (2.56 ml), pyridene (20 ml) in the presence of potassium carbonate (0.02 mol). The salt so produced is treated with the power alcohol to form gellified material.

3.6 Reaction sequence resulting from the fermentation of the sensible sources and formation of high power fuel.

$$S.S. \longrightarrow (C_6H_{10}O_5)_n \cdot nH_2O \qquad (i)$$

$$(C_6H_{10}O_5)_n \, nH_2O \longrightarrow n\, C_{12}H_{22}O_{11} + H_2O \qquad (ii)$$

$$n\, C_{12}H_{22}O_{11} + H_2O \longrightarrow n(2C_6H_{12}O_6) \qquad (iii)$$

$$C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3 \qquad (iv)$$

$$C_3H_6O_3 \longrightarrow CH_3COCOH + H_2O \qquad (v)$$

$$CH_3COCOH + \tfrac{1}{2}O_2 \longrightarrow CH_3COCOOH \qquad (vi)$$

$$CH_3COCOOH \longrightarrow CH_3COH + CO_2 \qquad (vii)$$

$$2CH_3COH + 2H_2 \longrightarrow 2CH_3\,CH_2OH \qquad (viii)$$

$$C_6H_{12}O_6 + 2(Me)_2CO \longrightarrow C_{12}H_{20}O_6 \qquad (ix)$$

$$(ix) + ClSO_3H \longrightarrow C_{12}H_{20}O_9S + HCl \qquad (x)$$

$$(x) + K_2CO_3 \longrightarrow C_{12}H_{19}O_8S\bar{O}K^+ + KHCO_3 \qquad (xi)$$

$$(xi) + nC_2H_5OH + nH_2O \longrightarrow C_{72}H_{230}O_{54}\,SK \qquad (xii)$$

In Sections 3.2, 3.3, 3.4 and 3.5 of this chapter were summarized the results of the study of the fermentation and formation of high power fuel. In this section these results will be considered in terms of possible reaction sequences.

Sensible sources involve in the conversion of starch $(n.C_6H_{10}O_5).nH_2O$ into sugar $(nC_{12}H_{22}O_{11})$ by diastase enzymic effect. The bio-degradation of sugar into glucose is caused by the maltase enzymic effect (i-iii). The pyruvic acid is an important intermediate of the degradation of carbohydrates by yeast at the

same rate as does the glucose (iv-vi). The reaction proceeds in acidic condition.

The glucose molecule is broken into two triose molecules which are hydrated to form methylglyoxal. The triose loses water to form glyoxal which in turn is oxidized to pyruvic acid. The pyruvic acid is decarboxylated to aldehyde which on reduction produced ethanol (vi-viii). Glucose is treated with acetone to convert it into its isoproplidene derivative and then these derivative are transferred into useful salts which changes power alcohol to high power fuel (ix-xii).

Ethanol molecules, C_2H_5OH experience hydrogen bonding between themselves in pure alcohol system. This tendency of alcohol shows an interesting transformation to produced high power fuel when ethanol comes in contact with the derivative of glucose salt it changes into gellified material. It is possible that gels are frequently described[54-57] as concentrated solution of a liquid in a solid. The liquid system in this case is a system of high viscosity and contains distinctive properties of three dimensional network (Fig. 1).

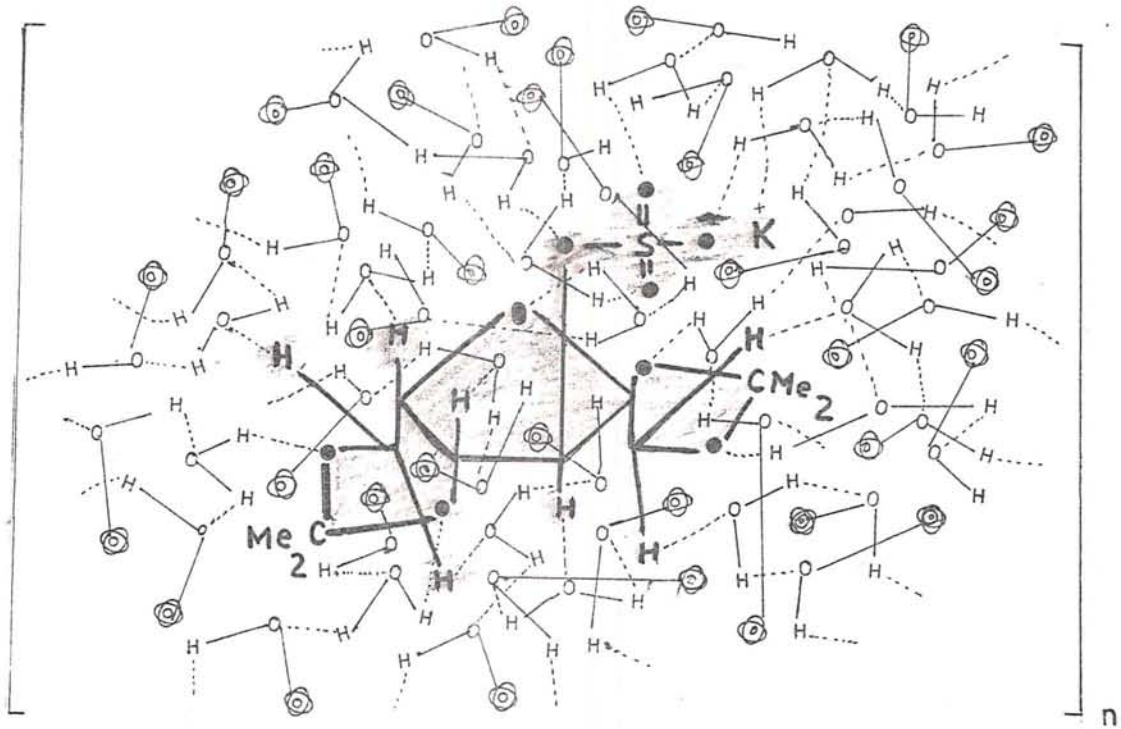


Fig.1 . Proposed Structure of High Power Fuel

3.7 Evidence in Favour of the Formation of High Power Fuel

The evidence for these proposed structure rest first on elemental analysis, C, 42.60; H, 11.29; O, 42.60; K, 1.92%. Thermal analysis results are reproduced in Fig. 2, TG shows loss in weight 80% corresponding to the elemination of volatile matter of ethanol present in the network. TG result is also supported by DTA which shows broad volatilization endotherm at 90°C. TG result however does not give interpreted results further to volatilization. DTA result shows two exothermic peaks at 301°C representing the decomposition of the residue indicating the breakdown of organic matter. Further peak at 358°C is an exothermic change due to the crystalline transition of the potassium salt. DSC result shows broad endothermic peak at 90°C and two exothermic peak at 357°C and 388°C respectively. Combine TG, DTA and DSC result support the existence of three dimensional network. IR result indicate the stretching frequency at 1425 due to the existence of SO_2-O-bond formation, the results are tabulated in Table 4.7. In the trace element study the result reveals the presence of potassium metal 31.72 mg%.

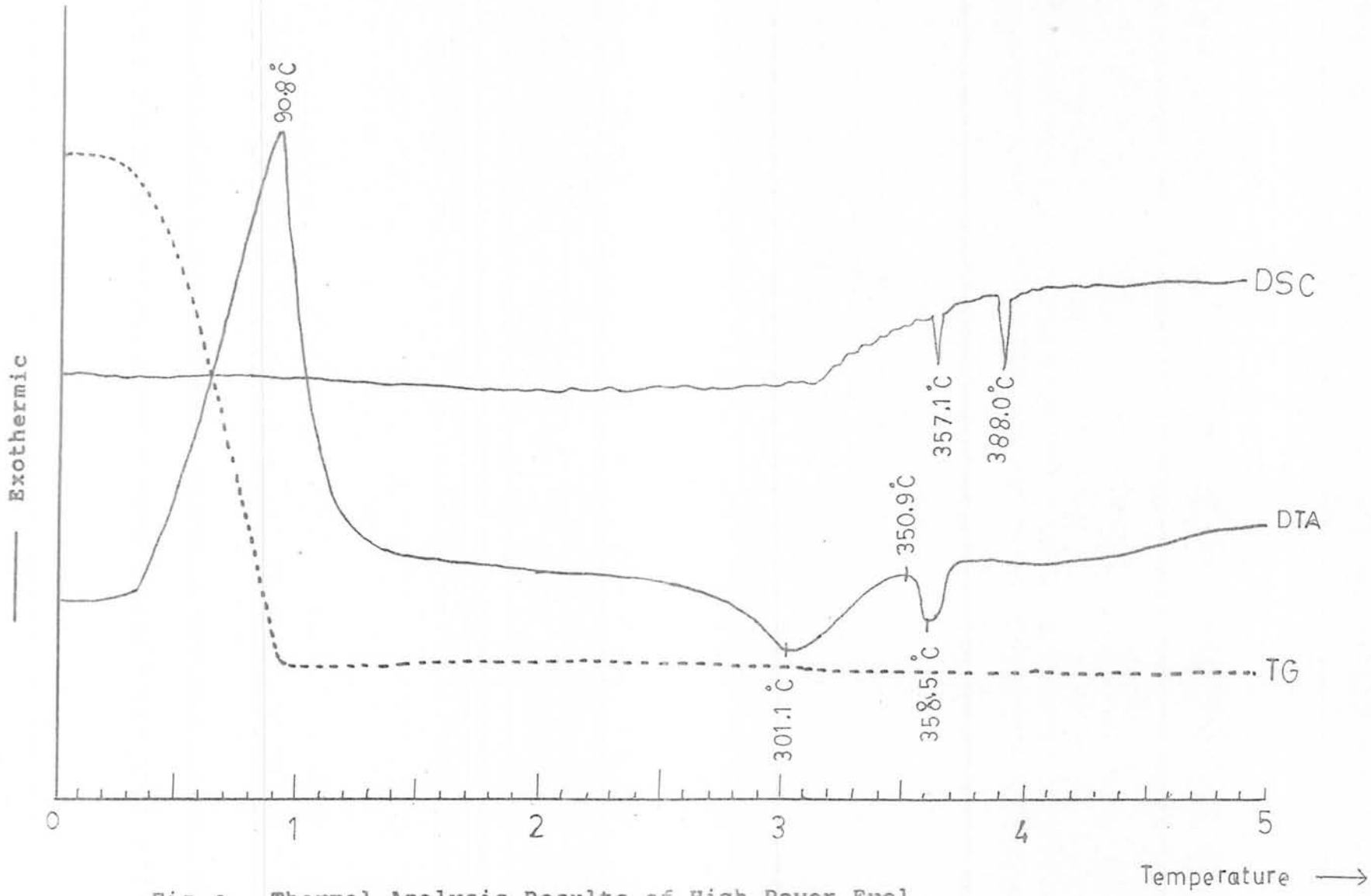


Fig. 2 Thermal Analysis Results of High Power Fuel

REFERENCES

REFERENCES

1. Baron, Stanley W., Brewed in America Boston (1962).
2. Grossman Harold J., Grossman's Guide to Wines, Spirits and Beers, rev. ed. New York (1964).
3. United States Brewers Foundation, A Beer Dispensing Hand Book, New York (1952).
4. Week's Morris, Jr., Beer and Brewing in America, New York (1949).
5. Prescott, S.C. and Dunn, Industrial Microbiology; 3rd ed. McGraw-Hill Book Company Inc. New York, p. 109 (1954).
6. The Encyclopedia Americana, Grolier Incorporated Danbury, U.S.A. (1987).
7. J.P.Arnold, Origin and History of Beer and Brewing (1911).
8. F.A.King, Beer Has A History (1947).
9. Alexander Findley, 'Chemistry in the Service of Man'. 8th ed. Western Printing Services (1964).
10. Goodrich, R.S., Chem. Eng. Prog. p. 29-34 (Jan. (1982).
11. Lipinsky et al., 'Sugar Crops as Source of Fuels', 11, Final Report. Battelle Columbus Division (Aug. 31, 1978).

12. Hough, Briggs, Stevens, Malting and Brewing Science, Chapter 19, Brewing Fermentations. Champman and Hall Ltd; London (1971).

13. H.L.Hind, Brewing Science and Practice Chapter 22, "Principles of Fermentation", Chapman and Hall Ltd., London (1950).

14. L.M.Christensen, p. 518, In R.W.Kerr, Chemistry and Industry of Starch, 2nd ed., Academic Press, New York (1950).

15. F.C.Webb, Biochemical Engineering, Van Nostrand (1964).

16. K.A.Underkofler, Industrial Fermentation, Chemical Publishing (1954).

17. M.Yudkin and R.Offord, "Comprehensible Biochemistry', Langman Group Ltd. (1973).

18. M.Yudkin and R.Offord, "Comprehensive Biochemistry," Longman Group Ltd. p. 228-232 (1973).

19. S.C.Prescott and C.G.Dunn, 'Industrial Microbiology', 3rd ed. McGraw-Hill Book Company Inc. New York p. 109 (1959).

20. L.L.Ingraham "Biochemical Mechanisms", John Wiley and Sons, Inc. New York (1962).

21. John R.Holum, "Introduction To Organic and Biological Chemistry". John Wiley and Sons, Inc. New York (1969).

22. David O.Johston, John T.Nettesille, James, Z.Wood, 'Chemistry, Man and Society', 4th ed. Saunders College Publishing, New York (1982).

23. K.Geiger, 'Brewing with Wheat Starch'. MBAA Technical Quarterly Vol. 9, No. 4, p. 195-199 (1972).

24. M.R.Sfat, 'Trends in brewing Materials', MBAA Technical Quarterly, Vol. 10, No. 4, p. 180-183 (1973).

25. T.M.Enari and M.Kinko, 'Unmalted Barley in Brewing, MBAA Technical Quarterly, Vol. 4, No. 3, p. 182-184 (1967).

26. Science and Technology Illustrated, Encylopaedia Britannica, Inc. Tokyo (1984).

27. McGraw-Hill Encyclopedia of Science and Technology 6th ed. McGraw-Hill Book Company New York (1987).

28. D.I.Wong et al., Fermentation and Enzyme Technology (1979).

29. The Microbiological Production of Industrial Chemicals, Sci. Amer., 245:159-178 (1981).

30. Under-Kofler and M.L.Wulf (eds.), Developments in Industrial Microbiology (1981).

31. J.W.Boyer and L.A.Underkofler, Chem. Met. Eng., 52, No. 12, 110 (1945).

32. W.H.Stark, R.E.Scalf, P.Kolachov and H.F.Willikie, Am. Soc. Brewing Chemists Proc., 49 (1941).

33. L.K.Arnold and L.A.Kremer, Corn as a Raw Material For Ethyl Alcohol, Iowa Eng. Exp. Sta., Bull. 167 (1950).

34. S.L.Adams, W.H.Stark and P.Kolachov, Cereal Chem., 20, 260 (1943).

35. H.F.Willkie, and J.A.Prochaska, Fundamentals of Distillery Practice, Louisville, Ky., Jos. E.Seagram and Sons (1943).

36. W.D.Gray, W.H.Stark and P.Kolachov, J.Bact., 43, 270 (1942).

37. M.C.Brockmann and T.J.B.Stier, J. Cellular Comp. Physiol., 29, 159 (1947).

38. J.M.Van Lanen, E.M.Le Mense, A.Anellis and J.Corman, Cereal Chem., 25, 325 (1948).

39. A.H.Cook, 'The Chemistry and Biology of Yeasts', Academic Press Inc., New York (1958).

40. H.A.Rose and J.S.Harrison, 'The Yeasts', Academic Press, London (1969).

41. A.M.Adams, Can. J. Research 27, 179 (1949).

42. G.C.Inskeep, A.J.Wiley, J.M.Holderby and L.P.Hughes, Ind. Eng. Chem., 43, 1702 (1951).

43. R.V.Givartovski, 'Edible Yeasts', Izvestiya Tzentral Nauch; Issledovatal Pischevoi Ukusovoi. Prom., Separate (1930).

44. R.V.Givartovski, "Edible Yeasts and Their Uses", Izvestiya Tzentral Nauch. Issledovated Inst. Pischevoi Prom., Separate (1930).

45. C.J.Alexopoulos and C.W.Mims, "Introductory Mycology" 3rd ed. John Wiley and Sons, New York (1979).

46. J.F.Hall, J. Inst. Brewing, 60, 482 (1954).

47. J.F.Hall, J. Inst. Brewing, 60, 486 (1954).

48. M.Ingram, 'Introduction to the Biology of Yeast', p. 169, Pitman, London (1955).

49. M.Ingram, 'Introduction to the Biology of Yeast', p. 157, Pitman, London (1955).

50. M.A.Rich and A.M.Stern, Can. J. Microbiol. 2, 453 (1956).

51. I.R.Joseph, P.E.Darrell and J.B.Donald, 'Essential of General Organic and Biochemistry', W.B.Saunders Company, Toronto (1969).

52. J.R.Francis, 'Concepts in Biochemistry', McGraw-Hill Book Company, Sydney (1967).

53. R.M.Henry and H.C.Eugene, 'Basic Biological Chemistry, Harper and Row, New York (1969).

54. H.G.Bungenberg de Jong, 'Colloid Science', Vol. 11, Elsevier Publishing co., Amesterdam, New York (1949).

55. J.D.Ferry, Advan. Protein Chem., 4, 1 (1948).

56. Yu. S.Lipatov and N.F.Proshlyakova, Usp. Khim. 30, 517 (1961); Russ. Chem. Rev. (Engl. Transl.), 30, 219 (1961).

57. K.J.Mysels, Introduction to Colloid Chemistry, Interscience Publishers, Inc., New York, p. 206 (1959).

58. T.G.Summit and M.L.Almquist, Borate Gel, U.S.Patent, 2, 645, 616 (1951).

59. H.Ohle and Jothmar, Alcohol Gel, U.S.Patent, 1, 783, 133 (1926).

60. M.Kirk, Utilization of Ethanol as a Farm Fuel (1983).

61. M.A.Clarke, Alternate Uses For Sugarcane and its product; Paper presented at 1979, Winter Meeting of ASAE.

62. Van Nostrand's Scientific Encyclopedia, 5th ed. Van Nostrand Reinhold Company, London (1976).

63. Vogel's Textbook of Organic Analysis, 4th ed, ELBS (1985).

64. R.N.Shreve, Chemical Process Industries', 3rd ed. McGraw-Hill Book company, Tokyo (1967).

65. Max. E.Rafelson and Stephen B.Binkley, 'Basic Biochemistry', 2nd ed. The Macmillan Company, London (1968).

66. Hourowitz, 'The Chemistry and Function of Protein' 2nd ed, Academic Press (1963).

67. Proteins and Amino Acids. In Biology Data Book. Altman and Ditmer (eds.), Fed. Am. Soc. Exper. Biol., pp. 388, 392 (1964).

68. The Proteins. Neurath and Bailey (eds.) 1st ed., 1953-1954; Neurath (ed.) 2nd ed. Vols. 1 and 2, 1963-1964, Academic Press.

69. A.Mazur and B.Harrow, 'Text Book of Biochemistry', W.B.Saunders Company, Philadelphia, London (1971).

70. G.R.Tristram and R.H.Smith: Amino Acid Composition of Proteins, Advan. Protein Chem., 18:227(1971).

71. N.S.Timasheff, and R.Townend, J. Am. Chem. Soc., 83, 464(1961); T.F.Kumosinski and S.N.Timasheff; J. Am. Chem. Soc., 886:5635(1966).

72. I.Fridovich, J. Biol. Chem., 238:592(1963).

73. S.J.Leach (ed.), Physical Principles and Techniques of Protein Chemistry, Part B, Academic Press, New York (1970).

74. R.L.Hill, Hydrolysis of Proteins, Advan. Protein Chem., 20:37(1965).

75. A.Meister, 'Biochemistry of the Amino Acid', Academic Press, New York (1965).

76. C.Tanford, Physical Chemistry of Macromolecules, Wiley, New York (1961).

77. J.Spragg, E.Schroder, J.M.Stewart, K.F.Austen and E.Haber, 'Biochemistry', 6:3933(1967).

78. F.F.Richards, R.W.Sloame, Jr. and E.Haber, Biochemistry, 6:476(1967).

79. M.Florkin,and E.H.Stolz (eds.), 'Comprehensive Biochemistry', Vols. 2,3,7 and 8, Elsevier, New York (1963).

80. J.S.Hough, D.E.Briggs and R.Stevens, 'Malting and Brewing Science', Champman and Hall, London (1971).

81. T.Wainwright, 'Biochemistry of Brewing', W.P.K. Findlay, ed., Macmillan, London via CRC Press, Cleveland (1971).

82. M.C.Meilgaard, 'Wort Composition', MBAA Tech. Quart. 13, 78(1976).

83. W.L.Owen, Blackstrap Molasses as Raw Material for Biochemical Industries, Russell Palmer, New York (1939).

84. I.R.Sherwood, Austral. Chem. Inst. J. and Proc., 14:221(1947).

85. A.A.Backhaus, U.S.Patent 1, 493, 183(1924).

86. A.A.Backhaus, U.S.Patent 1, 510, 373(1924).

87. G.T.Reich, U.S.Patent 2, 148, 579(1939).

88. Foods and Food Production Encyclopedia, Van Nostrand Reinhold Company, London (1982).

89. H.V.Harlam and M.L.Martine, 'Problems and Results in Barley Breeding', In USDA Year Book, pp. 303-346(1936).

90. J.H.Martin, H.L.Leonard and D.L.Stamp, 'Principles

of Field Crop Production', 3rd ed. Macmillan Publishing Co., Inc. New York (1976).

91. K.Ramiah, 'Rice Breeding and Genetics', Indian Council Agr. Res. Science Monograph 19, New Dehli (1953).

92. T.R.Standon, 'Superior Germ Plasm in Oats', USDA Year Book, pp. 347-413 (1936).

93. T.R.Stanton, 'New Products from an Old Crop', In Crops in Peace and War, USDA Year Book, pp. 341-344 (1950-51).

94. G.S.Khush, 'Cytogenetics and Evolutionary Studies in Secale:111 Cytogenetics of Weedy Ryes and Origin of Cultivated Rye, Econ. Bot., 17:60-71(1963).

95. G.B.Deodikar, 'Rye-Secale Cereale' L Indian Council Agr. Res., New Delhi, pp. 1-152(1963).

96. P.Weatherwax, 'Indian Corn in Old America', Macmillan, Inc. New York, pp. 1-253 (1954).

97. W.L.Dwen, 'Blackstrap Molasses as Raw Material for Biochemical Industries', Russell Palmer, New York (1939).

98. L.Sattler and F.W.Zerba, Ind. Eng. Chem., 41:1401 (1949).

99. Agricultural Statistics of Pakistan, Government of Pakistan, Ministry of Food, Agriculture and Co-operatives, Food & Agriculture Division (Economic

Wing), Islamabad (1988-89).

100. Official Methods of Analysis of the Association of Official Agricultural Chemists (AOAC) 3rd ed. Association of Official Agricultural Chemists, Washington (1965).

101. AACC Approved Methods, American Association of Cereal Chemists, St.Paul, Minnesota, USA (1976).

102. P.Pearson, 'The Chemical Analysis of Foods', Churchill Livingstone Criffiths 62:510(1937).

103. Burr and Barnes: Non Caloric Functions of Dietary Fats, Physical Rev., 23:256(1943).

104. Cohen: Protein Metabolism, Ann. Rev. Biochem., 14, 357(1945).

105. Cori and Cori: Carbohydrate Metabolism, Ann. Rev. Biochem., 15:193(1946).

106. Gogel's Text Book of Practical Organic Chemistry, Fourth Ed. English Language Book Society, Longman London (1986).

107. E.D.Oscen, 'Modern Optical Methods of Analysis', McGraw Hill Book Co. pp. 83-155; 487-578 (1975).

108. M.Davics, 'Infrared Spectroscopy and Molecular Structure, An Outline of the Principles', Edsvier Publishing Co., Amsterdam, pp. 17-18 (1963).

109. Dudley M.Williams and I.Flem, 'Spectroscopic Methods in Organic Chemistry', McGraw Hill Publishing Co. ltd., London, pp. 44, 78-80 (1969).

110. Gordon and C.Cambel, Anal. Chem., 32:271R(1960).

111. C.Duval, Anal. Chem., 23:1271(1953).

112. A.W.Coats and J.P.Rodfern, Analyst., 88:906(1963).

113. G.M.Lukaszesshi and J.P.Refern, Lab. Pract., 10:552(1961).

114. C.B.Murphy, Anal. Chem., 42:268R(1970).

115. C.B.Murphy, Anal. Chem., 44:513R(1972).

116. W.Z.Toursel, Far. Chem., 7:265(1967).

117. C.Duval, "Inorg. Thermogravimetic Analysis", Elsvier Amsterdam (1953).

118. W.W.Wendlandt and J.P.Smith, 'Thermal Properties of Transition Metal Amine complexes', Elsevier, Amsterdam (1967).

119. A.E.Newkirk and E.L.Simons, Talanta, 13, (1966).

120. C.B.Murphy, Anal. Chem., 40:380R(1968).

121. C.B.Murphy, Anal. Chem., 42:268R(1970).

122. C.B.Murphy, Anal. Chem., 44:513R(1972).

123. B.D.Mitchell and A.C.Birine, In DTA, R.C. Mackenie ed. Academic, London, Chap. 22 (1970).

124. M.Manning, Industrial Res., Feb. 18 (1966).

125. A.P.Gray, Am. Lab. Am., Jan. 43 (1971).

126. D.A.Vassallo, Anal. Chem., 34:132(1962).

127. E.M.Barrall, Thermochim. Acta, 5:377(1973).

128. H.L.Pederson, PaperC-2, International Symp. on Thermal Analysis, London, April (1965).

129. S.Takashima, T.Azumi and Y.Maeda, Nippon Kagaku Zarshi, 85:776(1964).

130. Rer. J.H.M. Nature, 206:85(1965).

131. T.L.Edrtom and I.C.Tech, Doc. Rept. WADDTR 61-72, Suppl. To Vol. X, AF Matals Lab. Wright-Paterson AFB, Ohio, August (1964).

132. T.Edrtom, T.C.Lewis, R.L.Raeicoat and C.F.Stout, Ibid, Vol. XXXII, May (1934).

133. I.C.Lewis and T.Edrtom, AF Matals Lab. Wright Patterson AFB, Ohio, Tech. Doc. Rept. WADD TR 61-72, Vol. XXVII, Nov. (1963).

134. M.A.Hughes and R.P.Sheldon, J. Appl. Polymers Sci. 81541 (1964).

135. L.H.Lee, J. Poly. Sci., 2A:2859(1964).

136. F.E.Karaxz and J.M.O'Reilly, Polym. Letters, 3: 361(1965).

137. I.Kirshenbaum, Z.W.Wilchinsky and B.J.Groten, Appl. Polym. Sci., 8:2723(1964).

138. J.P.Kennedy, J.J.Elliott and B.Groten, Makromal. Chem., 77:26(1964).

139. J.Boor and E.A.Youngman, Polymer. Letters, 2:903 (1964).

140. C.Geacintor, R.S.Schotland and R.B.Miles, J. Polym. Sci. Pt. C, 6:197(1964).

141. I.D.Rubin, Polym. Letters, 2:747(1964).

142. L.Busch and R.G.Cooks, Science, 218:247(1982).

143. B.Munson, Anal. Chem., 49:772A(1977).

144. B.Welz, 'Atomic Absorption Spectroscopy', Verlag Chemic., Weinheim, (1976).

145. R.R.Brooks and L.E.Smythe, Talanta, 22:495-505 (1975).

146. H.J.Issaq and E.W.Barr, Anal. Chem., 49:189-190 (1977).

147. S.D.Brown, Anal. Chem., 49(1977).

148. R.Stephens, CRC Crit. Rev. Anal. Chem., 9:167-195 (1980).

149. K.Yasuda, H.Koizumi, K.Ohishi, and T.Noda, Progr. Anal. At. Spectrose., 3:299-368(1980).

150. Atomic Absorption Methods Manual, Vol. 2, Flameless Operations, Instrumentation Laboratory, Wilmington, MA (1976).

151. H.Van Grunenberg, C.Bredt and W.Frendenberg, J. Am. Chem. Soc., 60:1507(1938).

152. A.Ault, W.N.Haworth and E.L.Hirst, J. Chem. Soc., 51(1935).

153. O.T.Schmidt and A.Simon, J. Prakt. Chem., 152:190 (1939).

154. F.Blindenbacher and T.Reichstein, Helv. Chim. Acta 31:1669(1948).

155. C.L.Mehltretter, B.H.Alexander, R.L.Mellies and C.E.Rist, J. Am. Chem. Soc., 73:2424 (1951).

156. A.L.Raymond and E.F.Schroeder, J. Am. Chem. Soc., 70:2785 (1948).

157. C.E.Ballou and H.C.L.Fischer, J. Am. Chem. Soc., 76:3188 (1948); C.E.Ballou, Biochem. Preparations, 5:67 (1957).

158. Food and Food Production Encyclopedia. Van Nostrand Company, London (1982).

159. J.H.Martin, H.L.Leonard and D.L.Stamp, 'Principles of Field Crop', Van Nostrand Reinhold Company, London (1981).

160. Winkler, Lehrbuch der Technischen Gasanalyse, Zte Auflage, P. 68 (1872).

161. Engelder, J. Phys. Chem., 21:679 (1917).

162. Krauskopf and Purdy, J. Ind. Eng. Chem., 12:158 (1920).

163. L.Lindet Annfals, 8:295 (1915).

164. A.Harden, J.Thompson and W.J.Young, Lister Inst. Biochem. J., 5:230-5 (1916).

165. L.M.Dennis and C.G.Edgar, 'Carbon Dioxide and Carbon Monoxide', Lehrbuch der Technischen Gasanalyse, Zte Anflage (1897).

166. L.Lindet Annfals, 8:295 (1915).

167. H.M.Winegarden, J. Biol. Chem., 74:189-202 (1927).

168. S.I.Ahmap, I.Ahmad and A.Pervez, 'Potential and Prospects of Power Alcohol Production from Molasses in Pakistna', NARC, Islamabad (1987).

169. Kirk, M., 'Utilization of Ethanol as a Farm Fuel', (1983).