Study Material for A.N.S.I. (S.T.) O C (T)

APRIL 7, 2020

ORGANIC CHEMISTRY DIVISION, NSI KANPUR

FIRST YEAR A.N.S.I. (S.T.) O C (T): By-Products

The process of sugar manufacture from sugar cane generates to following products besides sugar—

(a) bagasse -> 24-30% cane Applications: **Synthesis of furfural, and many more….**
(b) Molasses -> 3.5-4.5% cane Applications: **Isolation of Aconitic acid, and many more**….
(c) Filter cake -> 3.5-4% cane Applications: **Isolation of sugarcane wax, and many more….**

Synthesis of furfural from Sugarcane bagasse:

Lignocellulosic biomass: It is the most abundantly available raw material on the Earth for the production of biofuels, mainly bio-ethanol. It is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin). These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin. Lignocellulosic biomass can be broadly classified into virgin biomass, waste biomass and energy crops. Virgin biomass includes all naturally occurring terrestrial plants such as trees, bushes and grass. Waste biomass is produced as a low value byproduct of various industrial sectors such as agriculture (corn stover, sugarcane bagasse, straw etc.) and forestry (saw mill and paper mill discards). Energy crops are crops with high yield of lignocellulosic biomass produced to serve as a raw material for production of second generation biofuel; examples include switch grass(Panicum virgatum) and Elephant grass.

Sugarcane bagasse, a lignocellulosic biomass, which is the residue of the sugarcane, emerging from the grinding operation on extraction of juice has following composition;

(*i*) Water 45-50%
(*ii*) Dissolved solids including sugar 2.5-4%.
(iii Fibre 45-50%.

The water insoluble portion of bagasse is the fibre composed of cellulose, pentosans lignin and some mineral matter. The percentages of major components of dry bagasse vary widely in different countries as reported in literature and the averages are as under

(*i*) Cellulose 36-40% (Polysaccharide-Homo-Polymer of Glucose)
(ii) Pentosans/Xylan 21-28%b (Polysaccharide-Hetero-Polymer of Xylose (major)/arabinose, galactose, uronic acid)
(iii) Lignin 20-28.0 %
(Polymer of propyl phenol units, namely, coniferyl alcohol and sinapyl alcohol, with a minor quantity of *p*-coumaryl alcohol: these components is cross-linked together through carbon–carbon, ester, and ether linkages)
(iv) Ash 2.2-4.0 %
(v) Extractive *3-6* %

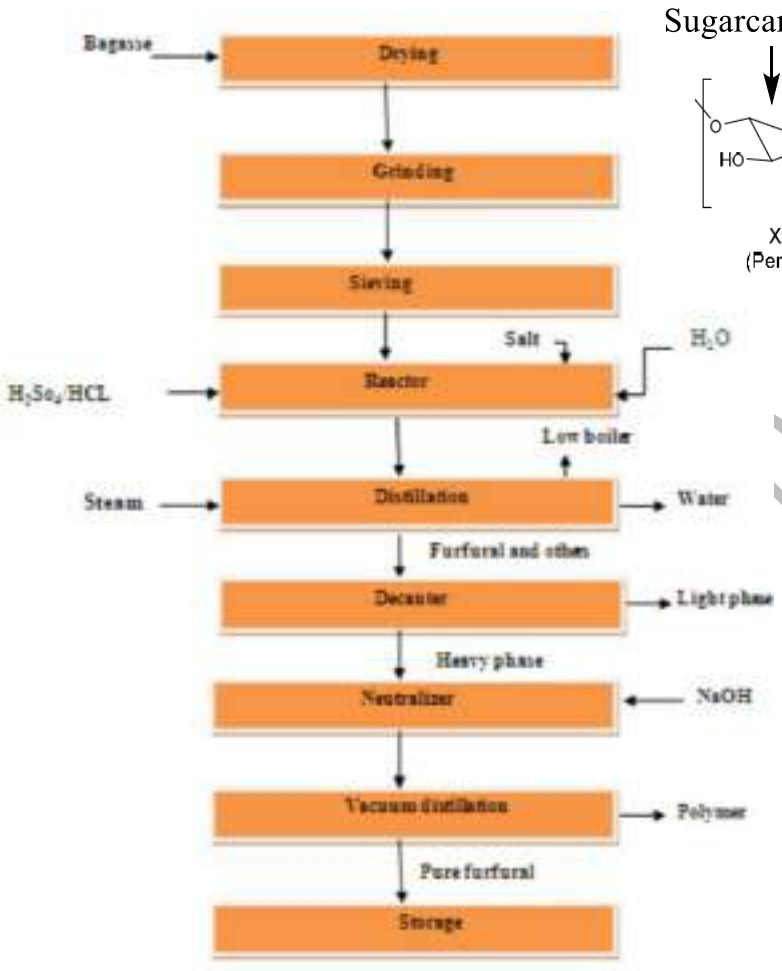
With major percentage of Xylan (> 90%) in the pentosans (21-28%) of bagasse it is ideally suited as raw material for furfural plant.

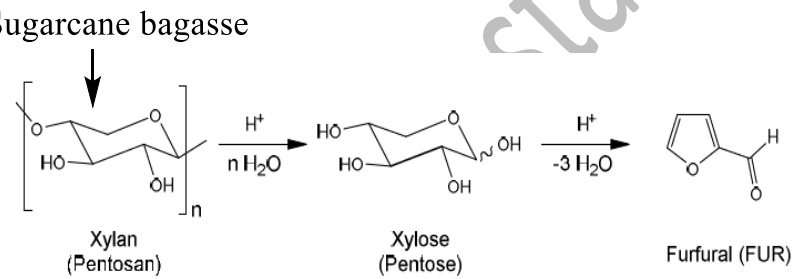
(FUR) has recently been emphasised as one of the top value-added chemicals derived from biomass,3 being identified as one of the key chemicals produced in the so-called lignocellulosic biorefineries. FUR is produced from renewable agricultural sources such as food crop residues and wood wastes. The synthesis of FUR from fossil based raw materials (e.g., *via* the catalytic oxidation of 1,3- dienes) is not economically competitive. The largest producer of FUR is China (~70% total production capacity). Other countries with significant FUR production include the Dominican Republic (Central Romana Corporation, 32 kTon/year, world's largest single producer) and South Africa (20 kTon/year). These three countries account for approximately 90% of the global FUR production capacity (280 kTon). The commercial utility of FUR was first discovered in around 1921. Thus, Furfural is an aldehyde produced from pentosans and serves as solvent in refining lubricants and for wood resin and can also be used in the manufacture of Nylon 6.6, THF production, Furfuryl-alcohol resins, Agricultural nematocide , Furan as building blocks for copolymers, Synthetic fuels (furanics) .

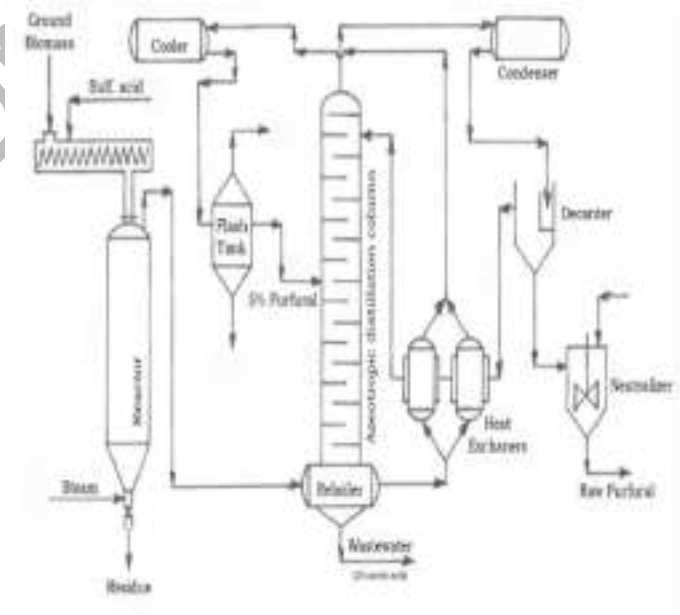
1922 : The Quacker Oats company start the industrial production of furfural from oats hulls in Iowa, USA, *rapidly distilling the aldehyde from the reaction chamber during the period of its formation.*
1923 : Durite Plastics Inc. is the first manufacturer of phenol-furfural resins.
1941: Furfural derivatives are tested as automotive fuel in blends with gasoline.
1949: Du Pont starts the production of adiponitrile for the manufacture of nylon 6.6 from furfural and THF.
1953: A.P.Dunlop, F.N.Peters, The Furans, ACS Mon. series published. Commercial availability of furfuryl alcohol.
1961: Du Pont abandons the furfural-THF-adiponitrile process opting for oil derivatives as starting materials.
1990: Furfural market around 270 kton/year, mainly to furfuryl alcohol and furan resin

At present, FUR is commercially produced through the acid-catalysed transformation of pentosan sugars present in sugarcane bagasse and biomass; the C5 polysaccharides are first hydrolysed by H_2SO_4/HCl to monosaccharides (primarily xylose), which are subsequently dehydrated to FUR . FUR is then recovered from the liquid phase by steam stripping to avoid further degradation, and purified by double distillation according to following scheme:

Synthesis of furfural from Sugarcane Bagasse (& other lignocellulosic materials):

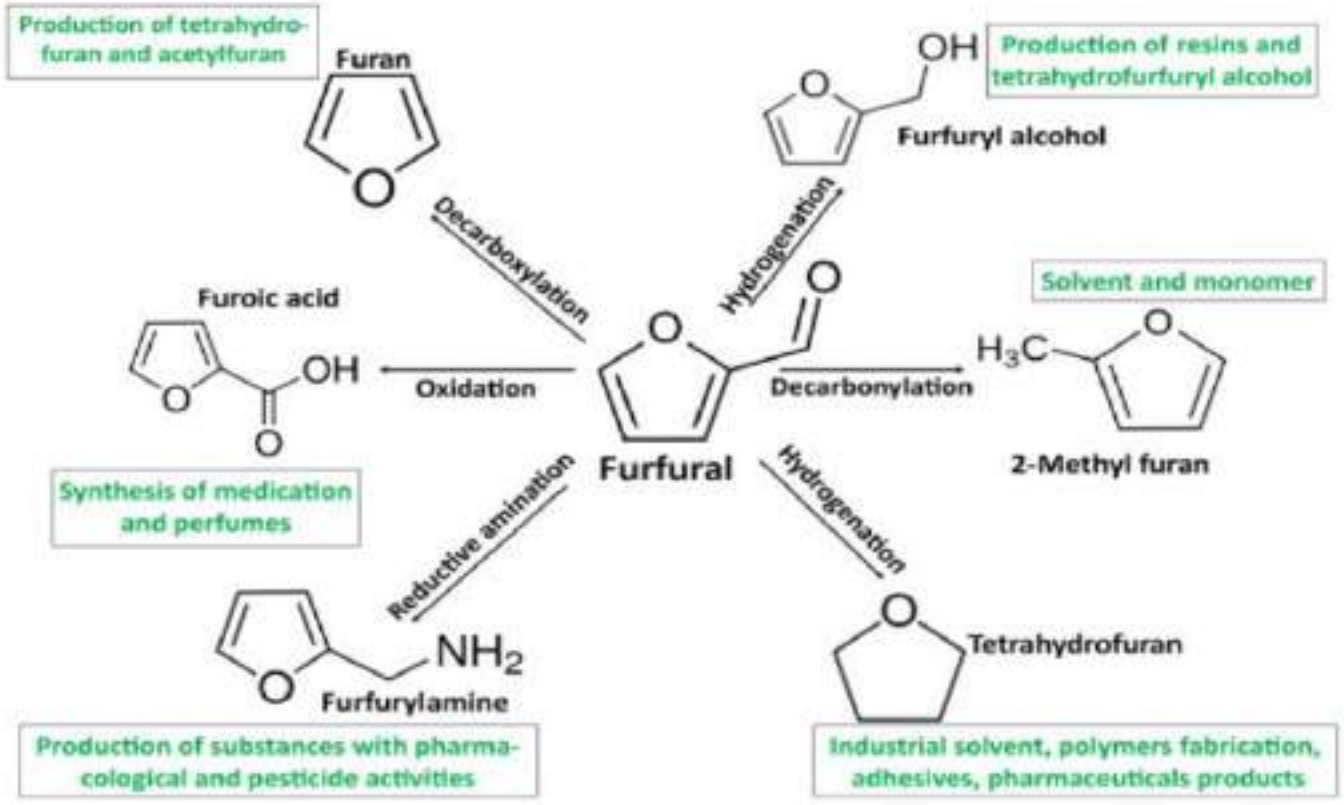






Low yields, approximately 50% of theoretical; **High energy use.** 20-50 ton of steam per ton of furfural. H**igh (sulfuric) acid usage**, roughly 20%wt of furfural output; **No integration** apart from residues incineration. Resaerch is ongoing to overcome these shortcomings.

Derivatives of furfural and its applications



Isolation of Aconitic acid from Molasses

Aconitic acid (1-propene-l:2:3 tricarboxylic acid) is a natural component and common metabolite of plants of two families viz. Gramineae and Ranunculaceae. Because of its functionality and environmental acceptability aconitic acid has a number of applications, notably in the plastic and synthetic rubber industries. At present, it is obtained by dehydration of citric acid which makes its supply limited and expensive. One of the principal uses for aconitic acid is in the production of high molecular weight esters which are used as plasticizer. The most common plasticizer is tributyl aconitate; other important ones include triamyl aconitate, triallyl aconitate, and tri(2-ethylhexyl) aconitate. Aconitic acid can be transformed into Itaconic acid, which is well known as a precursor for polymer synthesis and has been involved in industrial processes for decades.

Aconitic acid is the most abundant non-nitrogenous organic acid present in cane juice where its concentration is - thrice the concentration of acids other than amino acids. Although quantitatively it is a minor constituent of juice (1-1.5% on Brix solids), data on its distribution in sugar factory products apparently show that most of the aconitic acid which enters with the juice becomes concentrated in syrup and finally in molasses. It has been studied that factories reporting lower aconitic acid content in molasses generally exhibited lower molasses purity (i.e. better sugar recovery). This is consistent with the reported mellasigenic activity of aconitic acid which tends to form complex mellasigenic compounds with sugar and adversely affects its recovery. It has also quantitatively demonstrated that the rate of crystallisation of sucrose markedly decreased as aconitic acid concentration in the syrup increased. Some researchers has attributed this phenomenon to the increase in viscosity of sugar solutions due to aconitic acid, and contribution of the latter to viscosity development of molasses is also well documented. On account of pronounced solubility of calcium salts of these non-nitrogenous acids, they are considered as the principal contributors to the high calcium content of the clarified juice and eventually of molasses. Owing to their instability and reactivity, the said acids are also regarded as potential sources of mellasigenic substances and some of their salts e.g. calcium oxalate and aconitate are responsible for scaling on the process equipment heating surfaces and sediments in molasses storage tanks. It has observed that calcium and magnesium salts in molasses cause scaling of distillation columns in distilleries. It has been reported that, as the aconitic acid content in molasses increases, its fermentation efficiency goes

down. it is, therefore, advisable to separate aconitic acid from molasses before subjecting the latter to alcohol fermentation.

Aconitic acid content in blackstrap molasses varies considerably and is observed, for Louisiana molasses samples, to be in the range 0.96 to 6.13% based on brix solids. In some study carried out in India, it is observed that molasses samples, Aconitic acid content to be in the range 3 to 5 % based on brix solids.

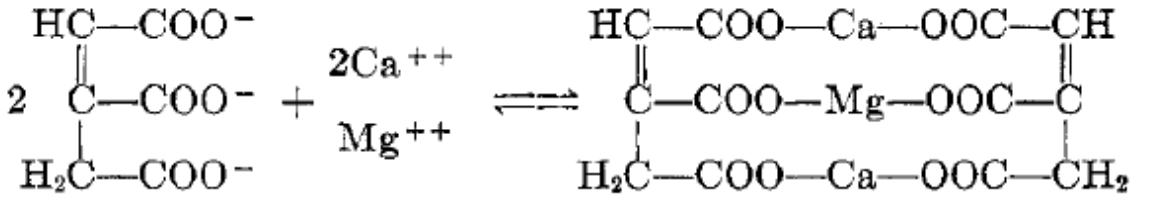
Extraction of aconitic acid from Molasses by precipitation includes dilution, liming, and crystallization:

First reported the, presence of aconitic acid in sugar cane products in 1877, it was not until 1944, when it was demonstrated by the Iberia Sugar Cooperative, Inc., in New Iberia, La., that commercial quantities of the acid could be obtained as alkaline earth salts from second or B molasses. Godchaux Sugars, Inc., at Raceland, La., is the only producer of dicalcium magnesium aconitate using a precipitation process based on the work of the Southern Regional Research Laboratory of the U. S. Department of Agriculture.

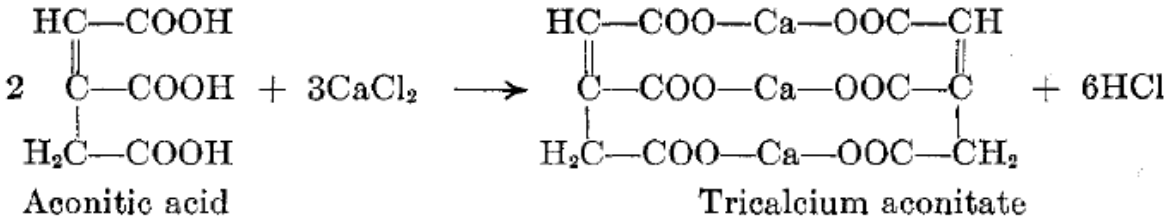
In essence the process consists of diluting the molasses to a suitable Brix (optimum dilution for the process is 50" to 55' Brix solids) in a holding tank where lime and calcium chloride are added, followed by a crystallizing step at elevated temperature to complete the formation and precipitation of the dicalcium magnesium aconitate. The aconitate is recovered by centrifuging, washing, recentrifuging, and drying; the molasses is re-concentrated. The obvious weakness of this process is that only about 40% of the available aconitic acid is recovered.
The optimum pH is around 6.5 to 6.8; greater alkalinity causes the solution to foam when heated. If lime is used for the precipitation, it should contain a minimum of carbonate, since the carbon dioxide liberated causes the solution to foam excessively. Technical grade calcium chloride and magnesium chloride are used to increase the calcium and magnesium content of the solution. A satisfactory addition is found to be 3 parts of anhydrous magnesium chloride for every 5 parts of aconitic acid. Larger quantities of salt have no effect on aconitate recovery; they unnecessarily increase the ash and chloride content of the molasses returned to the sugarhouse. Before chloride addition, the molasses was always diluted, limed, and heated to around 50 ^{0}C. Aconitate began to separate at about 82-83 ^{0}C, but the best yields were obtained at temperatures between 93-98 ^{0}C.

Chemistry involved…….

Fundamentally, the production of dicalcium magnesium aconitate involves very simple chemistry coupled with the necessary unit operations to effect recovery. Molasses usually contains some calcium ions, so that it is necessary only to make up the deficiency needed for precipitation of aconitic acid. There, is no need for additional magnesium; molasses normally contains sufficient quantity of these ions. The amount of calcium added depends on the type of molasses, but is generally within the range of 20 to 40% of the theoretical quantity needed to precipitate the tricalcium salt:



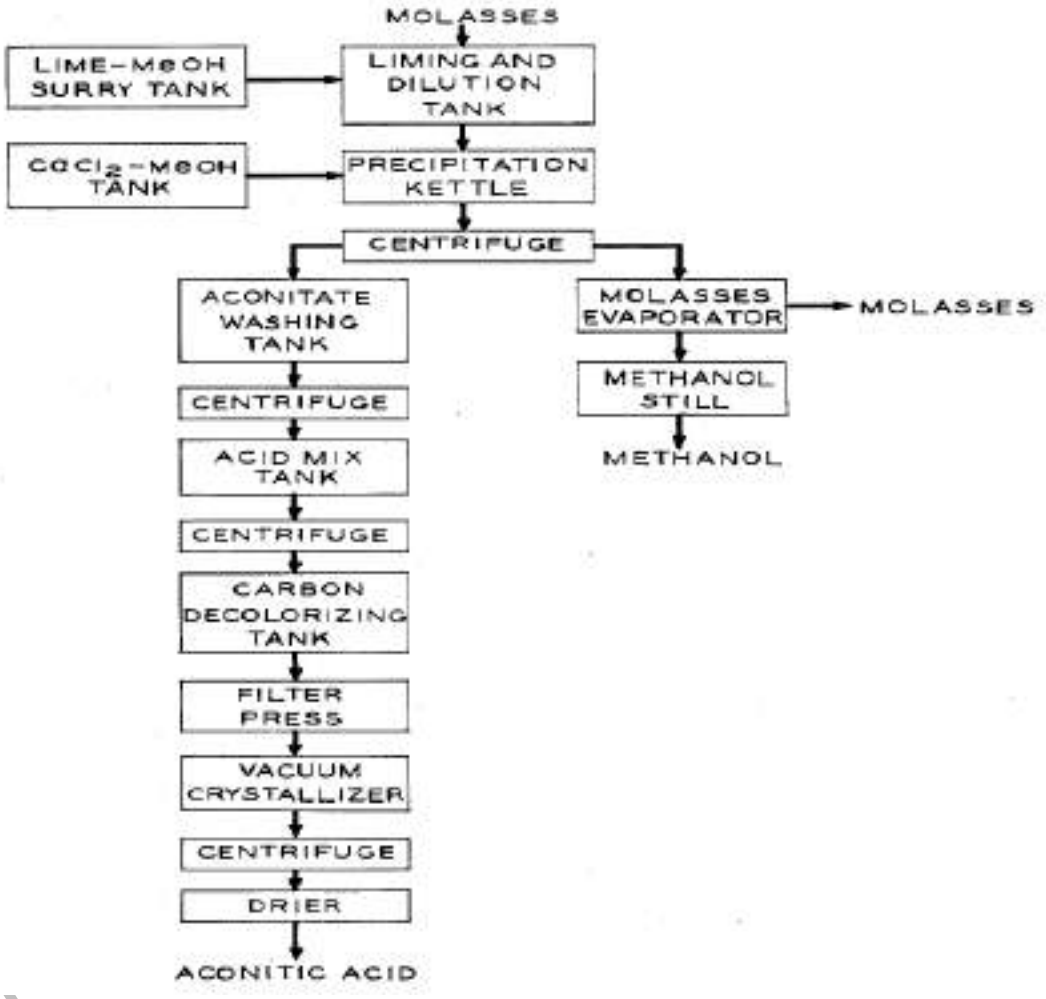
Aconitate ion — Dicalcium monomagnesium aconitate



Aconitic acid — Tricalcium aconitate

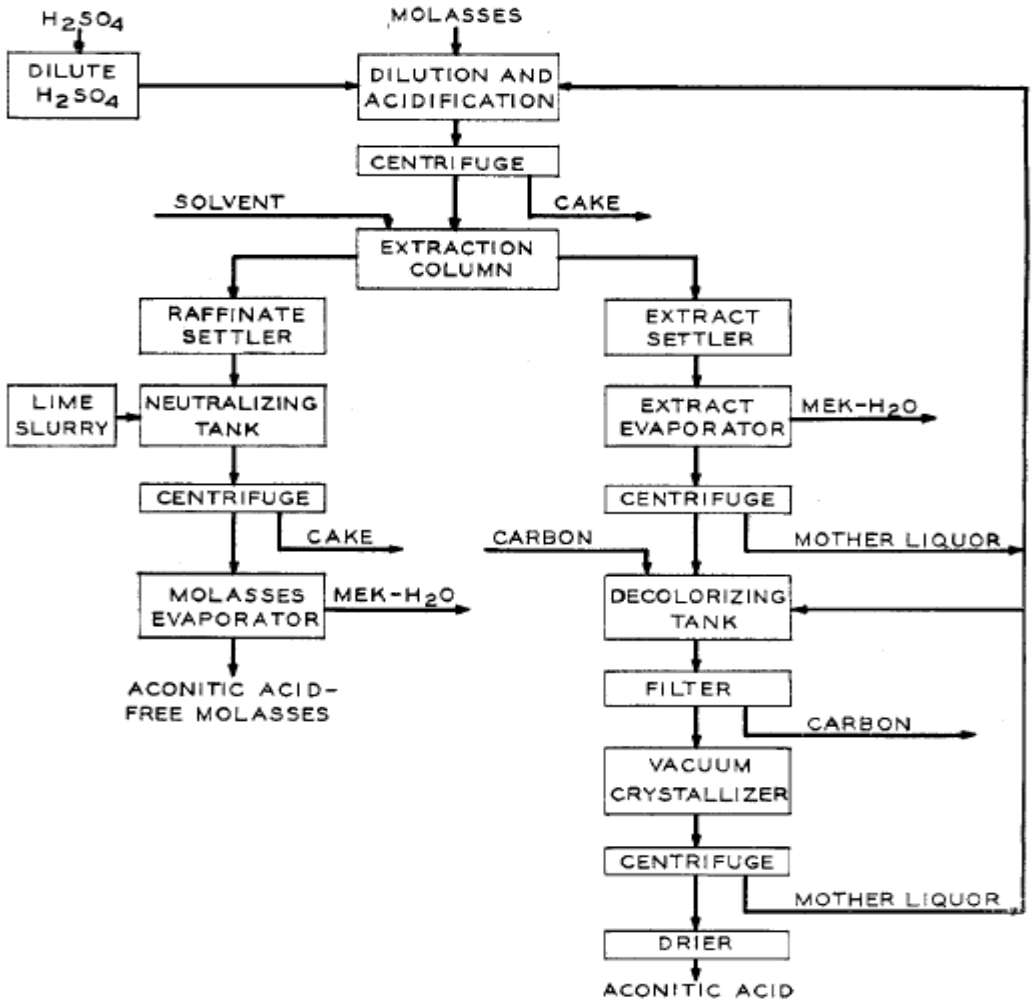
The extraction of aconitic acid from Molasses can be carried out by following three methods also:

Improved chemical precipitation method flow chart:



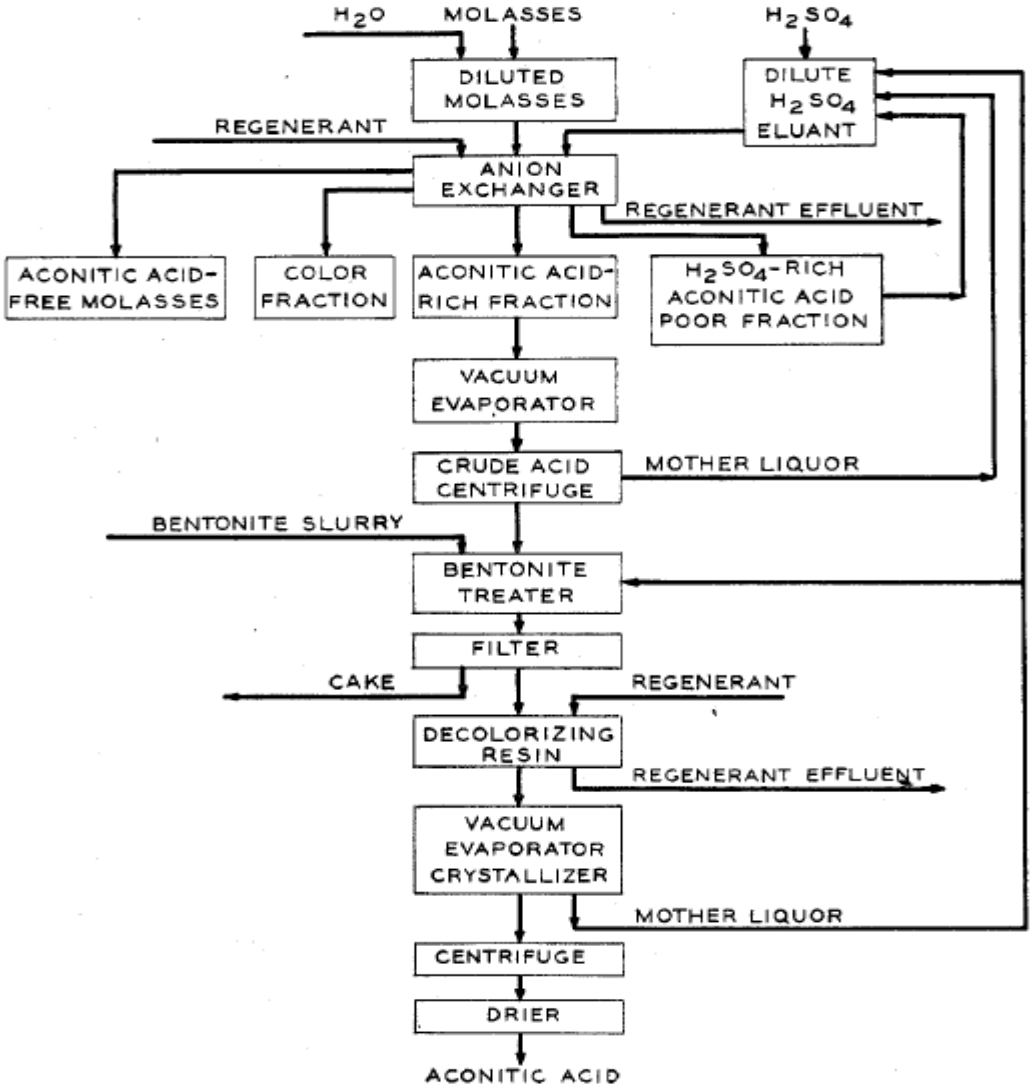
The overall yield above 70 %

Solvent extraction method flow chart:



The overall yield above 90 %

Ion exchange method flow chart:



the overall yield above 97 %

Isolation of sugarcane wax form pressmud

Waxes are a diverse class of organic compounds that are lipophilic, malleable solids near ambient temperatures. They include higher alkanes and lipids, may contain unsaturated bonds and include various functional groups such as fatty acids, primary and secondary alcohols, ketones, aldehydes and fatty acid esters, and aromatic compounds may also be present. Synthetic waxes often consist of homologous series of long-chain aliphatic hydrocarbons (alkanes or paraffins) that lack functional groups. They have typically with melting points above about 40 °C, melting to give low viscosity liquids. Waxes are insoluble in water but soluble in organic, nonpolar solvents. Some of the important varieties of waxes obtained from different sources are as follows:-

A MINERAL WAXES
- Paraffin wax
- Montan wax
- Lignite wax
- Ozocerite wax
- Ceresin wax
- Utah wax
- Peat wax

B VEGETABLE WAXES
- Bay Berry wax
- Candelilla wax
- Carnauba wax
- Cotton wax
- Esparto wax
- Fir wax
- Japan wax
- Ouricury wax
- Palm wax
- Rice oil wax
- Sugarcane wax
- Ucuhuba wax
- Cocoa butter wax

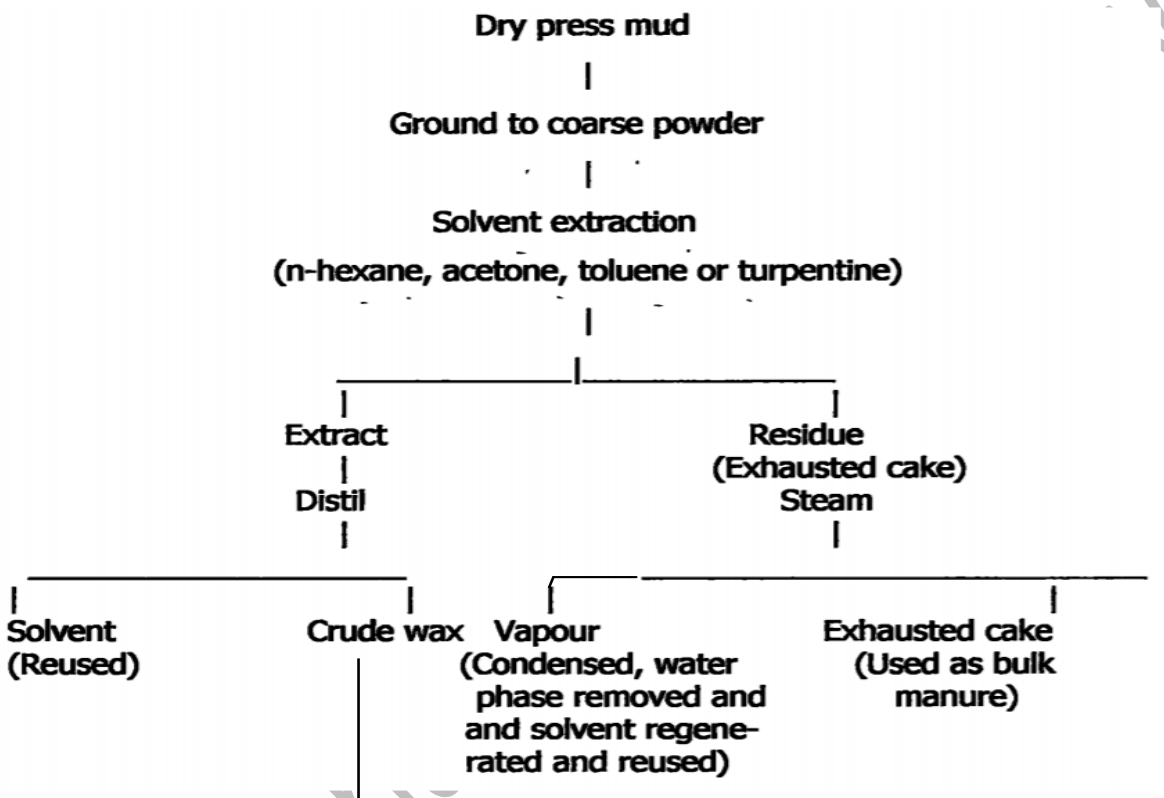
C ANIMAL WAXES
- Bees wax
- Chinese wax
- Shellac wax
- Spermaceti wax
- Wool wax

SUGARGANE WAX:

Sugarcane has an outer rind, which is very hard, perhaps nature's protective wall for arresting the evaporation of water from the plant material which consists of about **80%** water and **20%** cellulose. In addition to this hard rind, thin, layers of wax are present on the rind, particularly at the internodes, which is perhaps another natural phenomenon to reduce transpirational losses. The percentage of wax on sugarcane has not been estimated precisely yet, it has been

indicated that the wax forms about 0.12% on the weight of cane. Thus, sugarcane is the single largest source of vegetables wax. However, when sugarcane is normally crushed in the milling plants in the sugar factories, more than 50% of the wax contained on the rind finds its way into the bagasse which is subsequently burnt in the boilers as fuel. The remaining portion of the wax finds its way into the cane juice and as wax is water insoluble, it gets occluded by the inorganic salts and precipitated and accumulates in the filter cake or press mud. Wax content of the press mud obtained in the sugar factories following double-sulphitation process is more than that obtained in the factories following other process. Therefore, the press mud obtained in the sugar factories following double-sulphitation process is a better raw material for the extraction of wax. On an average, the press mud obtained in the sugar factories contains about 75% moisture and the wet cake % cane is of the order of 2.5 to 3.5% which again depends upon many factors. On an average, the production of press mud on dry basis is about 1% on the total quantity on sugarcane crushed.

The wax content of the press mud depends upon many factors like the variety of sugarcane crushed, the climatic, soil and agronomical conditions under which the sugarcane is grown, the milling and the clarification process adopted, etc. Crude Wax content of press mud in India is 8-18% on dry press mud. The process of recovering this wax is the solvent extraction system well established in extracting oils from oilcakes. The dewaxed cake can be a very good manure in the fields. Many organizations in different countries have also conducted considerable research work on the exact type of solvent required for extracting cane wax from press mud in a most economic way. Solvents like Benzene, Alcohol, Carbon Disuplhide, Carbon Tetrachloride, Petroleum, Mineral Turpentine were used and of all these solvents, mineral turpentine were found to be most economical and effective solvent. The process flow chart is depicted below:



Refining

(Vacuum distillation, fractional distillation with solvent/by chemical oxidation/treatment with charcoal)

Solvent process by IPA (rt to reflux)

Soft wax
(Dissolved in IPA @ rt; 25-30%)

True Wax
Liquid portion obtained after cooling hot solution of IPA, then evaporated and bleached & decolorization by bubbling air; 35-40%:)

Pitch
(Solid potion obtained after cooling hot solution of IPA; 28-32%)

The crude wax consisting of esters, fatty acids, alcohols and hydrocarbons contains about 50-60% hard portion the remaining being soft lipids. The hard wax has a melting point of 75°-80°C and can be used as a substitute for carnauba wax in industries like polish, carbon paper manufacture etc. The soft part can yield sterols and fatty acids, besides, being useful for fruit coating and preservation. The soft fatty fraction can be separated from crude wax by cold acetone treatment orabsolute alcohol as shown by work at Ravalgaon. Extraction of fatty portion from filtercake by cold petroleum solvent followed by high temperature treatment with the same solvent yield separately soft fatty wax and hard wax. Partial decomposition of fatty lipids is obtained by— (*i*) treatment of crude wax with high pressure steam and *(ii)* initial fermentation of filter cake before it is dried and further processed. The wax thus obtained is hard but also dark coloured and further treatment with nitric acid or potassium chromate with sulphuric acid with air blowing results in lightening the colour. Cane wax recovery from filter mud was established in Ravalgaon on commercial scale and continued for over three decades. Similar wax extraction units were set up in Cuba and Australia. This industry however could not be established in the world in a big way

SUGAR CANE WAX APPLICATION AND NEED FOR FURTHER R & D **WORK:**

Sugarcane wax consisted of many of compounds such as long-chain alkanes, alkenes, aromatic hydrocarbons, fatty acids, ketones, aldehydes, esters and long chain fatty alcohols. These long chain fatty alcohols (policosanol) has been reported to reduce platelet aggregation, endothelial damage, foam cell formation and lowering low-density lipoproteins (LDL) and increasing high- density lipoproteins (HDL). However, there is still debate continuing about the nutraceutical effect of policosanol. For instance, studies indicated that policosanol was ineffective in reducing blood cholesterol while others associated policosanol to mild-to-moderate lowering of LDL cholesterol among patients intolerant of statin therapy.

Industrial concerns manufacturing carbon Papers, Polishes, Coated Papers and Fabrics etc. in India normally use Carnauba wax. Some of these units used small quantities of crude cane wax and refined cane wax also produced in India. The general comments by the firms which used both Carnauba wax, as well as sugarcane wax in regard to the properties of both these waxes are indicated below:

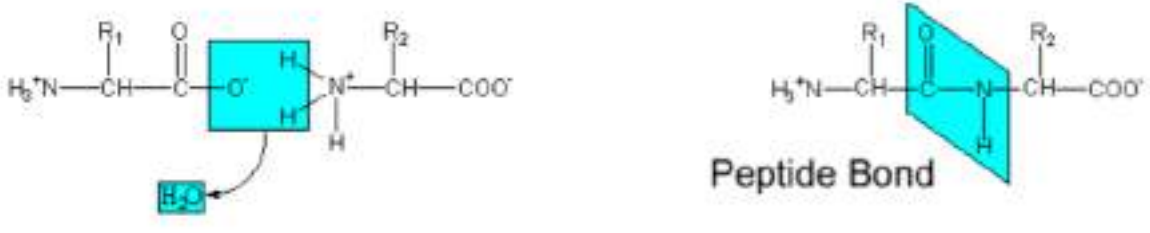
Property	Carnauba Wax	Sugarcane Wax
Color and small	Pale grey with sweet smell	Dark chocolate with unpleasant smell
Action of heat	Melts satisfactorily	Forms froth on melting
Melting point	80 to 84°C	78 to 80°C
Acid value	0 to 2	4 to 12
Oil retention and penetration	Very good	50% less
Pigment wetting	Very good	60% less

Study made in this direction by the author revealed that no commercial scale plant is working in any country except in India. Somehow or the other, the sugar technologists all over the world did not concentrate on the commercial production of cane wax from the press mud all these years and the plants already established in other countries had to be closed down due to low recovery of hard wax, suitable for industrial use and high loss of costly solvent. Further, the quality of crude wax and refined wax obtained was not consistent as to make the industrial consumers to standardize the process of manufacturing various products depending upon the quality of the wax obtained. Further, the availability of other waxes, particularly Carnauba wax did not induce the manufacturers to use the cane wax and suggest developments to be made therein. Further work is necessary to improve the hardness of the wax and its solvent retention properties. Recently, Godavari-biorefineries pioneered the commercialization of this natural, vegetable-based. The brand Naturowax is a vegetable wax that replaces Carnauba Wax.

FIRST YEAR A.N.S.I. (S.T.) O C (T): Proteins & Amino Acids

Proteins are complex, organic compounds composed of many amino acids linked together through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds and van der Waals forces. There is a greater diversity of chemical composition in proteins than in any other group of biologically active compounds. The proteins in the various animal and plant cells confer on these tissues their biological specificity.

Peptide bonds & Peptites: Amino acids are linked together by 'amide groups' called peptide bonds. During protein synthesis, the carboxyl group of amino acid at the end of the growing polypeptide chain reacts with the amino group of an incoming amino acid, releasing a molecule of water. The resulting bond between the amino acids is a peptide bond. The two amino acids can connect the other way as well, forming a structural isomer of the dipeptide, with a unique set of physical properties. Short chains are referred to as peptides, chains of up to about 50 amino acids are polypeptides, and chains of more than 50 amino acids are proteins. Amino acids in peptide chains are called amino acid residues. – The residue with a free amino group is called the N-terminal residue, and is written on the left end of the chain. – The residue with a free carboxylate group is called the C-terminal residue, and is written on the right end of the chain.



Size of Proteins

Proteins are very large polymers of amino acids with molecular weights that vary from 6000 amu to several million amu. – Glucose ($C_6H_{12}O_6$) = 180 amu – Hemoglobin ($C_{2952}H_{4664}O_{832}N_{812}S_8Fe_4$) = 65,000 amu

Proteins can be classified as:

Based on compositions:

(a) Simple proteins. On hydrolysis they yield only the amino acids and occasional small carbohydrate compounds. Examples are: albumins, globulins, glutelins, albuminoids, histones and protamines.

(b) Conjugated proteins. These are simple proteins combined with some non-protein material (prosthetic group) in the body. Examples are: nucleoproteins, glycoproteins, phosphoproteins, haemoglobins and lecithoproteins.

(c) Derived proteins. These are proteins derived from simple or conjugated proteins by physical or chemical means. Examples are: denatured proteins and peptides.

Based on Structures:

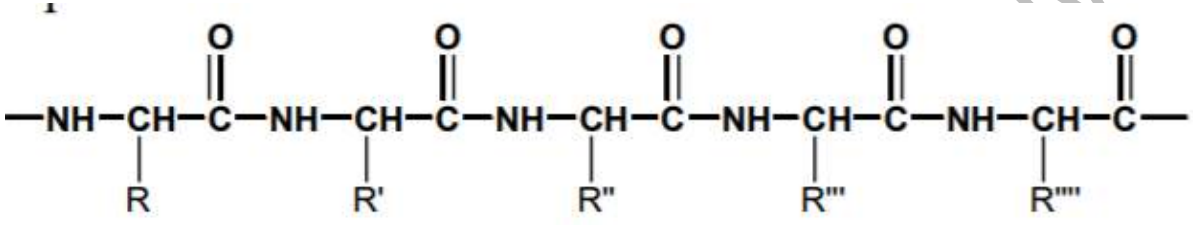
(a) Fibrous proteins are made up of long rod-shaped or stringlike molecules that can intertwine with one another and form strong fibers. – insoluble in water – major components of connective tissue, elastic tissue, hair, and skin – e.g., collagen, elastin, and keratin.
(b) Globular proteins are more spherical in shape – dissolve in water or form stable suspensions. – not found in structural tissue but are transport proteins, or proteins that may be moved easily through the body by the circularoty system – e.g., hemoglobin and transferrin; others-albumins

Protein Structure:

The structure of proteins is much more complex than that of simple organic molecules. – Many protein molecules consist of a chain of amino acids twisted and folded into a complex three-dimensional structure – The complex 3D structures of proteins impart unique features to proteins that allow them to function in diverse ways. There are four levels of organization in proteins structure: primary, secondary, tertiary, and quaternary.

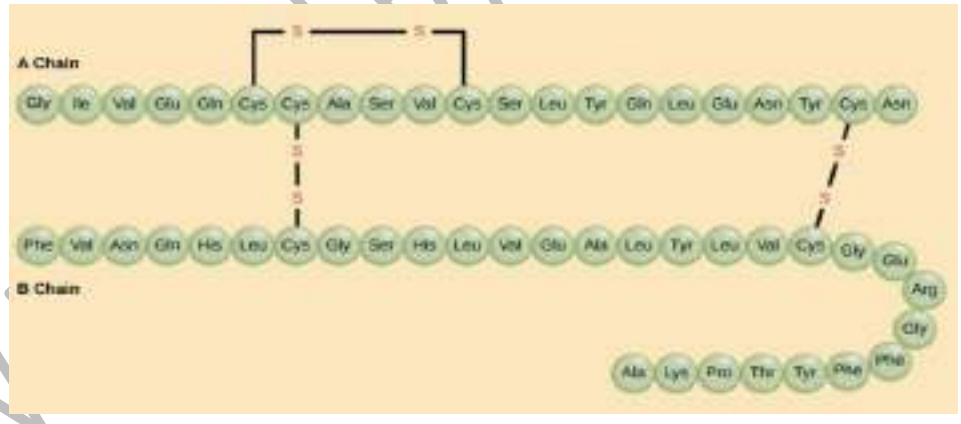
Primary Structure

The simplest level of protein structure, primary structure is simply the sequence of amino acids in a polypeptide chain. It gives the linear sequence of the side chains that are connected to the protein backbone. Each protein has a unique sequence of amino acid residues that cause it to fold into a distinctive shape that allows the protein to function properly.

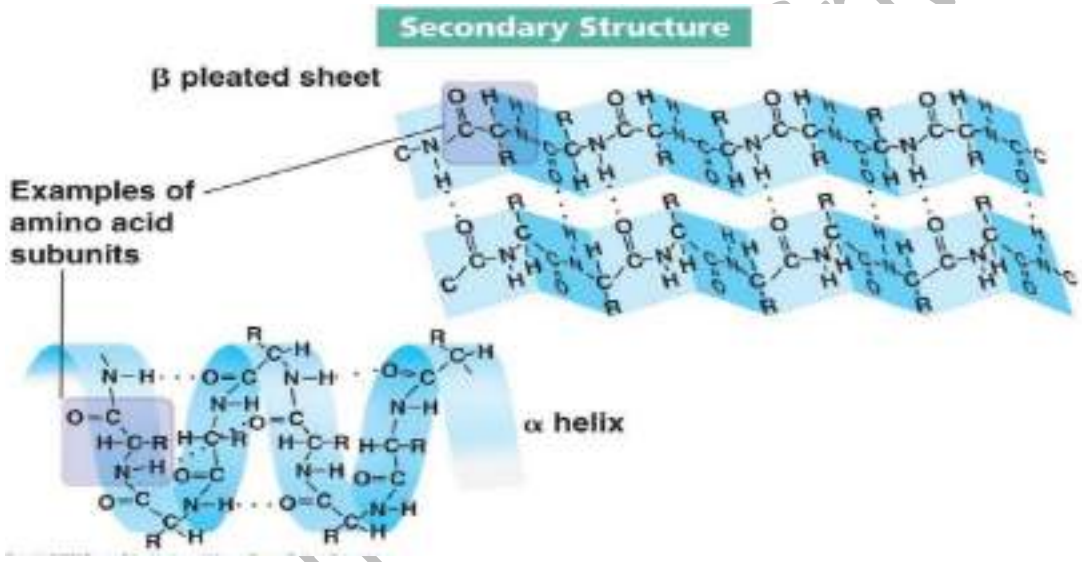


protein backbone

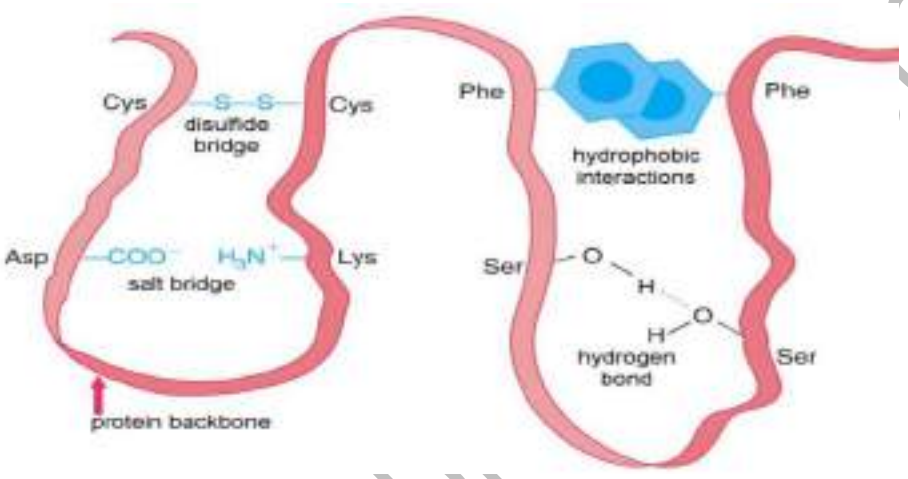
The hormone insulin has two polypeptide chains A, and B. The sequence of the A chain, and the sequence of the B chain can be considered as an example for primary structure.



Secondary structure: Secondary structure, refers to local folded structures that form within a polypeptide due to interactions between atoms. The most common types of secondary structures are the α helix and the β pleated sheet. Both structures are held in shape by hydrogen bonds, which form between the carbonyl O of one amino acid and the amino H of another.

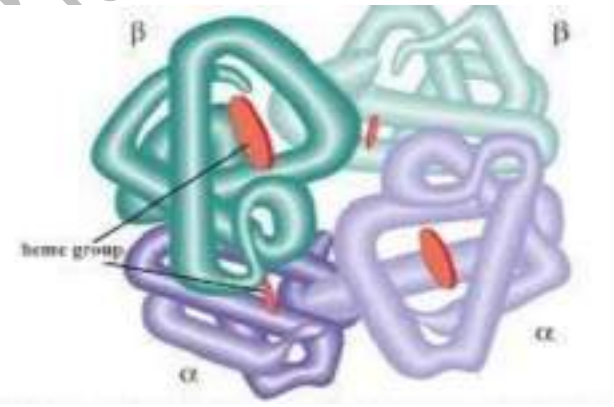


Tertiary structure: The overall three-dimensional structure of a polypeptide is called its tertiary structure. These structures result from four types of interactions between the R side chains of the amino acids residues: Disulfide bridges, Salt bridges, Hydrogen bonds & Hydrophobic interactions. The tertiary structure is primarily due to interactions between the R groups of the amino acids that make up the protein. Important to tertiary structure are ydrophobic interactions, in which amino acids with nonpolar, hydrophobic R groups cluster together on the inside of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules. Also, Disulfide bonds, covalent linkages between the sulfurcontaining side chains of cysteines, are much stronger than the other types of bonds



Quaternary Structure of Proteins:

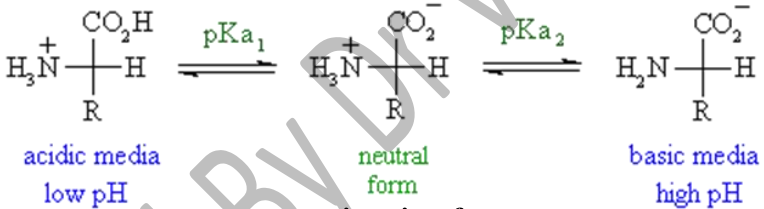
When two or more polypeptide chains are held together by disulfide bridges, salt bridges, hydrogen bond, or hydrophobic interactions, forming a larger protein complex. Each of the polypeptide subunits has its own primary, secondary, and tertiary structure. he arrangement of the subunits to form a larger protein is the quaternary structure of the protein.



Isoelectric point (pI):

The surface of a protein has a net charge that depends on the number and identities of the charged amino acids, and on pH. At a specific pH the positive and negative charges will balance and the net charge will be zero. This pH is called the isoelectric point, and for most proteins it occurs in the pH range of 5.5 to 8. The isoelectronic point or isoionic point is the pH at which the amino acid does not migrate in an electric field. This means it is the pH at which the amino acid is neutral, i.e. the zwitterion form is dominant. A protein has its lowest solubility at its isoelectric point. If there is a charge at the protein surface, the protein prefers to interact with water, rather than with other protein molecules. This charge makes it more soluble. Without a net charge, protein-protein interactions and precipitation are more likely.

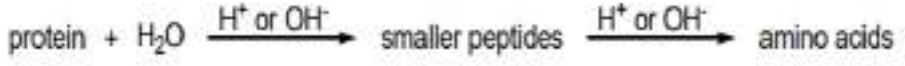
pH = pI



zwitterion form

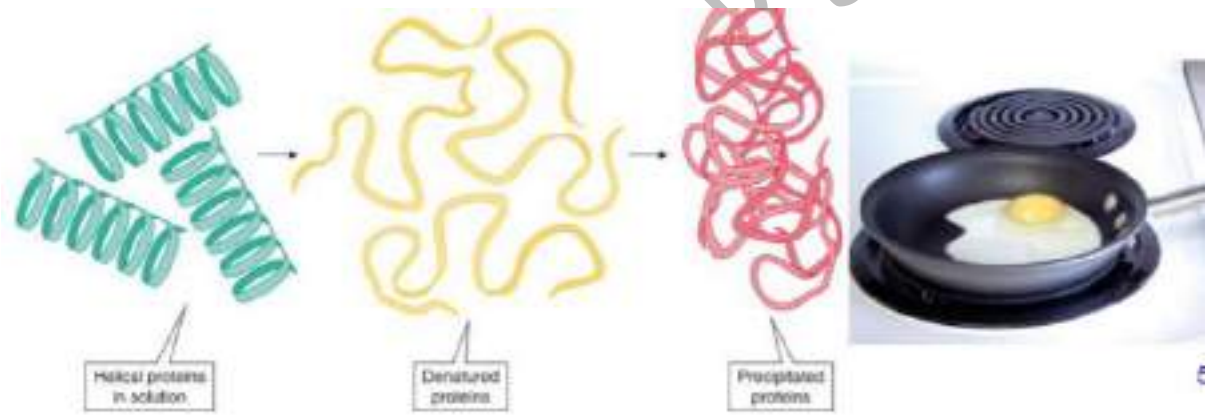
Protein Hydrolysis:

Amides can be hydrolyzed under acidic or basic conditions. The peptide bonds in proteins can be broken down under acidic or basic conditions into smaller peptides, or all the way to amino acids, depending on the hydrolysis time, temperature, and pH. The digestion of proteins involves hydrolysis reactions catalyzed by digestive enzymes. – Cellular proteins are constantly being broken down as the body resynthesizes molecules and tissues that it needs.



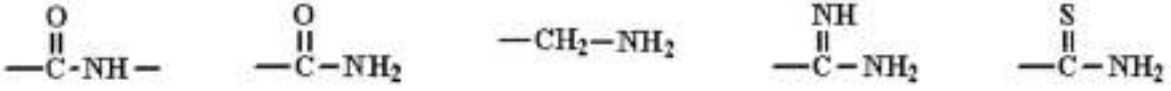
Denaturation and protein folding:

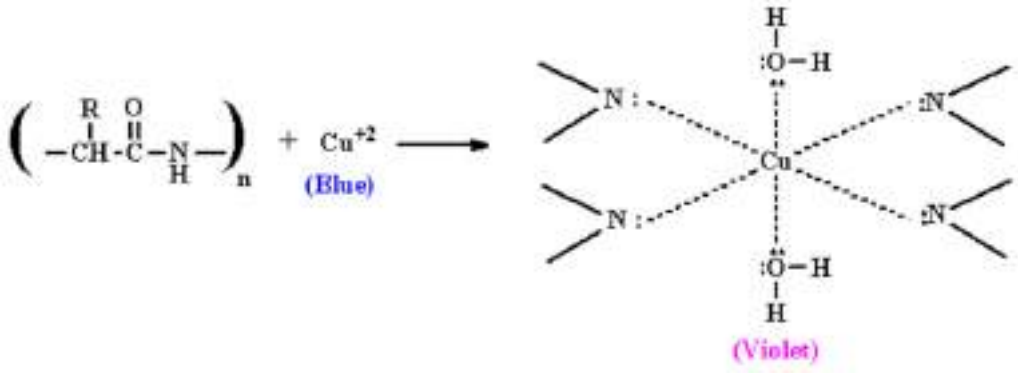
Each protein has its own unique shape. If the temperature or pH of a protein's environment is changed, or if it is exposed to chemicals, these interactions may be disrupted, causing the protein to lose its three-dimensional structure and turn back into an unstructured string of amino acids. When a protein loses its higher-order structure, but not its primary sequence, it is said to be denatured. Denatured proteins are usually nonfunctional. Most proteins are biologically active only over a temperature range of 0ºC to 40ºC. Denaturation is caused when the folded native structures break down because of extreme temps. or pH values, which disrupt the stabilizing structures. The structure becomes random and disorganized.



Protein Testing (Colour Reaction of Protein):

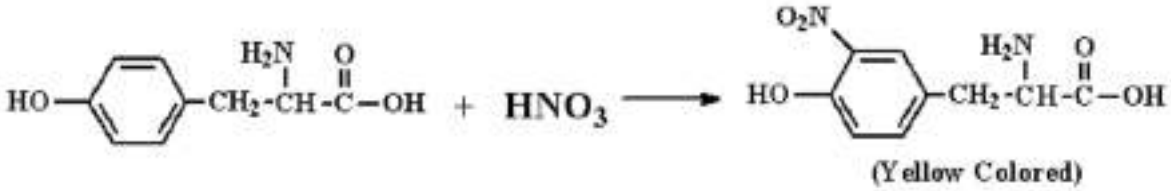
Biuret Test: The Biuret Test is a general test for proteins. When a protein reacts with copper(II) sulfate (blue), the positive test is the formation of a violet colored complex. The Biuret Test works for any compound containing two or more of the following groups:





Xanthoproteic test

Proteins on treatment with nitric acid give a yellow or orange color. Concentrated nitric acid is used for nitration. On the treatment of nitric acid, proteins give yellow precipitate which turns to orange color on treatment with alkali. The appearance of a yellow color solution confirms the presence of proteins. Phenyl rings containing an activating group can be nitrated producing a yellow product.

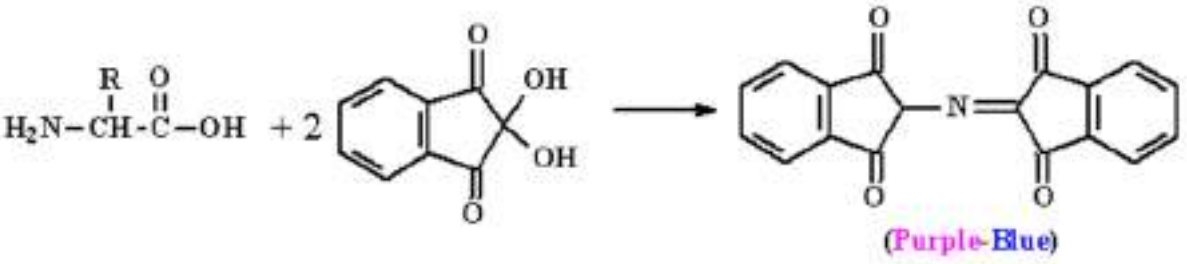


Millions test

Phenolic group of tyrosine of proteins reacts with mercuric sulfate in the presence of sodium nitrite and sulfuric acid to give red color. Millon's test is given by proteins containing phenolic amino acids. Gelatin does not give this test. First, a white precipitate is formed when proteins are treated with millions reagent and then turns to brick red color on boiling, this confirms the presence of proteins. The appearance of brick red color solution confirms the presence of proteins.

Ninhydrin test

Proteins react with pyridine solution of ninhydrin and change to a colored solution from a deep blue to violet-pink or sometimes even to a red color. Ninhydrin solution is prepared by dissolving 0.1gm of ninhydrin in about 100ml of distilled water. But this solution of ninhydrin is unstable and can be kept for two days. The appearance of violet color solution confirms the presence of proteins.



Role of Proteins and Amino Acids to Sugar Manufacturing Process

Although the total nonsugars in cane juice amount only to 0.7 - 1.5% of the juice, these substances have an important effect on the process of sugar manufacture. The nitrogenous substances present in the cane juice effect on the clarification and colour formation during sugar manufacturing process. Though nitrogen present is small of the order of 0.036-0.05% cane juice, there are two kinds of nitrogenous substances in cane juice.

1. Proteins which are complex high molecular weight substance.
   Three proteins: namely albumins (coagulable and soluble in pepsin), nucleins (coagulable but insoluble in pepsin) and peptones (not coagulable).
2. Amino-acids, simple unit of proteins:
   aspartic acid (predominate), glutamic acid, serine, glycine, alanine, γ-amino butyric acid, lysine, valine, leucine, arginine, phenylalanine, tyrosine, histidine, proline, methionine, tryptophan.
   amino acid amides: Asparagine, Glutamine

The amount of nitrogenous materials entering the juice line of the sugar factory will depend mainly on conditions, such as the variety of cane grown, and the procedure for its cultivation and fertilization, which are largely outside the control of the factory chemist, but it will be effected somewhat by the severity of milling, since it has been observed that the nodes of the cane contain more protein materials than the internodes and it is well known that the nodes require more severe treatment to crush them than the rest of the cane. The way in which the cane is harvested will of course also effect the nitrogen content of the juice, since if leaves are left on the cane, their higher nitrogen content will be partly transferred to the juice during milling and maceration. It has been reported that out that of the nonsugars in cane juice about 9% are proteins, 9.5% amino acids and 15.5% amino acid amides. Some of these substances, namely, the amino acids and their amides exist in true solution in cane juice, but the proteins are colloidal in this environment. When cane juice is clarified by lime and heat treatment some of the protein is coagulated and is removed, together with inorganic precipitates, during subsidation, but some remains in colloidal solution. The amino acids and their amides will tend to be removed as their calcium salts, but since these are not very insoluble there will be only a small removal of these substances in filter mud and hence they will be transported through the remainder of the manufacturing process and will contribute to molasses formation. It has been observed in sugar factories particularly in beet, it was aspartic and glutamic acids which were at least partially precipitated during defecation. The amides such as asparagine and glutamine will decompose during the lime-heat treatment with the formation of the corresponding amino acids and ammonia which, of course, provides the reason for the alkalinity of condensate water from the evaporators. Because these nitrogen compounds are not normally eliminated from the juice during clarification (some 60% of the nitrogen in cane juice could be referred to in this way) and they have been labelled as Objectionable nitrogen' by sugar technologists. Of this 50% is made up of amino acids and represents 55% of the total nitrogen compounds present in the cane. Thus, that nitrogen-containing nonsugars do accumulate in the molasses. For example: One sample of molasses of 85.7% total solids, 30.7% sucrose and 24.8 % reducing sugar content, obtained from a sugar factory, was found to contain 0.4 7 % nitrogen of which 0.135 % could be allocated to colloidal nitrogenous substances and 0.333% to nitrogen-containing substances capable of existing in true solution. Because the proteins in cane juice are colloids which exhibit a vast surface area, they possess an importance to sugar manufacture quite out of proportion to their mass. It has been calculated that, if 1 cubic centimeter of material is split up into particles of colloid dimensions, the total surface area

of these would be 1.5 acres. Thus, proteins in cane juice may well have a pronounced effect on colour formation during sugar manufacture and boiling house performance, crystallization and centrifuging the sugar, and possibly the amount of sugar which ultimately crystallizes. The presence of colloids in the massecuite will also tend to increase its viscosity so that the purging rate of the massecuite will be affected by the amount of colloidal material present. The presence of these materials could also affect the boiling house performance. Therefore, it is important to remove as much as possible of the proteins in cane juice at the defecation station. It is known that considerable amounts of colloidal nitrogenous material are in fact left in clarified juice. An estimate of the total colloid content of the juice before and after filtration showed that a 40-60 % removal had been effected (colloidal nitrogenous materials which become adsorbed onto the filter-aid). Some workers have considered how to increase the efficiency of protein removal from sugar juices and described the use of tannins for removing proteins from sugar juices or from molasses. Since the isoelectric point of albumin is at pH 5.5 and that of amino acids present in juice at lower pH values, and alkaline earths exhibit a strong inhibitory effect on the heatcoagulation of albumin, the optimum precipitation of this protein could not take place when limed juice was heated and suggested that better coagulation would be effected by first heating juicem and then liming it. Furthermore, it is known that both albumins and nucleins, which comprise about a fifth of the nitrogenous materials in cane juice, are melassigenic, they were even more melassigenic than alkalis. Though, the precipitation of albumin during defecation is advantageous since this type of precipitate will occlude other materials onto it and thus aid the removal of other nonsugars. Because they can only be very imperfectly removed by the defecation process, they must contribute to molasses formation and, despite the small quantities involved, it is probable that they are important melassigenic factors. This is because they combine with reducing sugars to form dark coloured products of high molecular weight termed melanoidins. The nitrogen content of sugarcane juice amounts to only a few hundredths of one percent, of which about half is present in forms potentially active in color forming reactions. The initial step of the Maillard reaction (Maillard browning) is believed to be formation of N-(D-glucosyl) amino acids, which are converted to D-fructose amino acids by the Amadori rearrangement. There is considerable evidence for free radical participation in an accompanying oxidative process. Amino acids do not react in their cationic forms, only slightly in their zwitterion forms, and fully only in their anionic forms. If the amino compound is in fact an amino acid, Strecker degradation usually takes place, whereby CO2 is liberated and an aldehyde or ketone is formed with one carbon atom

fewer than the original amino acid. The browning can be inhibited by sulfite addition. These are in fact materials capable of forming colloidal dispersions and hence small quantities may be quite effective in inhibiting crystallization. These dark coloured products also contribute not inconsiderably to the colour of raw sugar massecuites. It is responsible for the foaming- froth fermentation that occurs in molasses storage tanks, since it is known that the reaction is accompanied by the evolution of carbon dioxide. This may well continue to take place when the molasses has been in storage for some time. However, the froth fermentation may also be due to the decomposition of products formed by the action of lime on reducing sugars. The best method and probably the only method of removing amino acids or amino acid amides from sugarcane juice is by the use of ion-exchange resins (removal of nonsugar solids: that of the total nitrogen removable by an ion-exchange process, 70-80 % is removed by the cation bed and 20-30 % by the anion bed). The amino acids in cane juice are optically active and it might be thought that they would affect the Polarimetrie analysis for sugars in factory products, but the amounts present are so small that this is not likely to be the case. Moreover, the rather drastic treatment to which they are subjected during the sugar manufacturing process will doubtless tend to racemize them and hence destroy their optical activity.

ENZYMES:

Enzymes are biological catalysts, consisting of protein molecules and are industrially produced from microorganisms. They are specific in their action and leave no undesirable residue in the final product. In sugar manufacture in the strictest sense they are not employed in juice purification process, nonetheless their addition in the stages preceding or succeeding clarification to overcome the problems in processing created by the presence starch and dextran, is essential.

1. **AMYLASE:**

Starch is a polysaccharide composed of amylose and amylopectin in the ratio of 1:4 out of which the amylose exerts depressing effect on the filterability of raw sugars. The starch granules are solubilized at 70°C and very little of it is eliminated in purification process either by defecation of Sulphitation. Because of it melassigenic effect and the contribution to increase in viscosity in pan boiling starch content above certain level-viz. 400-500 ppm is considered undesirable. Moreover much of the starch finds its way into the sucrose

crystals and starch content in sugar beyond 150 ppm. Creates problems in filtration in the refining of raw sugar. Enzyme amylase hydrolyses starch at temperature 60°C - 70°C and PH 6.5, in to oligosaccharides and other polysaccharides according to studies conducted in South African factories. An amylase termanmyl of Novo A/c enzyme is recommended to be added at the entry point of the second body of the evaporator since it is suitable and effective at 90°C - 95°C. The enzyme is diluted ten times before use. If a particular type of amylase is effective at 70°C - 80°C in its action on starch it is added at the entry to the last but one vessel of evaporator. Perk recommended withdrawal of syrup from 3rd body of evaporator in to a tank with thermostatic control and retention of the same in contact with enzyme for 20 minutes for efficient starch hydrolysis before the syrup is taken in to last vessel of the evaporator.

DEXTRANASE:

Dextran is produced by bacteria Leuconostoc Mesenteriodes in cane which are crushed after prolonged postharvest delay and even in the mills. Dextran is a long chain polymer of glucose molecules joined together by Alpha 1:6 linkage and possesses very high molecular weight ($5x10^6$). It increases viscosity in boiling and contributes to higher loss of recoverable sugar. At the crystallization station dextran inhibits the growth along B axis of the sucrose crystals which elongate along the 'C' axis. According to the findings of filbury elongation of sucrose crystals of 2.3 is noticed in 'C' massecuite boiling with dextran level of 1.3% solid, as against normal 1-1.2. Use of specifica enzyme dextranase results in partial reduction of dextran concentration and the residual dextran is of lower molecular weight which does not affect the crystallization by increasing viscosity of suppressing the growth of crystals along B-axis. The dextranase enzyme is effective at PH 4.5-5.5 and temperatures 50-60°C conditions which make its addition essential at raw juice stage before heating. The optimum reaction time is 15-20 minutes. While the dosage is 10-20 ppm. of juice for hydrolysis of dextran.

The dextranase is commercially produced from strain of Penicillium, and its use of choice should be governed by consideration of cost. This enzyme can solve the problems emanating from introduction of dextran generated from staling of cut cane at the expense of sugar in the process and it is essential that greater emphasis is laid on preventing this dextran formation by control of post-harvest delay in processing and good mill sanitation.

FIRST YEAR A.N.S.I. (S.T.) O C (T): Non-nitrogenous Organic Acid

Organic acids represent a significant percentage of the total soluble non-sugars of sugarcane; are originally present in cane juice which varies significantly with the period of maturity and climate as well as the nature of soil and fertilization. The organic acids have an important role in the fundamental life processes of plants and exist as metablic intermediate. The organic acids are found in abundant (0.7 to 1.8 % on solid in juice) which comes out during milling of the cane they are responsible for most of the titratable acidity of the juice, which measures the total concentration of hydrogen ions. Most of the organic acids are present in a comparatively low concentrations, but Aconitic acid is present in abundance (existing at an average level of about 1.50 % on solid in juice or approximately three times higher than the level of other acids-citric, malic, oxalic, glycolic, mesaconic, tartaric, succinic, fumaric and syringic). Theses acid are responsible for the acidity in the juice due to the organic acids and their salts, it also solves the purpose of regulating the acidity and alkalinity by it buffering action on the juice. Acetic acid and lactic acid are not naturally found in cane, their presence is product of microbial infection. These acids increase their concentration in frozen cane. Levulinic acid by the action of heat on sugars & levulose in underlimed and overlimed juice in acid media. Glucic acid is produced by action of heat on sugars in alkaline media. Some organic acid may come from plant e. g. vanillic acid. These acids and their alkali salts are quite soluble in water and they have important effects on the liming action and clarification processes on the recovery of sugar.

Effect of Organic Acids on Clarification:

It is generally agreed that the principal reaction in clarification by liming is the formation and flocculating of insoluble calcium phosphates. The minimum effect of the organic acids is to compete for the lime and increase the amount required to attain the necessary final pH. Organic acids affect the clarification process due to the sensitivity of this process to variation in hydrogen concentrations. These acids compete for the lime with phosphoric acid, and due to their buffer capacity (the ability to absorb large quantities of lime or other base with a small change in pH) increase the amount of lime required. These acids are able to participate in complex reactions with sugars and other organic constituents of the juice; they are associated with the formation of mellasigenic compounds (dark colour formation) during sugar processing. The solubilities of the salts of these

organic acids, with few exceptions, are sufficiently high that the amounts present remain dissolved in the clarified juice and are carried through subsequent steps of the process to accumulate in the molasses. Only the calcium salts of aconitic and oxalic acids are either insoluble or concentrated enough to separate to some extent during evaporation. Non-nitrogenous organic acids of this class are comparatively stable compounds which are not eliminated by decomposition at the temperatures of clarification and evaporation used in sugar manufacture. Deposition of aconitates and oxalate in scales at heaters and evaporators surfaces and delayed precipitation of aconitates in molasses are due to the peculiar properties of the complex calcium and magnesium salts of the acid. Salts of organic acids have been shown to affect the rate of crystallization of sugar significantly. Soluble calcium salts of organic acids are primarily responsible for a smaller rise in purity in the clarification of juices. Precipitation of phosphate is also adversely affected, either by the solubilizing effect of the organic calcium salts on calcium phosphate, or by failure to lime completely in the highly buffered juice.

Problem in clarification of juices containing high concentrations of aconitate relative to phosphate: The principal competitor of phosphoric acid in this reaction is the aconitic acid which is now known to be the most abundant of these compounds in the juice, but the proportions of some of the others may not be negligible. The minimum phosphate requirement for good clarification has been shown to be 300 mg/liter, and the percentages are equivalent to concentrations of 8 mg/liter of malic, and 4 mg/liter of oxalic acids. The concentration of aconitic acid is always at least of the same order as that of phosphoric and, on an equivalent basis, it probably exceeds the phosphate in most juices. At some places, the aconitic acid concentration averages approximately 1500 mg/liter, and in terms of chemical equivalents it exceeds the phosphate in a ratio of 3/2, or more. This preponderance occurs also in juices of low aconitic acid content, as such juices are low in phosphate and total ash as well. The buffering capacity of the organic acid facilitates the control of pH by acting as a safeguard against sudden changes to excessive alkalinity, the presence of the organic acid buffer is desirable. Large amounts have the disadvantages not only of increasing the lime requirement, but of holding calcium combined in a soluble form. Soluble calcium salts of organic acids are primarily responsible for a smaller rise in purity in the clarification of juices. Precipitation of phosphate is also adversely affected, either by the solubilizing effect of the organic calcium salts on calcium phosphate, or by failure to lime completely in the highly buffered juice. The practical results of the influence of aconitic acid can be strikingly demonstrated by considering following example of clarification study in which it was found that the percentage of raw juice phosphate eliminated was correlated inversely with the concentration of aconitic acid in the juices. The extreme examples were a juice containing 0.552% P205 and 1.52% aconitic acid on dry solids, from which the elimination of phosphate was 8 7 % , and one containing 0.521% P205 and 2.15 % aconitic acid, from which only 72 % of the phosphate was eliminated.