

Lactose (milk sugar; O-β-D-galactopyranosyl-D-glucopyranose) is the principal carbohydrate found in significant quantities in milk.

Trace amounts of other sugars are glucose (50 mg/l) and fructose and glucosamine, galactosamine and N-acetyl neuraminic acid as components of glycoproteins and glycolipids.

Human milk contains highest lactose content (7.0%) while the average lactose content of normal bovine milk is 4.8% .

The lactose content of cows milk varies with the breed of cow, individual animals, udder infection (mastitis depresses secretion of lactose) and stage of lactation.

Lactose and lipids act as sources of energy and lactose along with sodium, potassium and chloride ions, plays a major role in maintaining the osmotic pressure in the mammary system.

Importance in fermented and ripened dairy products.

It contributes to the nutritive value of milk and milk products, it is related to the texture and solubility of certain stored dairy products, and it plays an essential role in the color and flavor of highly heated (caramelized) dairy products.

It affects the texture of certain concentrated and frozen products.

It is involved in heat-induced changes in the colour and flavour of highly heated milk products.

Its changes in state (amorphous vs. crystalline) have major implications for the production and stability of many dehydrated milk products.

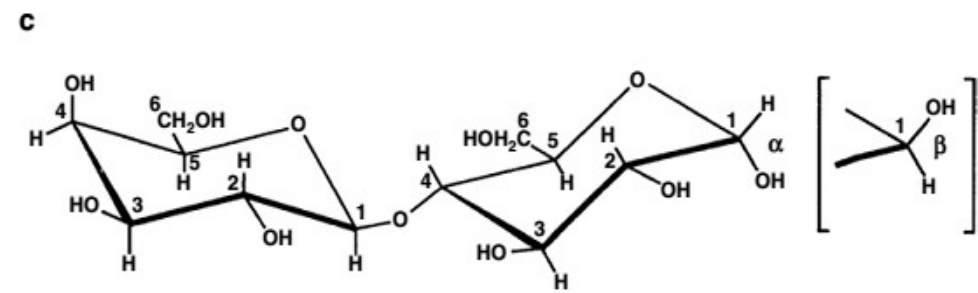
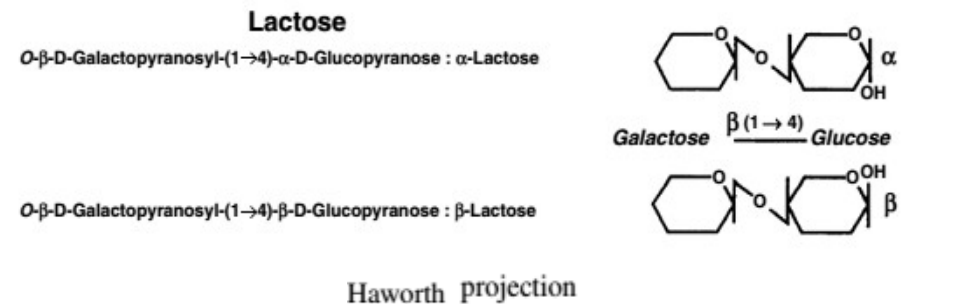
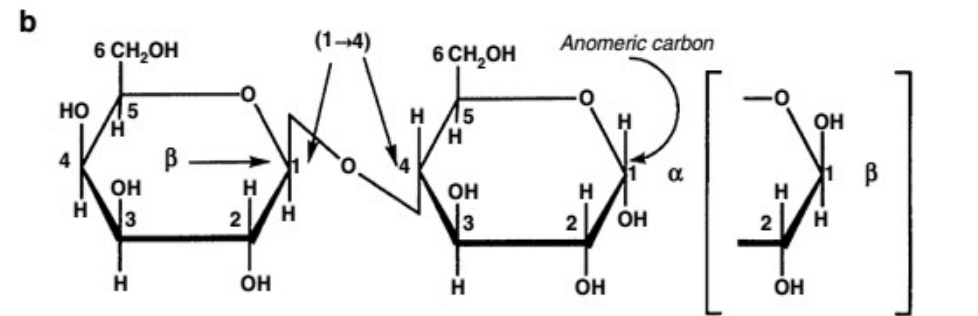
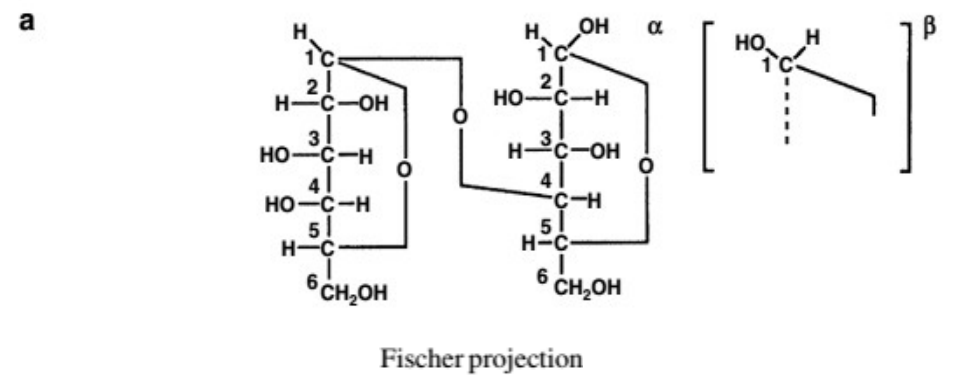
Lactose is a disaccharide composed of D-glucose and D-galactose; the aldehyde group of galactose is linked to glucose, through β -1,4-glycosylic linkage.

Lactose exists in two basic isomeric forms i.e. α and β (designations refer to the substituents on the number one carbon of the glucose moiety).

The rings can open and re-close, allowing rotation to occur about the carbon bearing the reactive carbonyl yielding two distinct configurations (α and β) of the hemiacetals (addition of alcohol to an aldehyde). The carbon about which this rotation occurs is the anomeric carbon and the two forms are termed anomers. Carbohydrates can change spontaneously between the α and β configurations: a process known as mutarotation.

An axial OH on the anomeric carbon makes the sugar α and equatorial OH on the anomeric carbon makes the sugar β .

Lactose normally occurs naturally in either of two crystalline form α -monohydrate and anhydrous β or as an amorphous “glass” mixture of α - and β -lactose. The difference is mainly in their solubility, optic rotation and melting point.



The configuration around the anomeric C1 of glucose is not stable and can readily change (mutarotate) from the α - to the β -form and vice versa when the sugar is in solution until equilibrium is established.

Ordinary commercial lactose is α -lactose monohydrate. It is prepared by concentrating an aqueous lactose solution to super saturation and allow for crystallization to take place at a modern rate below 93.5 C. That α -hydrate is the stable solid form at ordinary temperatures is indicated by the fact that the other solid forms change to the hydrate in the presence of a small amount of water below 93.5 C.

β -lactose: This is the other isomeric form lactose above 93.5 C crystallization or drying of lactose solutions yields β anhydrate.

All of these forms of lactose undergo mutarotation in aqueous solution, yielding a specific rotation $+55.4^\circ$ (anhydrous basis) at equilibrium.

The specific rotation of a chemical compound $[\alpha]$ is defined as the observed angle of optical rotation a , when plane-polarized light is passed through a sample with a path length of one decimeter and a sample concentration of one gram per millilitre.

Specific rotation: $[\alpha] = 100 a/lc$

Where;

$[\alpha]$ = specific rotation at 20C

a = degrees of angular rotation

l = length of tube in decimeter

c = concentration of substance in grams per 100 ml of solution

Specific rotation of a pure material: Wavelength, temperature, solvent in which the material was dissolved.

Formal unit for specific rotation values is $\text{deg dm}^{-1}\text{cm}^3\text{per g}$

a is the form with greater optical rotation.

Regardless of the form used in the preparation of solution, the specific rotation will continue to change until +55.4°C is reached at equilibrium. This is equivalent to the 37.3 % in α form and 62.7 % in β form. Since equilibrium rotation is the sum of the individual mix of α and β forms.

Rate of mutarotation depends upon temperature (75C), pH, combination of salts (citrates and phosphates) and high levels of sucrose.

The composition of the mixture at equilibrium may be calculated as follows:

Specific rotation: $[\alpha]_D^{20}$
α -form +89.4°
β -form +35.0°
Equilibrium mixture +55.4°
Let equilibrium mixture = 100
Let x% of the lactose be in the α -form
Then (100 - x)% is the β -form
At equilibrium:
$89.4x + 35(100 - x) = 55.4 \times 100$
$x = 37.5$
$100 - x = 62.5$

Solubility increases with increasing temperature.

Coarse lactose crystals dissolve much slower than tiny lactose particles.

α form is converted to β form



Unsaturated with respect to a, and more α -hydrate dissolves



This process continues until equilibrium is established between α and β and no more α -hydrate can dissolve, thus establishing the final solubility.



This solution is saturated with respect to a, but a great deal of β -lactose powder can be dissolved in it (greater initial solubility of the β form)



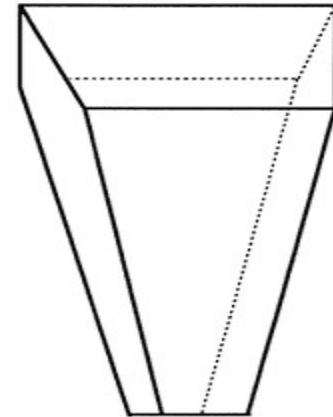
The solution becomes saturated with along before the saturation point of β is reached. However, additional β dissolving in such a solution upsets the equilibrium, and mutarotation takes place. Since the solution was already saturated with a, a formed by mutarotation will crystallize to reestablish equilibrium.

- High pressure leads to crystal form of prisms.
- Low precipitation pressure, crystal form changes to diamond-shape plates, then to pyramids and tomahawks and finally, slow crystallization to the fully developed crystal.
- Low pH (<1) increases the rate of lactose crystallization.
- Some carbohydrates actively inhibit the crystallization of lactose.
- Methanol and ethanol accelerate crystallization by as much as 30 to 60% even at low (1%) concentrations.
- The rate of crystal growth increases rapidly as supersaturation (precipitation pressure) is increased.
- In dairy products, impurities or additives (types or concentrations) interfere with the crystalline habit and prevent lactose crystallization.
- Gelatin is an example of a crystallization inhibitor. In highly supersaturated lactose solutions, however, gelatin cannot suppress nucleation, which explains its ineffectiveness in preventing sandiness in ice-cream, marine and vegetable gums are currently in wide use in ice cream formulations.
- Mutarotation, which is slow at low temperatures

α -Hydrate: α -Lactose crystallizes as a monohydrate containing 5% water of crystallization and can be prepared by concentrating an aqueous lactose solution to supersaturation and allowing crystallization to occur below 93.5 °C. The α -hydrate is the stable solid form at ambient temperatures and in the presence of small amounts of water below 93.5°C, all other forms change to it. The α -monohydrate has a specific rotation in water at 20 °C of +89.4°. It is soluble only to the extent of 7 g per 100 g water at 20°C. It forms a number of crystal shapes, depending on the conditions of crystallization; the most common type on fully developed is tomahawk-shaped.

α -Anhydrous: Anhydrous α -lactose may be prepared by dehydrating α -hydrate in vacuo at a temperature between 65 and 93.5 °C; it is stable only in the absence of moisture.

β -Anhydride: Since β -lactose is less soluble than the α -isomer >93.5 °C, the crystals formed from aqueous solutions at a temperature above 93.5 °C are β -lactose which are anhydrous and have a specific rotation of 35°.



Also called as amorphous noncrystalline glass.

When a lactose solution is dried rapidly, its viscosity increases so quickly that crystallization cannot take place. The dry lactose with removed water is “concentrated syrup” or an “amorphous”(noncrystalline) glass.

Lactose glass is stable if protected from moisture, but since it is very hygroscopic, it rapidly takes up moisture from the air and becomes sticky. When the moisture content reaches about 8% or a relative vapour pressure near 0.5, the lactose achieves a maximum weight; a discontinuity is observed in the sorption isotherm, and water is desorbed from the lactose.

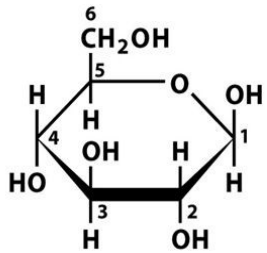
Variations in the densities of the various lactose crystals

- α -hydrate form: 1.540
- Anhydrous β : 1.589
- Anhydrous α formed by dehydration under vacuum: 1.544

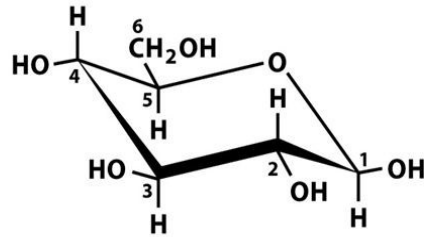
- At certain concentrations, the relative sweetness of sugars varies. Lactose is relatively sweeter at higher concentrations than at lower concentrations.
- β -lactose is sweeter than α -lactose but β form of lactose is not appreciably sweeter than the equilibrium mixture except when the concentration of lactose solution equals or $>7\%$.
- Since there is approximately 62.7% of β form in the equilibrium mixture, a β -lactose solution differs less in sweetness from a solution in equilibrium.
- β -lactose appears to be somewhat sweeter than α -lactose due to that β -lactose dissolves somewhat quicker in the saliva of the mouth than α -lactose, reaching a higher concentration in the same period of time and thus giving rise to a higher sweetness sensation.

SPATIAL RELATIONSHIPS

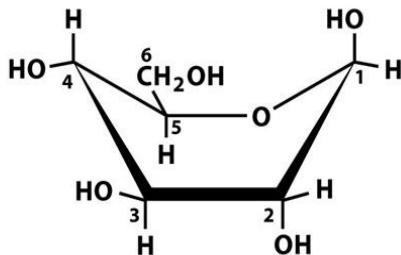
- The spatial relationships of the atoms of the furanose and pyranose ring structures are more correctly described by the two conformations identified as the chair form (more stable) and the boat form. The pyranose ring is formed by the reaction of the hydroxyl group on carbon 5 (C-5) of a sugar with the aldehyde at carbon 1. This forms an intramolecular hemiacetal. If reaction is between the C-4 hydroxyl and the aldehyde, a furanose is formed.
- Substituents that extend parallel to the ring's threefold rotation axis are "axial" and those extend outward from symmetry axis are "equatorial". In the chair conformation, the orientation of the OH group about the anomeric carbon of α -D-glucose is axial and equatorial in β -D-glucose.



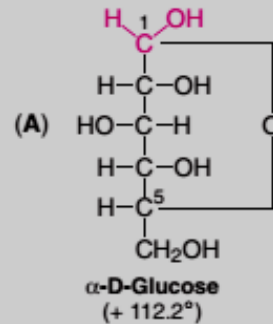
Haworth projection



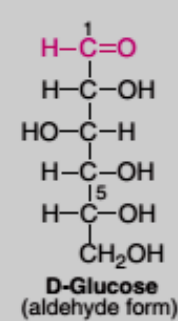
Chair conformation



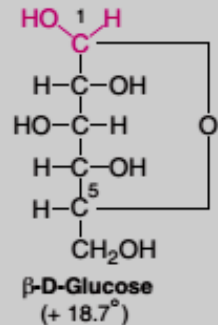
Boat conformation



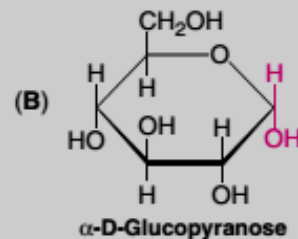
α -D-Glucose
(+ 112.2°)



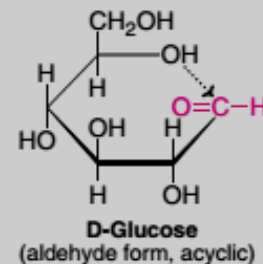
D-Glucose
(aldehyde form)



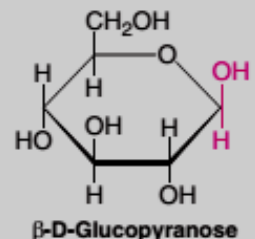
β -D-Glucose
(+ 18.7°)



α -D-Glucopyranose



D-Glucose
(aldehyde form, acyclic)



β -D-Glucopyranose

MILK SALT COMPOSITION DURING UDDER INFECTION

Milk from cow with mastitic infections contains a low level of total solids, especially lactose and high levels of sodium and chloride, the concentration of which are directly related.

The sodium and chloride ions come from blood to compensate osmotically for the depressed lactose synthesis or vice versa. These are related by the Koestler number:

$$\text{Koestler Number} = \% \text{Cl} \div \% \text{Lactose} \times 100$$

(Normally 1.5 - 3.0 but increases on mastitic infection and has been used as an index of such infections. The pH of milk increases (e.g. 6.8-6.9) to approach that of blood (7.4) during mastitic infection due to the influx of constituent from blood).

CHEMICAL PROPERTIES OF LACTOSE

The hemiacetal linkages between carbon 1 and 5 of the glucose moiety

β -1,4 glycosidic linkages between the two sugars

The hydroxyl groups of both the glucose and galactose units

The carbon to carbon bonds

OXIDATION:

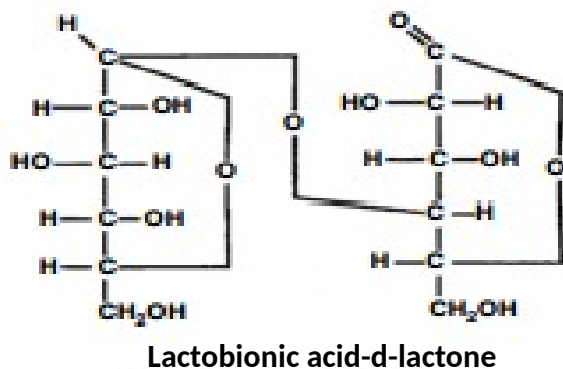
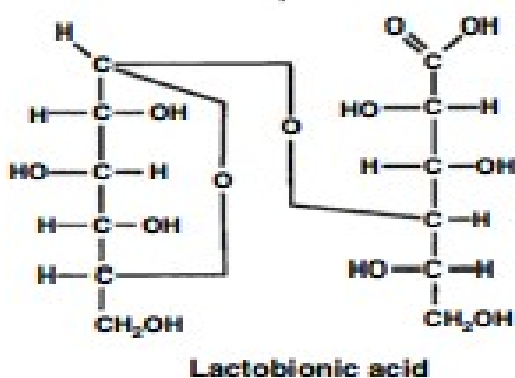
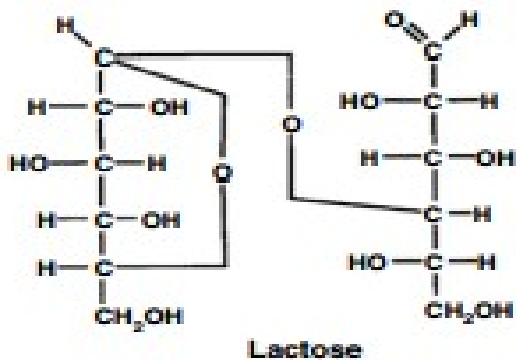
Vigorous Oxidation: Oxidation of lactose with dilute nitric acid ruptures the glycosidic linkage and produces dicarboxylic acid derivatives of the two sugars.

Lactose \longrightarrow D-glucaric acid + D-galactaric acid
(Saccharic acid) (Mucic acid)

Biological oxidation:

Lactose \longrightarrow CO₂ and water

(Mixed cultures of bacteria and protozoa obtained from sewage sludge. Such processes are useful in decomposing lactose-containing wastes from dairy factories.)



Mild Oxidation: Determine reducing sugars quantitatively

Lactose \rightarrow Lactobionic acid (The free aldehyde group of lactose can be oxidized to a carboxylic acid or lactobionic acid).

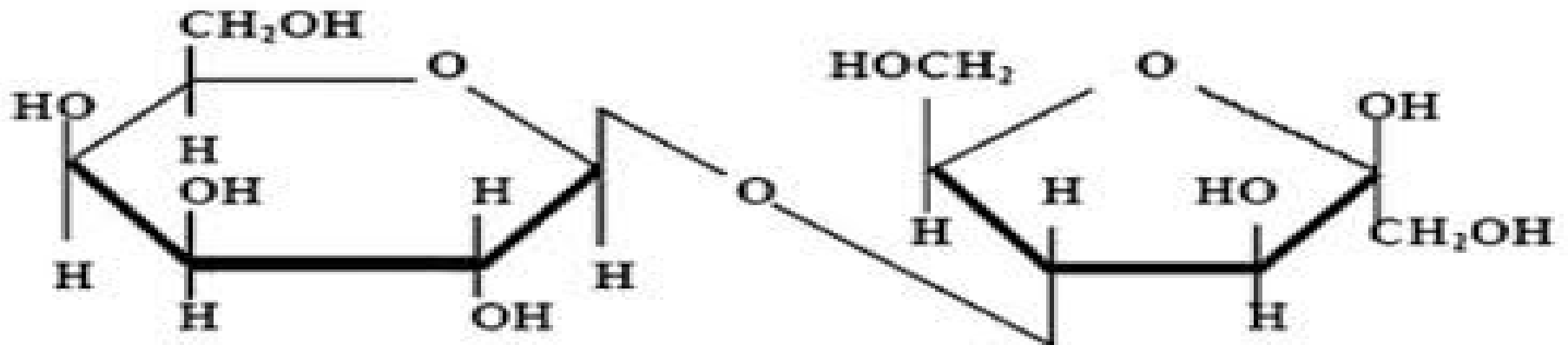
Lactose also can be oxidized to lactobionic acid by lactose dehydrogenase (*Pseudomonas*) and lactobionic acid have a profound tendency to form lactones.

LACTULOSE: (4-O- β -D-Galactopyranosyl-D-Fructose)

Isomerized lactose consisting of galactose and fructose. Lactulose is an epimer of lactose in which the glucose moiety is isomerized to fructose. It can be produced under mild alkaline conditions via the Lobry de Bruyn-Alberda van Ekenstein reaction and at a low yield as a by-product of β -galactosidase action on lactose.

It is produced on heating milk to sterilizing conditions and is a commonly used index of the severity of the heat treatment to which milk has been subjected, e.g., to differentiate in-container sterilized milk from UHT milk, it is not present in raw or HTST pasteurized milk.

A method used to prepare lactulose nearly 90% yield is by treatment of lactose with boric acid in an aqueous solution made basic by tertiary amines.



Lactulose is extremely soluble in water and methanol and difficult to crystallize.

Lactulose is unstable in alkaline solution.

Lactulose is sweeter than lactose and about 60% as sweet as sucrose.

This laxative is not metabolized by oral bacteria and not hydrolyzed by intestinal β -galactosidase and hence reaches the large intestine where it can be metabolized by lactic acid bacteria, including *Bifidobacterium* spp. and serves as a bifidus factor. So, lactulose has attracted considerable attention as a means of modifying the intestinal microflora, reducing intestinal pH and preventing the growth of undesirable putrefactive bacteria.

Added to infant formulae to simulate the bifidogenic properties.

ACID HYDROLYSIS:

Lactose is resistant to acid hydrolysis compared to other disaccharides.

• The speed of hydrolysis of lactose varies with time, temperature, and concentration of the reactant; 5-40% lactose solutions (w/w) can readily be hydrolyzed with 1 to 3 N hydrochloric acid or sulfuric acid. 90% of the lactose could be hydrolyzed to the constituent monosaccharides at relatively low temperatures (60 C) and long reaction times (up to 36 h).

Lactose hydrolysis can also be brought about with 0.1 N HCl in short reaction times at 121 C.

Sulfonic acid-type ion exchange resins have been used to catalyze lactose hydrolysis (90-98°C). The advantages of this method are continuous operation, short reaction times, and no mineral acid to be removed from the hydrolyzed product.

ENZYMATIC HYDROLYSIS:

3 major approaches to enzymatic hydrolysis -

- ✓ “Single use” or “throwaway” lactase systems
- ✓ Lactase recovery systems based on membranes to retain the lactase for reuse
- ✓ Immobilized systems in which the enzyme is physically or chemically bound to a solid matrix.

Lactases are suitable for industrial processing of whey or lactose. Prepared from the yeast *Kluyveromyces lactis* has a optimum pH 4.8 and temperature of about 50 C. A batch processing operation is the simplest method of achieving enzymatic lactose hydrolysis but suffers from the disadvantage that a large amount of recoverable enzyme is needed.

Membrane reactor systems in which the enzyme is recovered by ultra filtration of the reaction mixture after hydrolysis is complete.

Lactose hydrolysis with immobilized systems is preferred when regular production of hydrolyzed syrups on a large scale. Corning immobilized system uses lactase (*Aspergillus niger*) covalently bound to a controlled-poresilica carrier. The rate of hydrolysis is dependent on the mineral, lactose, galactose concentrations, temperature and pH.

For immobilized systems, adequate techniques such as back flushing with water, acetic acid, milk alkali and detergents with bactericidal activity must be developed to ensure sanitary operations.

Galactose is primarily involved in the formation of the oligosaccharides, which accounts for the lower concentration of free galactose than of free glucose during hydrolysis.

The use of hydrolyzed lactose syrups has been proposed as an alternative sweetener to corn syrup solids.

LACTOSE FERMENTATION

In dairy background, the most important is lactic acid bacterial fermentation.

Lactic acid bacteria is important in the preparation of cultured products, butter and cheese.

The bacteria are classified as homofermentative if they produce only lactic acid and heterofermentative if they produce lactic acid, acetic acid, alcohol and carbon dioxide.