Chapter 2
Lactose

2.1 Introduction

Lactose is the principal carbohydrate in the milk of most mammals, exceptions are the California sea lion and the hooded seal, which are the only significant sources. Milk contains only trace amounts of other sugars, including glucose (50 mg/l) and fructose and glucosamine, galactosamine and *N*-acetyl neuraminic acid as components of glycoproteins and glycolipids. The milk of all species that have been studied contain oligosaccharides which are major constituents of the milk of some species, including human. This chapter will concentrate on the chemistry and properties of lactose with a short section on oligosaccharides.

The concentration of lactose in milk varies widely between species (Table 2.1). The lactose content of cows' milk varies with the breed of cow, individual animals, udder infection (mastitis) and stage of lactation. The concentration of lactose decreases progressively and significantly during lactation (Fig. 2.1); this behaviour contrasts with the trends for lipids and proteins, which, after decreasing during early lactation, increase strongly during the second half of lactation. The concentration of lactose in milk is inversely related to the concentrations of lipids and proteins (Fig 2.2) (Jenness and Sloan 1970; Jenness and Holt 1987). The principal function of lactose and lipids is as sources of energy; since lipids are ~2.2 times more energy-dense than lactose, when a highly caloric milk is required, e.g., by animals in a cold environment (marine mammals and polar bears), this is achieved by increasing the fat content of the milk. The inverse relationship between the concentrations of lactose and lipids and protein reflects the fact that the synthesis of lactose draws water into the Golgi vesicles, thereby diluting the concentrations of proteins and lipids (Jenness and Holt 1987).

Mastitis causes an increased level of NaCl in milk and depresses the secretion of lactose. Lactose, along with sodium, potassium and chloride ions, plays a major role in maintaining the osmotic pressure in the mammary system. Thus, any increase or decrease in lactose content (a secreted constituent, i.e., formed within the mammary gland, which is isotonic with blood) is compensated for by an increase or decrease

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Table 2.1 Concentration (%) of lactose in the milk of selected species

Species	Lactose	Species	Lactose	Species	Lactose
California sea lion	0.0	Mouse (house)	3.0	Cat (domestic)	4.8
Hooded seal	0.0	Guinea pig	3.0	Pig	5.5
Black bear	0.4	Dog (domestic)	3.1	Horse	6.2
Dolphin	0.6	Sika deer	3.4	Chimpanzee	7.0
Echidna	0.9	Goat	4.1	Rhesus monkey	7.0
Blue whale	1.3	Elephant (Indian)	4.7	Man	7.0
Rabbit	2.1	Cow	4.8	Donkey	7.4
Red deer	2.6	Sheep	4.8	Zebra	7.4
Grey seal	2.6	Water buffalo	4.8	Green monkey	10.2
Rat (Norwegian)	2.6				

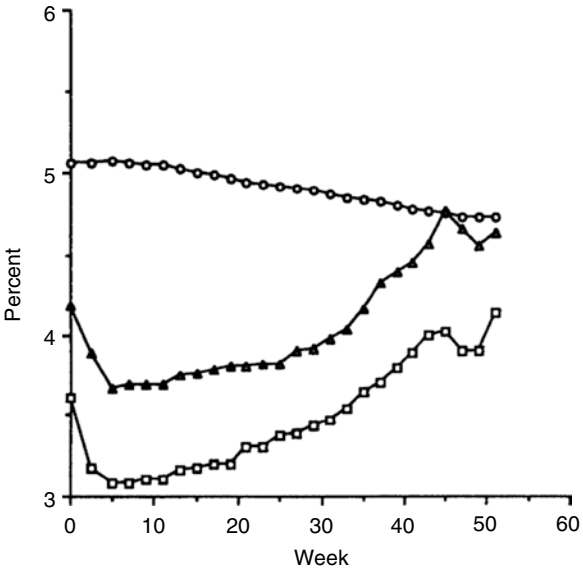


Fig. 2.1 Changes in the concentrations of fat (*closed triangle*), protein (*empty square*) and lactose (*open circle*) in milk during lactation

in the soluble salt constituents (excreted) (Fig. 2.3). This osmotic relationship partly explains why certain milks with a high lactose content have a low ash content and *vice versa* (Table 2.2).

Similarly, there is an inverse relationship between the concentrations of lactose and chloride, which is the basis of Koestler's chloride-lactose test for abnormal milk:

$$\text{Koestler Number} = \frac{\%\text{Chloride} \times 100}{\%\text{Lactose}}$$

A Koestler Number <2 indicates normal milk while a value >3 is considered abnormal.

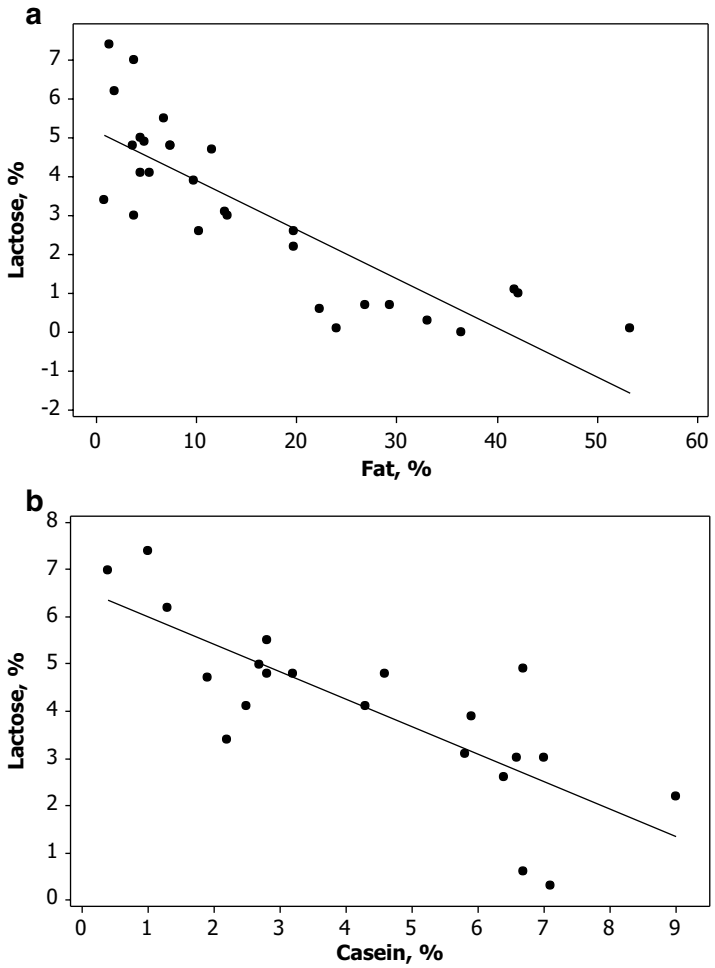


Fig. 2.2 Correlation between lactose and fat (**a**) and casein (**b**) in the milk of 23 species (based on the data of Jenness and Sloan 1970)

Lactose plays an important role in milk and milk products:

1. It is an essential constituent in the production of fermented dairy products.
2. It contributes to the nutritive value of milk and its products; however, many non-Europeans have limited or zero ability to digest lactose in adulthood, leading to **lactose intolerance**.
3. It affects the texture of certain concentrated and frozen products.
4. It is involved in heat-induced changes in the colour and flavour of highly heated milk products.
5. Its changes in state (amorphous vs. crystalline) have major implications for the production and stability of many dehydrated milk products.

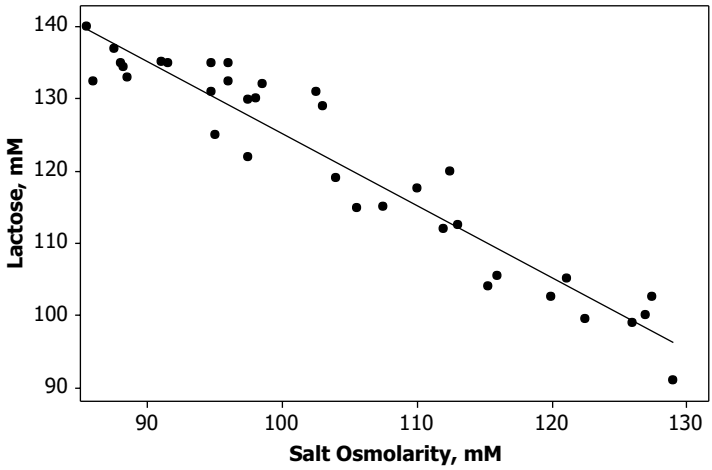


Fig. 2.3 Relationship between the concentration of lactose (mM) and osmolarity (mM) due to salts (redrawn from the data of Holt 1985)

Table 2.2 Average concentration (%) of lactose and ash in the milk of some mammals

Species	Water	Lactose	Ash
Human	87.4	6.9	0.21
Cow	87.2	4.9	0.70
Goat	87.0	4.2	0.86
Camel	87.6	3.26	0.70
Mare	89.0	6.14	0.51
Reindeer	63.3	2.5	1.40

2.2 Chemical and Physical Properties of Lactose

2.2.1 Structure of Lactose

Lactose is a disaccharide consisting of galactose and glucose, linked by a β1-4 glycosidic bond (Fig. 2.4). Its systematic name is 0-β-D-galactopyranosyl-(1-4)-α-D-glucopyranose (α-lactose) or 0-β-D-galactopyranosyl-(1-4)-β-D-glucopyranose (β-lactose). The hemiacetal group of the glucose moiety is potentially free (i.e., lactose is a **reducing** sugar) and may exist as an α- or β-anomer. In the structural formula of the α-form, the hydroxyl group on the C_1 of glucose is *cis* to the hydroxyl group at C_2 (oriented downward).

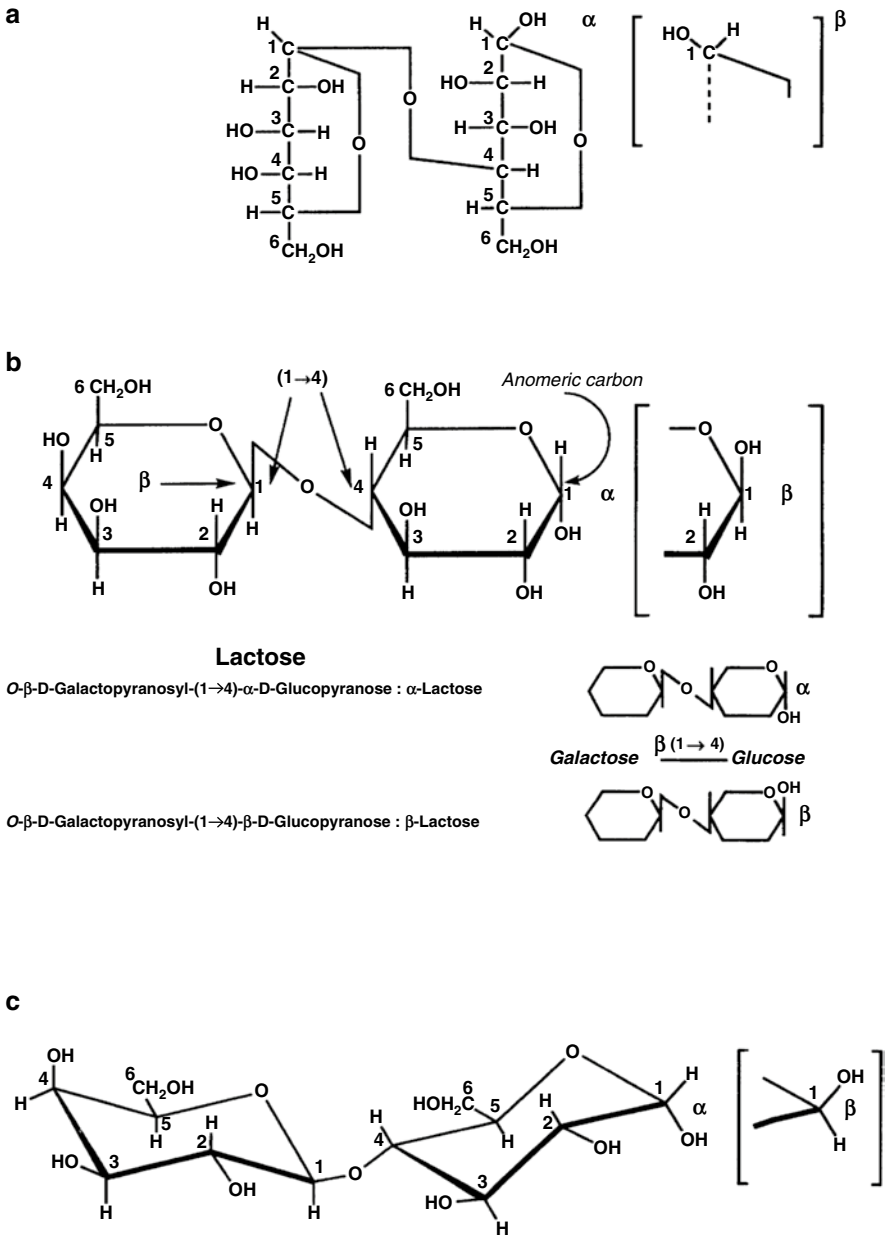


Fig. 2.4 Structural formulae of α- and β-lactose (**a**) Open chain, (**b**) Fischer projection, (**c**) Haworth projection and (**d**) conformational formula

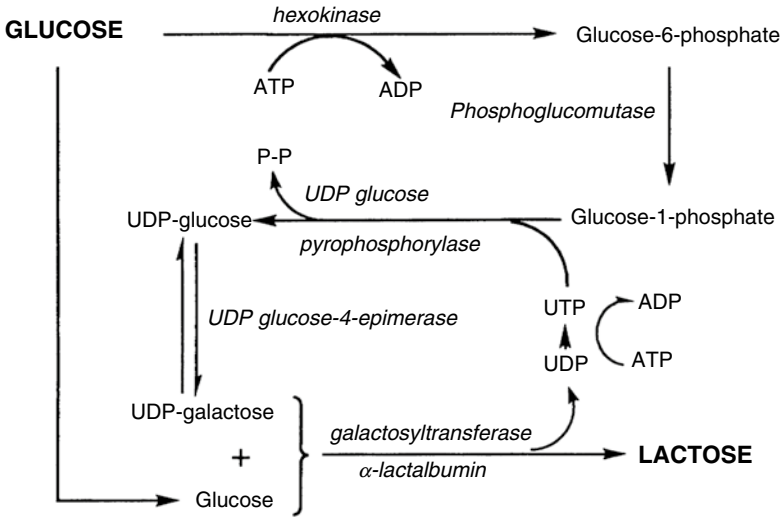


Fig. 2.5 Pathway for lactose synthesis

2.2.2 *Biosynthesis of Lactose*

Lactose is unique to mammary secretions. It is synthesized from glucose absorbed from blood. One molecule of glucose is isomerized to UDP-galactose *via* the 4-enzyme Leloir pathway (Fig. 2.5). UDP-Gal is then linked to another molecule of glucose in a reaction catalysed by the enzyme, lactose synthetase, a 2-component enzyme. Component A is a non-specific galactosyl transferase (EC 2.4.1.22) which transfers the galactose from UDP-gal to a number of acceptors. In the presence of the B component, which is the whey protein, α-lactalbumin, the transferase becomes highly specific for glucose (its K_M is decreased 1,000-fold), leading to the synthesis of lactose. Thus, α-lactalbumin is an enzyme modifier and its concentration in milk is directly related to the concentration of lactose (Fig. 2.6); the milk of some marine mammals contain neither α-lactalbumin nor lactose.

The presumed significance of this control mechanism is to enable mammals to terminate the synthesis of lactose when necessary, i.e., to regulate and control osmotic pressure when there is an influx of NaCl, e.g., during mastitis or in late lactation (lactose and NaCl are the major determinants of the osmotic pressure of milk, which is isotonic with blood, the osmotic pressure of which is essentially constant). The ability to control osmotic pressure is sufficiently important to justify an elaborate control mechanism and "wastage" of the enzyme modifier.

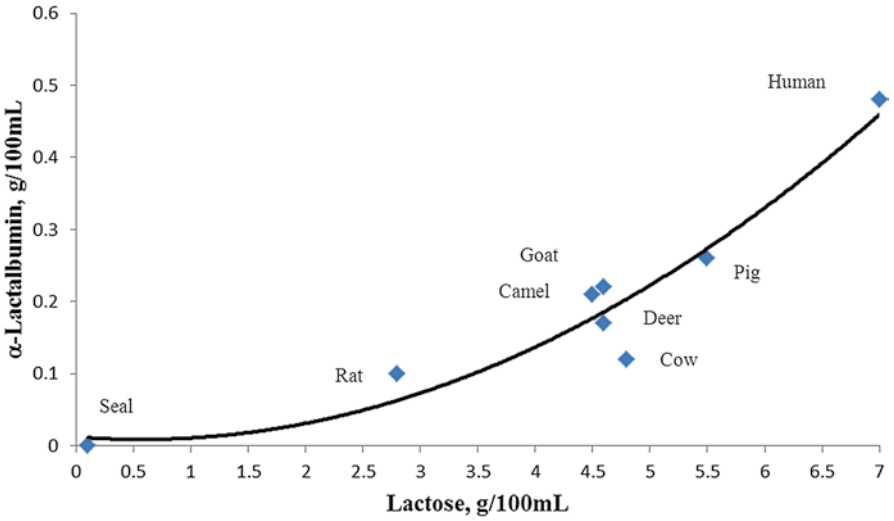


Fig. 2.6 Correlation between lactose and α-lactalbumin concentrations in the milk of eight species (adapted from Ley and Jenness 1970)

2.2.3 Lactose Equilibrium in Solution

The configuration around the C_1 of glucose (i.e., the anomeric C) is not stable and can readily change (**mutarotate**) from the α- to the β-form and *vice versa* when the sugar is in solution as a consequence of the fact that the hemiacetal form is in equilibrium with the open chain aldehyde form which can be converted into either of the two isomeric forms (Fig. 2.4).

When either isomer is dissolved in water, there is a gradual change from one form to the other until equilibrium is established, i.e., mutarotation occurs. These changes may be followed by measuring the change in optical rotation with time until, at equilibrium, the specific rotation is +55.4°.

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:
$89.4x + 35(100 - x) = 55.4 \times 100$
$x = 37.5$
$100 - x = 62.5$

Thus, the equilibrium mixture at 20 °C is composed of 62.7 % β and 37.3 % α-lactose. The equilibrium constant, β/α, is 1.68 at 20 °C. The proportion of lactose in the α-form increases as the temperature is increased and the equilibrium constant consequently decreases. The equilibrium constant is not influenced by pH, but the rate of mutarotation is dependent on both temperature and pH. The change from α- to β- is 51.1, 17.5 and 3.4 % complete at 25, 15 and 0 °C, respectively, in 1 h and is almost instantaneous at about 75 °C.

The rate of mutarotation is slowest at pH 5.0, increasing rapidly at more acid or alkaline values; equilibrium is established in a few minutes at pH 9.0.

2.2.4 Significance of Mutarotation

The α and β forms of lactose differ with respect to:

Solubility
Crystal shape and size
Hydration of the crystalline form, which leads to **hygroscopicity**
Specific rotation
Sweetness

Many of these characteristics are discussed in the following sections.

2.2.5 Solubility of Lactose

The solubility characteristics of the α- and β-isomers are distinctly different. When α-lactose is added in excess to water at 20 °C, about 7 g per 100 g water dissolve immediately. Some α-lactose mutarotates to the β anomer to establish the equilibrium ratio 62.7β:37.3α; therefore, the solution becomes unsaturated with respect to α and more α-lactose dissolves and some mutarotetes to β-lactose. These two processes (mutarotation and solubilization of α-lactose) continue until two criteria are met: ~7 g α-lactose are in solution and the β/α ratio is 1.6:1.0. Since the β/α ratio at equilibrium is about 1.6 at 20 °C, the final solubility is $7\ g + (1.6 \times 7)\ g = 18.2$ g per 100 g water.

When β-lactose is dissolved in water, the initial solubility is ~50 g per 100 g water at 20 °C. Some β-lactose mutarotates to α to establish a ratio of 1.6:1. At equilibrium, the solution would contain 30.8 g β and 19.2 g α/100 ml; therefore, the solution is supersaturated with α-lactose, some of which crystallizes, upsetting the equilibrium and leading to further mutarotation of β to α. These two events, i.e., crystallization of α-lactose and mutarotation of β, continue until the same two criteria are met, i.e., ~7 g of α-lactose in solution and a β/α ratio of 1.6:1. Again, the final solubility is ~18.2 g lactose per 100 g water. Since β-lactose is much more soluble than α and mutarotation is slow, it is possible to form more highly concentrated solutions by dissolving β- rather than α-lactose. In either case, the final solubility of lactose is the same (18.2 g/100 g of water).

The solubility of lactose as a function of temperature is summarized in Fig. 2.7. The solubility of α-lactose is more temperature dependent than that of β-lactose and the solubility curves intersect at 93.5 °C. A solution at 60 °C contains approximately 59 g lactose per 100 g water. Suppose that a 50 % solution of lactose (~30 g β- and 20 g α-) at 60 °C is cooled to 15 °C. At this temperature, the solution can contain only 7 g of α- or a total of 18.2 g of lactose per 100 g water at equilibrium. Therefore, lactose will crystallize very slowly out of solution as irregularly-sized crystals which may give rise to a sandy, gritty texture.

2.2.6 Crystallization of Lactose

As discussed in Sect. 2.2.5, the solubility of lactose is temperature dependent and solutions are capable of being highly supersaturated before spontaneous crystallization occurs and even then, crystallization may be slow. In general, supersolubility at any temperature equals the saturation (solubility) value at a temperature 30 °C higher. The insolubility of lactose, coupled with its capacity to form supersaturated solutions, is of considerable practical importance in the manufacture of concentrated milk products.

In the absence of nuclei and agitation, solutions of lactose are capable of being highly supersaturated before spontaneous crystallization occurs. Even in such solutions, crystallization occurs with difficulty. Solubility curves for lactose are shown in Fig. 2.8 and are divided into unsaturated, metastable and labile zones. Cooling a saturated solution or continued concentration beyond the saturation point, leads to supersaturation and produces a metastable area where crystallization does not occur readily. At higher levels of supersaturation, a labile area is observed where crystallization occurs readily. The pertinent points regarding supersaturation and crystallization are:

1. Neither nucleation nor crystal growth occurs in the unsaturated region.
2. Growth of crystals can occur in both the metastable and labile areas.
3. Nucleation occurs in the metastable area only if seeds (centres for crystal growth) are added.
4. Spontaneous crystallization can occur in the labile area without the addition of seeding material.

The rate of nucleation is slow at low levels of supersaturation and in highly supersaturated solutions owing to the high viscosity of the solution. The stability of a lactose "glass" (see Sect. 2.2.6.4) is due to the low probability of nuclei forming at very high concentrations.

Once a sufficient number of nuclei have formed, crystal growth occurs at a rate influenced by:

(a) Degree of supersaturation.
(b) Surface area available for deposition.
(c) Viscosity.
(d) Agitation.
(e) Temperature.
(f) Mutarotation, which is slow at low temperatures.

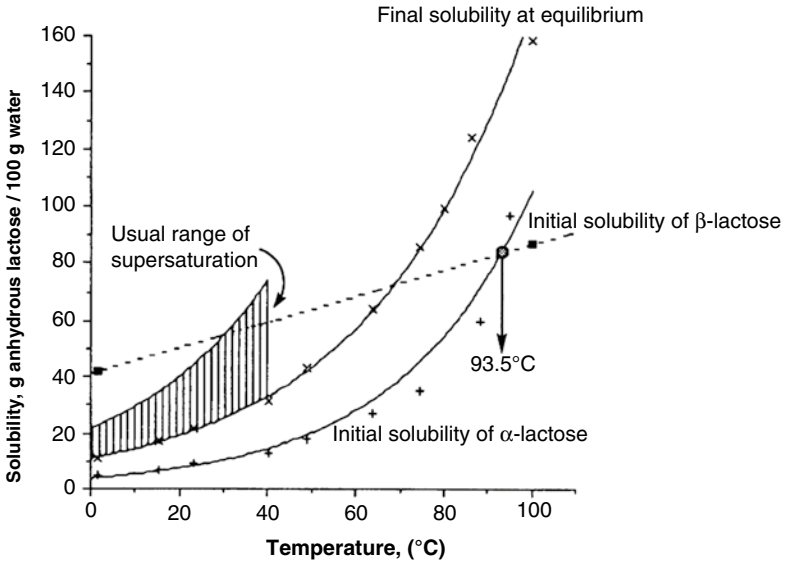


Fig. 2.7 Solubility of lactose in water (modified from Jenness and Patton 1959)

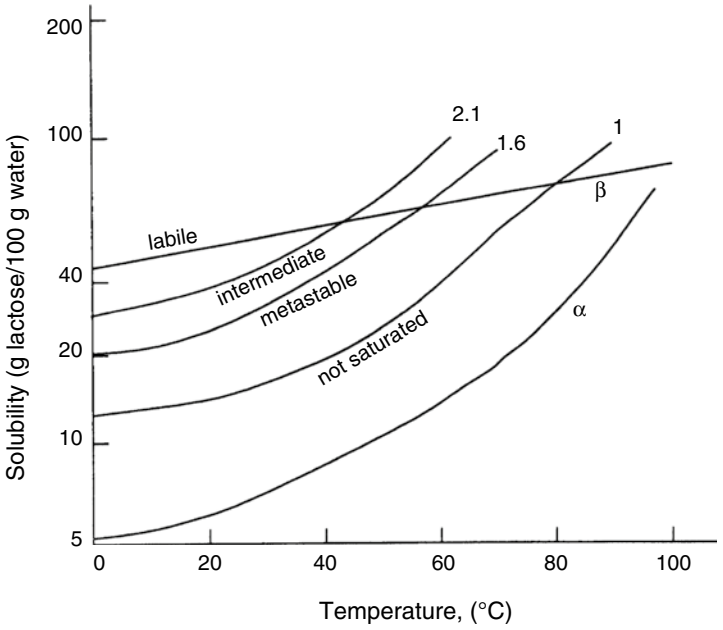


Fig. 2.8 Initial solubility of α-lactose and β-lactose, final solubility at equilibrium (*line 1*), and supersaturation by a factor 1.6 and 2.1 (α-lactose excluding water of crystallization) (modified from Walstra and Jenness 1984)

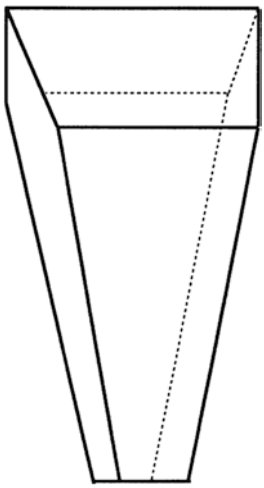


Fig. 2.9 The most common crystal form of α-lactose hydrate

2.2.6.1 α-Hydrate

α-Lactose crystallises as a monohydrate containing 5 % water of crystallization and can be prepared by concentrating an aqueous lactose solutions 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 when fully developed is tomahawk-shaped (Fig. 2.9). Crystals of lactose are hard and dissolve slowly. In the mouth, crystals less than 10 μm are undetectable, but above 16 μm they feel gritty or "sandy" and at 30 μm, a definite gritty texture is perceptible. The term "sandy" or “sandiness” is used to describe the defect in condensed milk, ice cream or processed cheese spreads where, due to poor manufacturing techniques, large lactose crystals are formed.

2.2.6.2 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.

2.2.6.3 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°. β-Lactose is sweeter than α-lactose, but is not

Table 2.3 Some physical properties of the two common forms of lactose

Property	α-Hydrate	β-Anhydride
Melting point[a], °C	202	252
Specific rotation, $[\alpha]_D^{20}$	+89.4°	+35°
Solubility in water (g/100 ml) at 20 °C	8	55
Specific gravity (20 °C)	1.54	1.59
Specific heat	0.299	0.285
Heat of combustion (kJ mol^{-1})	5,687	5,946

[a]Decomposes; values vary with rate of heating, α-hydrate loses H_2O at 120 °C
Values on anhydrous basis, both forms mutarotate to +55.4°

appreciably sweeter than the equilibrium mixture of α- and β- normally found in solution.

Some properties of α- and β-lactose are summarized in Table 2.3. Mixed α/β crystals, e.g., $\alpha_5\beta_3$, can be formed under certain conditions. The relationship between the different crystalline forms of lactose is shown in Fig. 2.10.

2.2.6.4 Lactose Glass

When a lactose solution is dried rapidly (e.g., spray drying lactose-containing concentrates), viscosity increases so quickly that there is insufficient time for crystallization to occur. A non-crystalline amorphous form is produced containing α- and β-forms in the ratio at which they exist in solution. Lactose in spray dried milk exists as a concentrated syrup or amorphous glass which is stable if protected from air, but is very hygroscopic and absorbs water rapidly from the atmosphere, becoming sticky.

2.2.7 Problems related to Lactose Crystallization

The tendency of lactose to form supersaturated solutions that do not crystallize readily causes problems in many dairy products unless adequate controls are exercised. The problems are due primarily to the formation of large crystals, which cause sandiness, or to the formation of a lactose glass, which leads to hygroscopicity and caking (Fig. 2.11).

2.2.7.1 Dried Milk and Whey

Lactose is the major component of dried milk products: whole milk powder, skim milk powder and whey powder contain ~30, 50 and ~70 % lactose, respectively. Protein, fat and air are dispersed in a continuous phase of amorphous solid lactose.

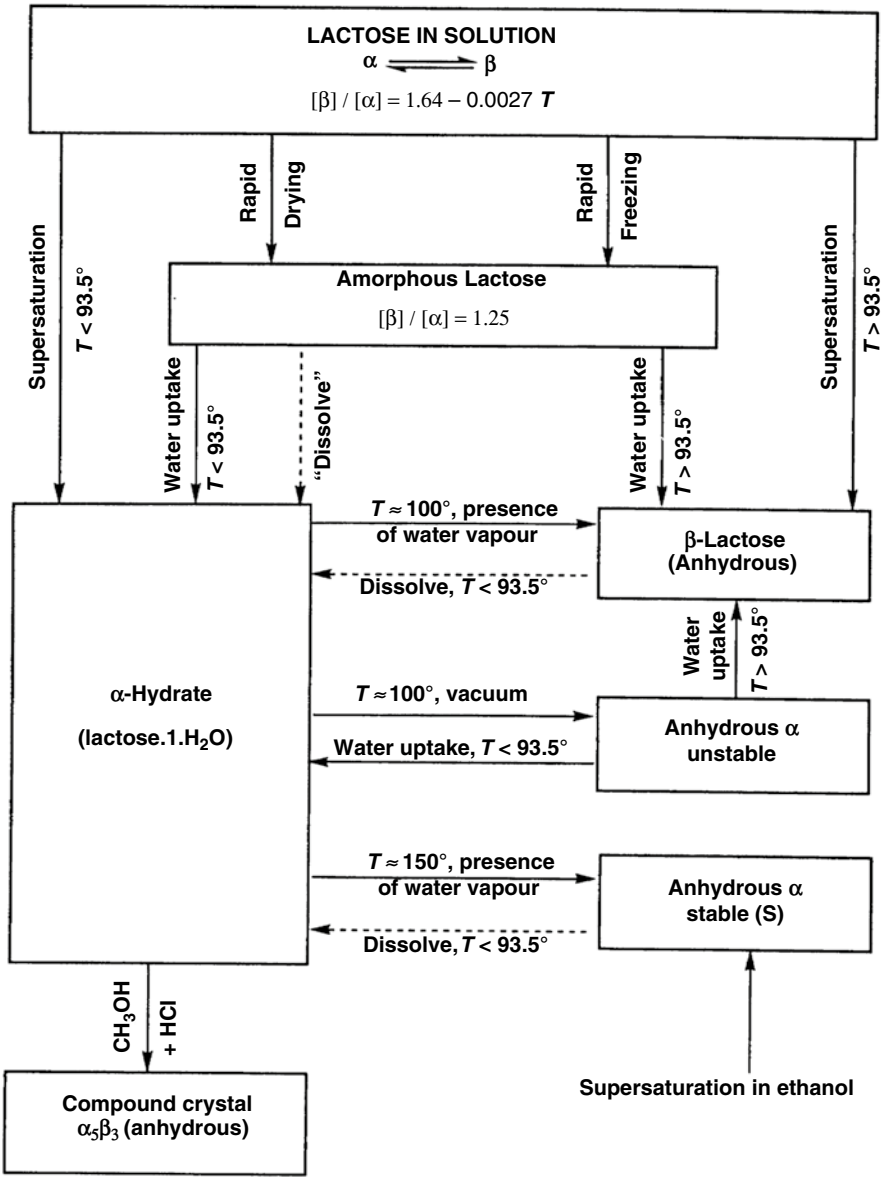


Fig. 2.10 Modifications of lactose (T, temperature in °C) from Walstra and Jenness 1984)

Consequently, the behaviour of lactose has a major impact on the properties of dried milk products (Schuck 2011).

In freshly-made powder, lactose is in an amorphous state with an α:β ratio of 1:1.6. This amorphous lactose glass is a highly concentrated syrup since there is not sufficient time during drying for crystallization to proceed normally. The glass has a

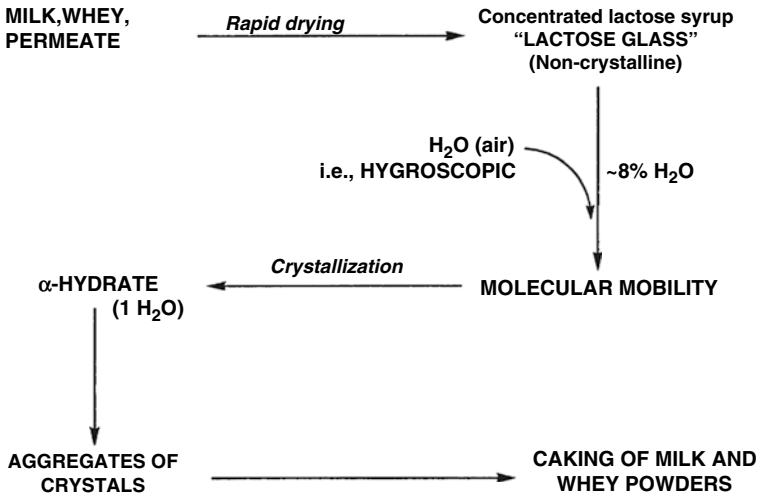


Fig. 2.11 Formation and crystallization of lactose glass

low vapour pressure and is hygroscopic, taking up moisture very rapidly when exposed to the atmosphere. On the uptake of moisture, dilution of the lactose occurs and the molecules acquire sufficient mobility and space to arrange themselves into crystals of α-lactose monohydrate. These crystals are small, usually with dimensions of <1 μm. Crevices and cracks exist along the edges of the crystals, into which other components are expelled. In these spaces, favourable conditions exist for the coagulation of casein because of the close packing of the micelles and the destabilizing action of concentrated salt systems. The fat globule membrane may be damaged by mechanical action, and Maillard browning, involving lactose and amino groups of protein, proceeds rapidly when crystallization has occurred.

Crystallization of lactose in dried milk particles causes "caking" of the powder into a hard mass. If a considerable portion of lactose in the freshly-dried product is in the crystalline state, caking of the powder on contact with moisture is prevented, thereby maintaining the dispersibility of the powder. Lactose crystallization is achieved by rehydrating freshly-dried powder to ~10 % H_2O, by exposure to moisture-saturated air, and redrying it or by removing powder from the main drying chamber before it has been completely dried and completing drying in a fluidized bed. This process is used commercially for the production of "instantized" milk powders. Clustering of the particles into loose, spongy aggregates occurs; these agglomerates are readily wettable and dispersible. They exhibit good capillary action and water readily penetrates the particles, allowing them to sink and disperse whereas the particles in non-instantized powder float due to their low density which contributes to their inability to overcome surface tension. Also, because of the small size of the particles in conventional spray-dried powders, close packing results in the formation of inadequate space for capillary action between the particles, thereby preventing uniform wetting. As a result, large masses of material are wetted on the outside, forming a barrier of highly concentrated product which prevents internal wetting and results in large undispersed

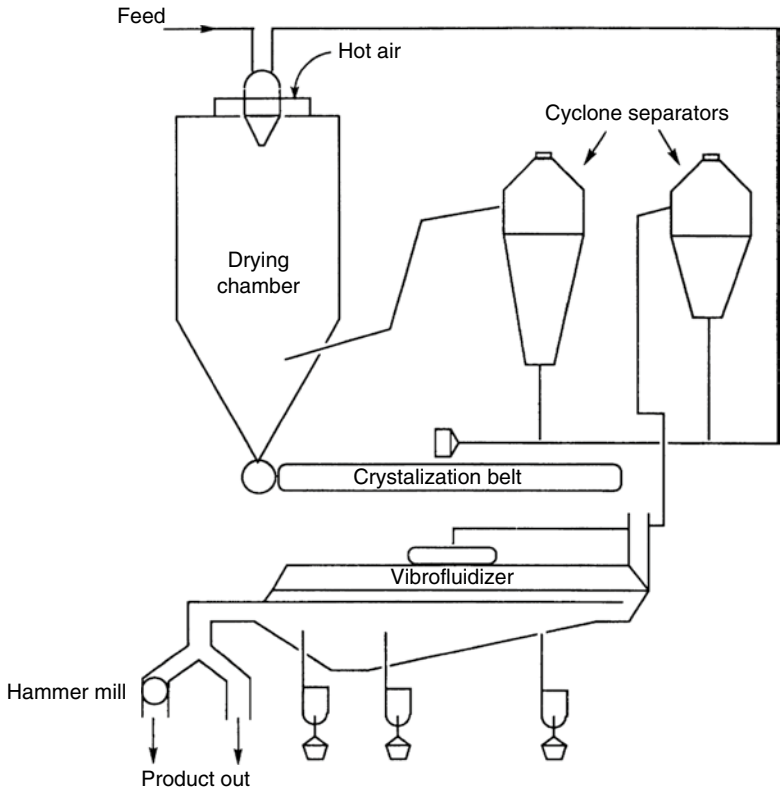


Fig. 2.12 Schematic representation of a low temperature drying plant for whey (modified from Hynd 1980)

clumps of powder. This problem is overcome by agglomeration and in this respect, lactose crystallization is important since it facilitates the formation of large sponge-like aggregates, with good capillary action and wettability.

The state of lactose has a major effect on the properties of spray dried whey powder manufactured by conventional methods, i.e., preheating, condensing to about 50 % total solids and drying to <4 % moisture. The powder is dusty and very hygroscopic and when exposed to ambient air, it has a pronounced tendency to cake owing to its very high lactose content (~70 %).

Problems arising from the crystallization of lactose in milk and whey powders may also be avoided or controlled by pre-crystallizing the lactose. Essentially, this involves adding finely-divided lactose powder which acts as nuclei on which the supersaturated lactose crystallises. Addition of 0.5 kg finely-ground lactose to the amount of concentrated product (whole milk, skim milk or whey) containing 1 tonne of lactose will induce the formation of ~10^6 crystals/ml, ~95 % of which will have dimensions <10 μm and 100 % <15 μm, i.e., too small to cause textural defects.

Diagrams of spray driers with instantizers attached are shown in Figs. 2.12 and 2.13.

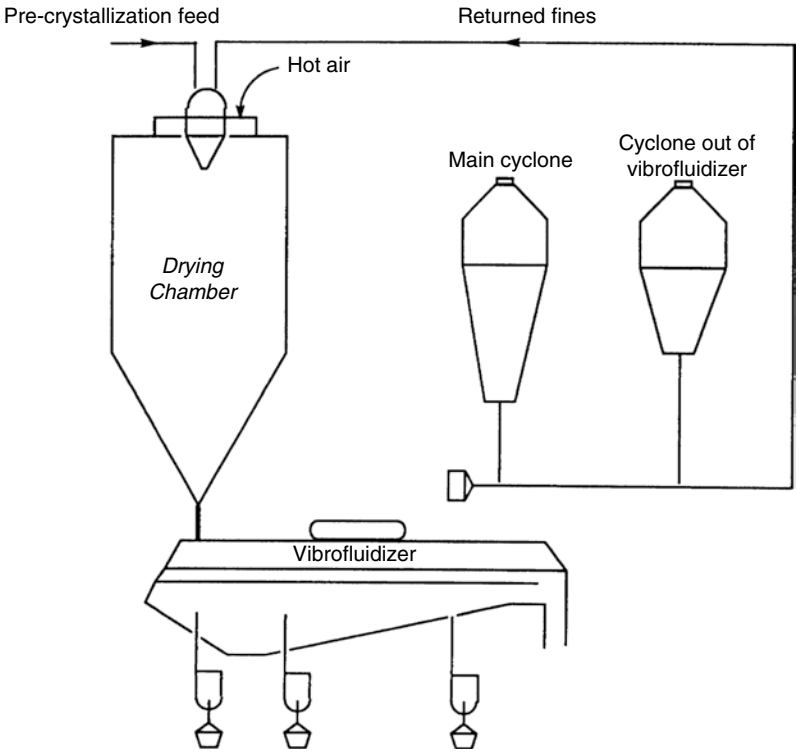


Fig. 2.13 Schematic representation of a straight through drying plant for whey (modified from Hynd 1980)

2.2.7.2 Thermoplasticity of Lactose

Unless certain precautions are taken during the drying of whey or other solutions containing a high concentration of lactose, the hot, semi-dry powder may adhere to the metal surfaces of the dryer, forming deposits, a phenomenon referred to as thermoplasticity. The principal factors which influence the temperature at which thermoplasticity occurs ("sticking temperature") are the concentrations of lactic acid, amorphous lactose and moisture in the whey powder.

Increasing the concentration of lactic acid from 0 to 16 % causes a linear decrease in sticking temperature (Fig. 2.14). The degree of pre-crystallization of lactose affects sticking temperature: a product containing 45 % pre-crystallized lactose has a sticking temperature of 60 °C while the same product with 80 % pre-crystallization sticks at 78 °C (Fig. 2.15). Pre-crystallization of the concentrate feed to the dryer thus permits considerably higher feed concentrations and drying temperatures. Pre-crystallization is routinely used in the drying of high-lactose products such as whey powder and demineralized whey powder.

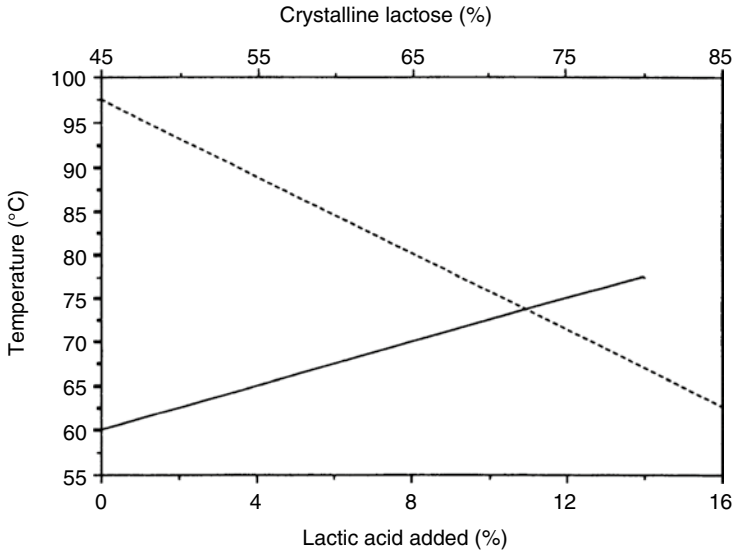


Fig. 2.14 Effect of added lactic acid (*dashed lines*) and degree of lactose crystallization (*dotted lines*) on the sticking temperature of whey powder (1.5–3.5 % moisture)

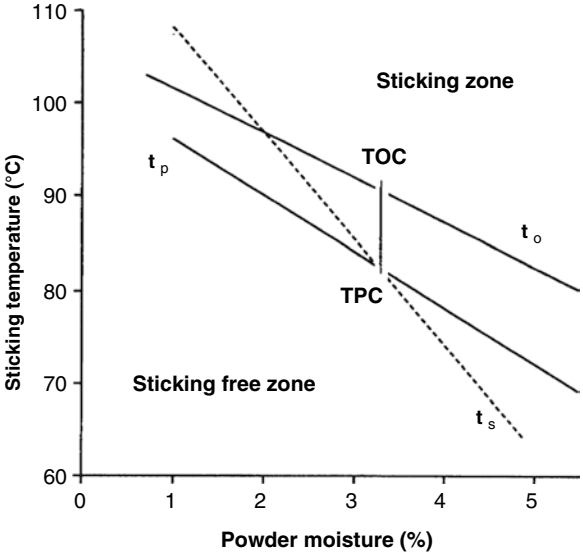


Fig. 2.15 Influence of moisture content on the temperature of powder in a spray dryer (t_p), dryer outlet temperature (t_o) and sticking temperature (t_s). The minimum product temperature required to avoid problems with sticking is at TPC with the corresponding dryer outlet temperature TOC (modified from Hynd 1980)

In practice, the most easily controlled factor is the moisture content of the whey powder, which is determined by the outlet temperature of the dryer (t_o, Fig. 2.15). However, as a result of evaporative cooling, the temperature of the particles in the dryer is lower than the outlet temperature (t_p, Fig. 2.15) and the difference between t_o and t_p increases with increasing moisture content. The sticking temperature for a given whey powder decreases with increasing moisture content (t_s, Fig. 2.15) and where the two curves (t_s and t_p) intersect (point TPC, Fig. 2.15) is the maximum product moisture content at which the dryer can be operated without product sticking during drying. The corresponding point on the outlet temperature curve (TOC) represents the maximum dryer outlet temperature which may be used without causing sticking.

2.2.7.3 Sweetened Condensed Milk

Crystallization of lactose occurs in sweetened condensed milk (SCM) and crystal size must be controlled if a product with a desirable texture is to be produced. As it comes from the evaporators, SCM is almost saturated with lactose. When cooled to 15–20 °C, 40–60 % of the lactose will eventually crystallize as α-lactose hydrate. There are 40–47 parts of lactose per 100 parts of water in SCM, consisting of about 40 % α- and 60 % β- (ex-evaporator). To obtain a smooth texture, crystals with dimensions <10 μm are desirable. The optimum temperature for crystallization is 26–36 °C. Pulverized α-lactose, or preferably lactose "glass", is used as seed. Continuous vacuum cooling, combined with seeding, gives the best product.

2.2.7.4 Ice Cream

Crystallization of lactose in ice cream causes a sandy texture. In freshly hardened ice cream, the equilibrium mixture of α- and β-lactose is in the "glass" state and is stable as long as the temperature remains low and constant. During the freezing of ice cream, the lactose solution passes through the labile zone so rapidly and at such a low temperature that little lactose crystallization occurs.

If ice cream is warmed or the temperature fluctuates, some ice will melt, and an infinite variety of lactose concentrations will emerge, some of which will be in the labile zone where spontaneous crystallization occurs while others will be in the metastable zone where crystallization can occur if suitable nuclei, e.g., lactose crystals, are present. At the low temperature, crystallization tendency is low and extensive crystallization usually does not occur. However, the nuclei formed act as seed for further crystallization when the opportunity arises and they tend to grow slowly with time, eventually causing a sandy texture. The defect is controlled by limiting the milk solids content or by using β-galactosidase to hydrolyse lactose.

2.2.7.5 Other Frozen Dairy Products

Although milk may become frozen inadvertently, freezing is not a common commercial practice. However, concentrated or unconcentrated milk is sometimes frozen commercially, e.g., to supply remote locations (as an alternative to dried or UHT milk), to store sheep's or goats' milk, production of which is seasonal, or human milk for infant feeding in emergencies (milk banks).

As will be discussed in Chap. 3, freezing damages the milk fat globule membrane, resulting in the release of "free fat". The casein system is also destabilized due to a decrease in pH and an increase in Ca^{2+} concentration, both caused by the precipitation of soluble CaH_2PO_4 and/or Ca_2HPO_4 as $Ca_3(PO_4)_2$, with the release of H^+ (see Chap. 5); precipitation of $Ca_3(PO_4)_2$ occurs on freezing because pure water crystallises, causing an increase in soluble calcium phosphate, with which milk is already saturated. Crystallization of lactose as α-hydrate during frozen storage aggravates the problem by reducing the amount of solvent water available.

In frozen milk products, lactose crystallization causes instability of the casein system. On freezing, supersaturated solutions of lactose are formed: e.g., in concentrated milk at −8 °C, 25 % of the water is unfrozen and it contains 80 g lactose per 100 g, whereas the solubility of lactose at −8 °C is only ~7 %. During storage at a low temperature, lactose crystallizes slowly as a monohydrate and consequently the amount of free water in the product is reduced.

The formation of supersaturated lactose solutions inhibits freezing, and consequently stabilizes the concentration of solutes in solution. However, when lactose crystallizes, water freezes and the concentration of other solutes increases markedly (Table 2.4).

The increase in calcium and phosphate leads to precipitation of calcium phosphate and a decrease in pH:

$$3\,Ca^{2+} + 2\,H_2PO_{4^-} \leftrightarrow Ca_3\left(PO_4\right)_2 + 4\,H^+$$

These changes in the concentration of Ca^{2+} and pH lead to destabilization of the casein micelles.

Table 2.4 Comparison of ultrafiltrate from liquid and frozen skim milk

Constituent	Ultrafiltrate of skim milk	Ultrafiltrate of liquid portion of frozen concentrated milk
pH	6.7	5.8
Chloride, mM	34.9	459
Citrate, mM	8.0	89
Phosphate, mM	10.5	84
Sodium, mM	19.7	218
Potassium, mM	38.5	393
Calcium, mM	9.1	59

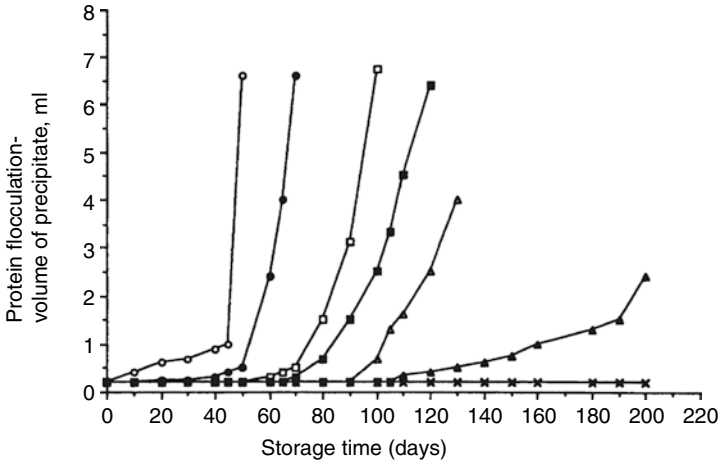


Fig. 2.16 Effect of lactose hydrolysis on the stability of milk to freezing (modified from Tumerman et al. 1954)

Any factor that accelerates the crystallization of lactose shortens the storage life of the product. At very low temperatures (<–23 °C), neither lactose crystallization nor casein flocculation occurs, even after long periods. Enzymatic hydrolysis of lactose by β-galactosidase before freezing retards or prevents lactose crystallization and casein precipitation in proportion to the extent of the hydrolysis (Fig. 2.16).

2.3 Production of Lactose

In comparison with sucrose (the annual production of which is 175×10^6 tonnes, US Department of Agriculture) and glucose or glucose-fructose syrups, only relatively small quantities of lactose are produced. However, it attracts commercial interest because it has some interesting properties and is readily available from whey, a by-product in the production of cheese or casein. World production of cheese is ~19×10^6 tonnes, the whey from which contains ~8×10^6 tonnes of lactose; ~0.3×10^6 tonnes of lactose are contained in the whey produced during casein manufacture. According to Affertsholt-Allen (2007), only about 325,000 tonnes of lactose are used annually in the EU and 130,000 tonnes in the USA, i.e., only ~7 % of that potentially available. Much larger amounts are used in whey and demineralized whey powders.

Production of lactose essentially involves concentrating whey or UF permeate under vacuum, crystallization of lactose from the concentrate, recovery of the crystals by centrifugation and drying of the crystals (Fig. 2.17). The first-crop crystals are

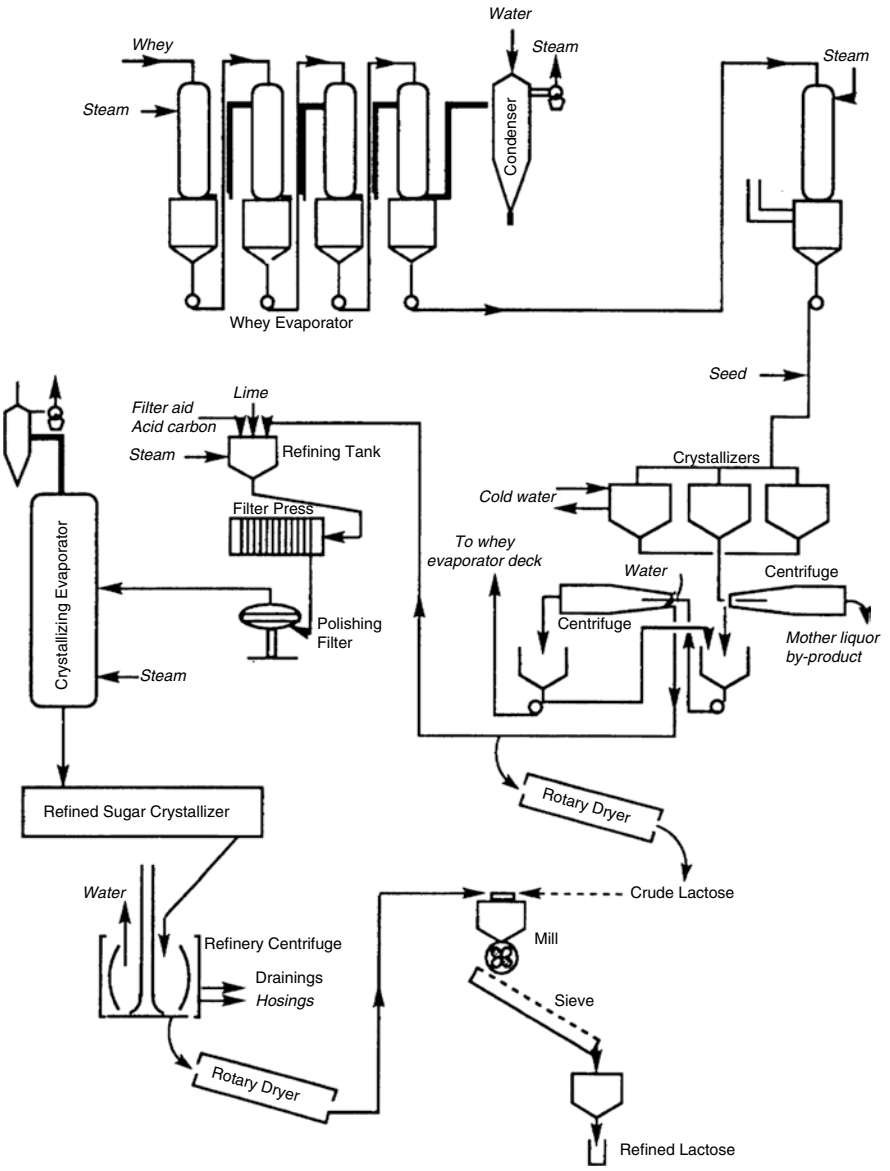


Fig. 2.17 Schematic representation of plant for the manufacture of crude and refined lactose from sweet whey

usually contaminated with riboflavin and are therefore yellowish; a higher grade, and hence more valuable, lactose is produced by redissolving and recrystallizing the crude lactose (Table 2.5). Lactose may also be recovered by precipitation with $Ca(OH)_2$, especially in the presence of ethanol, methanol or acetone (Paterson 2009, 2011).

Table 2.5 Some typical physical and chemical data for various grades of lactose (from Nickerson 1974)

Analysis	Fermentation	Crude	Edible	U.S.P.[b]
Lactose (%)	98.0	98.4	99.0	99.85
Moisture, non-hydrate (%)	0.35	0.3	0.5	0.1
Protein (%)	1.0	0.8	0.1	0.01
Ash (%)	0.45	0.40	0.2	0.03
Lipid (%)	0.2	0.1	0.1	0.001
Acidity, as lactic acid (%)	0.4	0.4	0.06	0.04
Specific rotation $[\alpha]D^{20}$	[a]	[a]	52.4°	52.4°

[a]Not normally determined

[b]*USP* US Pharmacopoeia grade

Table 2.6 Food applications of lactose

Humanized baby foods
Demineralized whey powder or lactose
Instantizing/free-flowing agent in foods
Agglomeration due to lactose crystallization
Confectionery products
Improves functionality of shortenings
Anticaking agent at high relative humidity
Certain types of icing
Maillard browning, if desired
Accentuates other flavours (chocolate)
Flavour adsorbant
Flavour volatiles
Flavour enhancement
Sauces, pickles, salad dressings, pie fillings

Lactose has several applications in food products (Table 2.6), the most important of which is probably in the manufacture of humanized infant formulae. It is used also as a diluent for the tableting of drugs in the pharmaceutical industry (which requires further purification and high quality extra pure, and therefore is more expensive) and as the base for plastics.

Among sugars, lactose has a low level of sweetness (Table 2.7), which is generally a disadvantage but is advantageous in certain applications. When properly crystallized, lactose has low hygroscopicity (Table 2.8), which makes it an attractive sugar for use in icings for confectionary products.

Table 2.7 Relative sweetness of sugars (concentration, %, required to give equivalent sweetness) (from Nickerson 1974)

Sucrose	Glucose	Fructose	Lactose
0.5	0.9	0.4	1.9
1.0	1.8	0.8	3.5
2.0	3.6	1.7	6.5
2.0	3.8	–	6.5
2.0	3.2	–	6.0
5.0	8.3	4.2	15.7
5.0	8.3	4.6	14.9
5.0	7.2	4.5	13.1
10.0	13.9	8.6	25.9
10.0	12.7	8.7	20.7
15.0	17.2	12.8	27.8
15.0	20.0	13.0	34.6
20.0	21.8	16.7	33.3

Table 2.8 Relative humectancy of sucrose, glucose and lactose (% moisture absorbed at 20 °C)

	Relative humidity		
Sugar	60 %		100 %
	1 h	9 days	25 days
Lactose	0.54	1.23	1.38
Glucose	0.29	9.00	47.14
Sucrose	0.04	0.03	18.35

2.4 Derivatives of Lactose

Although the demand for lactose has been strong in recent years, it is unlikely that a profitable market exists for all the lactose potentially available. Since the disposal of whey or UF permeate by dumping into waterways is no longer permitted, ways of utilizing lactose have been sought for several years. For many years, the most promising of these was considered to be hydrolysis to glucose and galactose, but other modifications are attracting increasing attention.

2.4.1 Enzymatic Modification of Lactose

Lactose may be hydrolysed to glucose and galactose by enzymes (β-galactosidases, commonly called lactase) or by acids. Commercial sources of β-galactosidase are moulds (especially *Aspergillus* spp.), the enzymes from which have acid pH optima, and yeasts (*Kluyveromyces*) which produce enzymes with neutral pH optima. When β-galactosidases became commercially available, they were considered to have considerable commercial potential as a solution to the "whey problem" and for the treatment of lactose intolerance (see Sect. 2.6.1), but for various reasons their commercialization has not been as great as expected. The very extensive literature on various aspects of β-galactosidases and on their application in free or immobilized

form has been reviewed by Mahoney (1997) and Playne and Crittenden (2009). Technological challenges in the production of glucose-galactose syrups have been overcome but the process is not very successful commercially. Glucose-galactose syrups are not economically competitive with glucose or glucose-fructose syrups produced by hydrolysis of maize starch, unless the latter are heavily taxed. As discussed in Sect. 2.6.1, an estimated 70 % of the adult human population have inadequate intestinal β-galactosidase activity and are therefore lactose intolerant; the problem is particularly acute among Asians and Africans. Pre-hydrolysis of lactose was considered to offer the potential to develop new markets for dairy products in those countries. Various protocols are available: addition of β-galactosidase to milk in the home, pre-treatment of milk at the factory with free or immobilized enzyme or aseptic addition of sterilized free β-galactosidase to UHT milk, which appears to be particularly successful. However, the method is not used widely and it is now considered that the treatment of milk with β-galactosidase will be commercially successful only in niche markets.

Glucose-galactose syrups are about three times sweeter than lactose (70 % as sweet as sucrose) and hence lactose-hydrolysed milk could be used in the production of ice-cream, yoghurt or other sweetened dairy products, permitting the use of less sucrose and reducing caloric content. However, such applications have had limited commercial success.

The glucose moiety can be isomerized to fructose by the well-established glucose isomerization process to yield a galactose-glucose-fructose syrup with increased sweetness. Another possible variation would involve the isomerization of lactose to lactulose (galactose-fructose) which can be hydrolysed to galactose and fructose by some β-galactosidases.

β-Galactosidase has transferase as well as hydrolase activity and produces oligosaccharides (galactooligosaccharides, Fig. 2.18) which are later hydrolysed (Fig. 2.19). This property may be a disadvantage since the oligosaccharides are not digestible by humans and reach the large intestine where they are fermented by bacteria, leading to the same problem caused by lactose. However, they stimulate the growth of *Bifidobacterium* in the lower intestine; a product (oligonate, 6′galactosyl lactose) is produced commercially by the Yokult Company in Japan for addition to infant formulae. Other commercial preparations of galacto-oligosaccharides (GOS) include Vivinal® GOS, which is manufactured by Friesland Campina, the Netherlands, and when combined with fructo-oligosaccharides (FOS) has been clinically-proven to have health benefits such as aiding in the relief of eczema, allergies and gastrointestinal discomfort. Generally similar GOS-based products are available from Clasado Biosciences, UK. Some galactooligosaccharides have interesting functional properties and may find commercial applications (see Ganzle 2011b).

2.4.2 Chemical Modifications

Several interesting derivatives can be produced from lactose (see Ganzle 2011a).

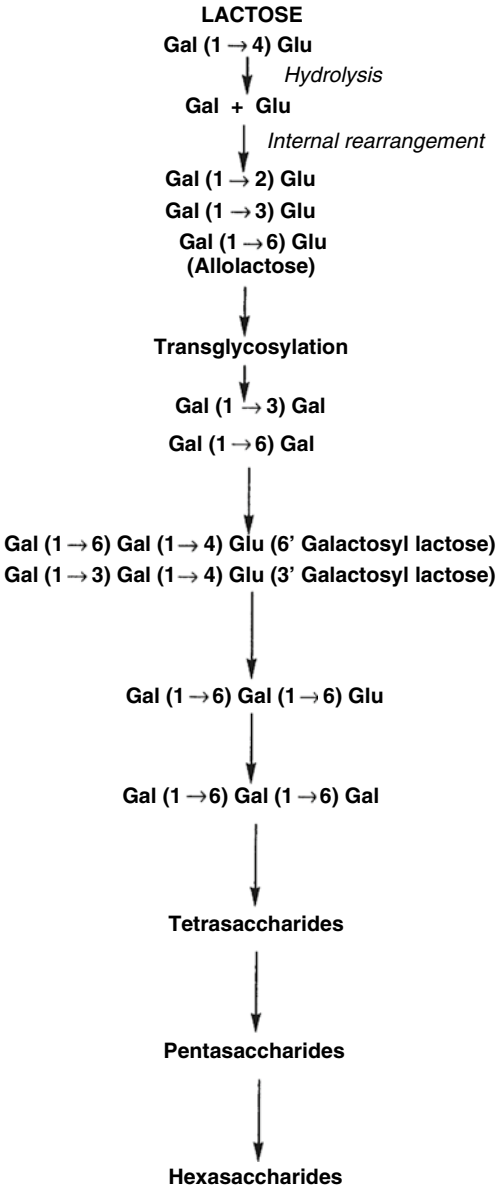


Fig. 2.18 Possible reaction products from the action of β-galactosidase on lactose (from Smart 1993)

2.4.2.1 Lactulose

Lactulose is an epimer of lactose in which the glucose moiety is isomerized to fructose (Fig. 2.20). The sugar does not occur naturally and was first synthesized by Montgomery and Hudson in 1930. It can be produced under mild alkaline conditions via the Lobry de Bruyn-Alberda van Ekenstein reaction and at a low yield as

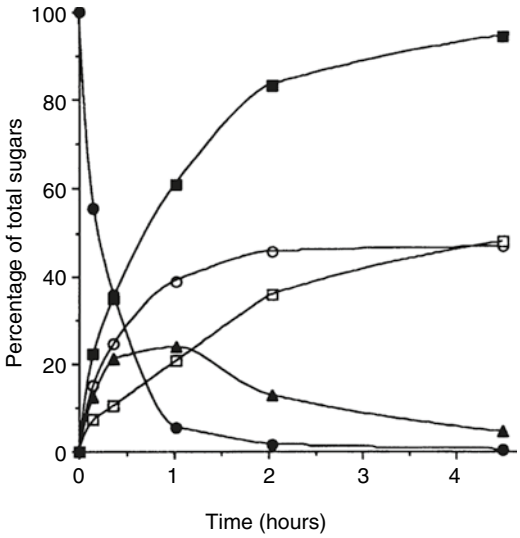


Fig. 2.19 Production of oligosaccharides during the hydrolysis of lactose by β-galactosidase (modified from Mahoney 1997)

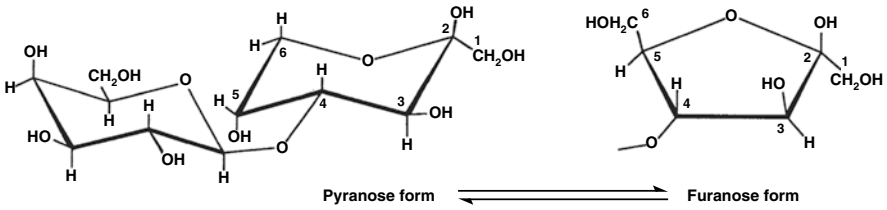


Fig. 2.20 Chemical structure of lactulose

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 (Fig. 2.21); it is not present in raw or HTST pasteurized milk.

Lactulose is sweeter than lactose and about 60 % as sweet as sucrose. It is not metabolized by oral bacteria and hence is not cariogenic. It is not hydrolysed by intestinal β-galactosidase and hence reaches the large intestine where it can be metabolised by lactic acid bacteria, including *Bifidobacterium* spp. and serves as a bifidus factor. For this reason, lactulose has attracted considerable attention as a means of modifying the intestinal microflora, reducing intestinal pH and preventing the growth of undesirable putrefactive bacteria (Fig. 2.22). It is now commonly added to infant formulae to simulate the bifidogenic properties of human milk—apparently, 20,000 tonnes per annum are now used for this and similar applications. Lactulose is also reported to suppress the growth of certain tumour cells (Tamura et al. 1993).

Lactulose is usually used as a 50 % syrup but a crystalline trihydrate, which has very low hygroscopicity, is available.

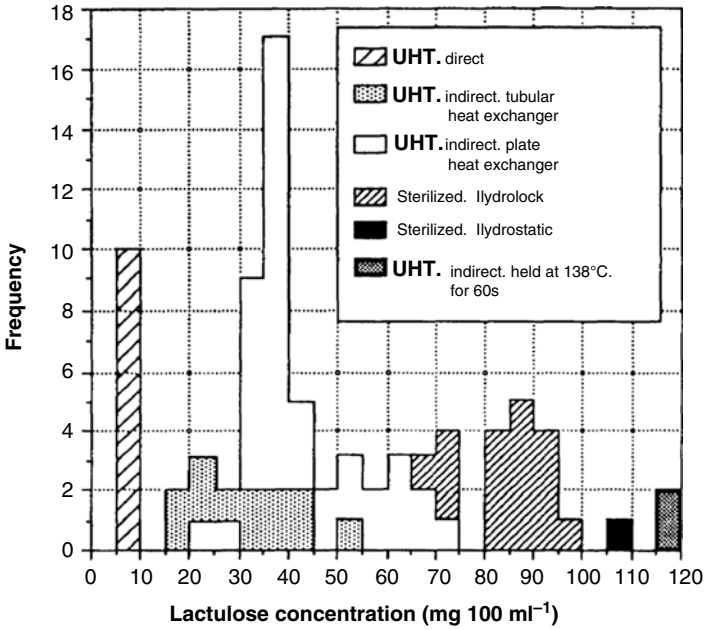


Fig. 2.21 Concentration of lactulose in heated milk products (modified from Andrews 1989)

2.4.2.2 Lactitol

Lactitol (4-O-β-D-galactopyranosyl-D-sorbitol), is a sugar alcohol produced on reduction of lactose (Fig. 2.23), usually using Raney nickel; it does not occur naturally. It can be crystallized as a mono- or di-hydrate. Lactitol is not metabolized by higher animals; it is relatively sweet and hence has potential as a non-nutritive sweetener. It is claimed that lactitol reduces the absorption of sucrose, reduces blood and liver cholesterol levels and is anti-cariogenic. It has applications in low-calorie foods (jams, marmalade, chocolate, baked goods); it is non-hygroscopic and can be used to coat moisture-sensitive foods, e.g., candies.

It can be esterified with 1 or more fatty acids (Fig. 2.23) to yield a family of food emulsifiers, analogous to the sorbitans produced from sorbitol.

2.4.2.3 Lactobionic Acid

This derivative is produced by oxidation of the free carbonyl group of lactose (Fig. 2.24), chemically (Pt, Pd or Bi), electrolytically, enzymatically or by fermentation. It has a sweet taste, which is very unusal for an acid. Its lactone crystallizes readily. Lactobionic acid has found only limited application; its lactone could be used as an acidogen but it is probably not cost-competitive with gluconic acid-δ-lactone. It is used in preservation solutions for organs (to prevent swelling) prior to transplantation, and in skin-care products.

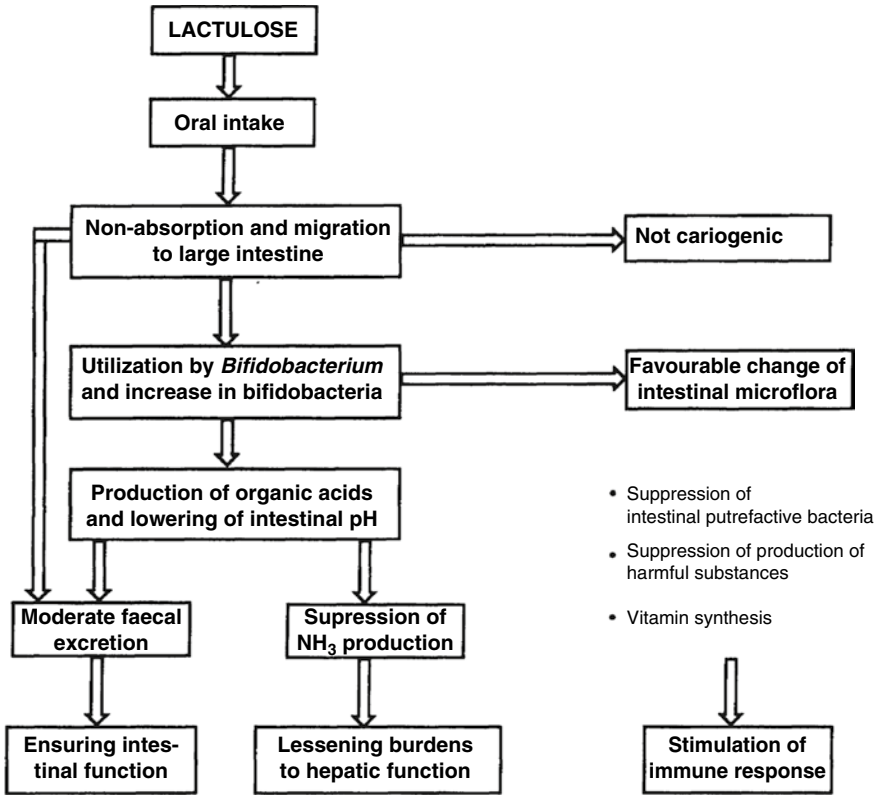


Fig. 2.22 Significance of lactulose in health (modified from Tamura et al. 1993)

2.4.2.4 Lactosyl Urea

Urea can serve as a cheap source of nitrogen for cattle but its use is limited because NH_3 is released too quickly, leading to a toxic level of NH_3 in the blood. Reaction of urea with lactose yields lactosyl urea (Fig. 2.25), from which NH_3 is released more slowly.

2.4.3 Fermentation Products

Lactose is readily fermented by lactic acid bacteria, especially *Lactococcus* spp. and *Lactobacillus* spp., to lactic acid, and by some species of yeast, e.g., *Kluyveromyces*, to ethanol (Fig. 2.26). Lactic acid may be used as a food acidulant, as a feed-stock

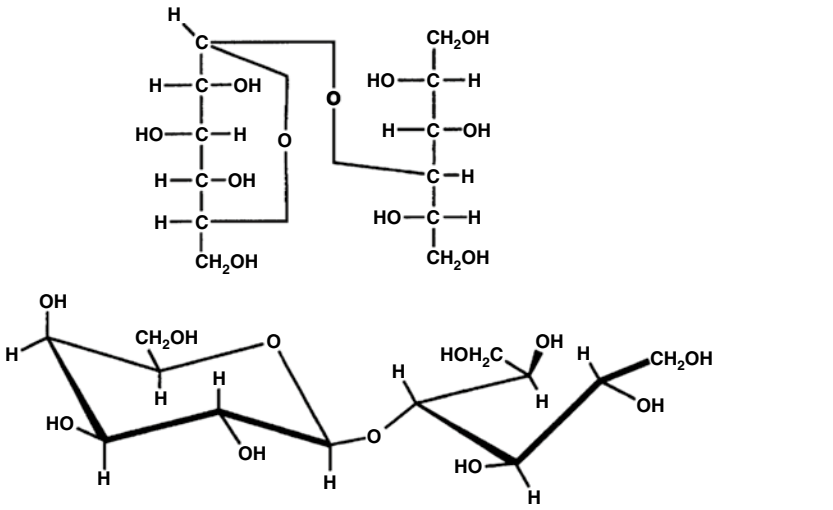


Fig. 2.23 Structure of lactitol and its conversion to lactyl palmitate

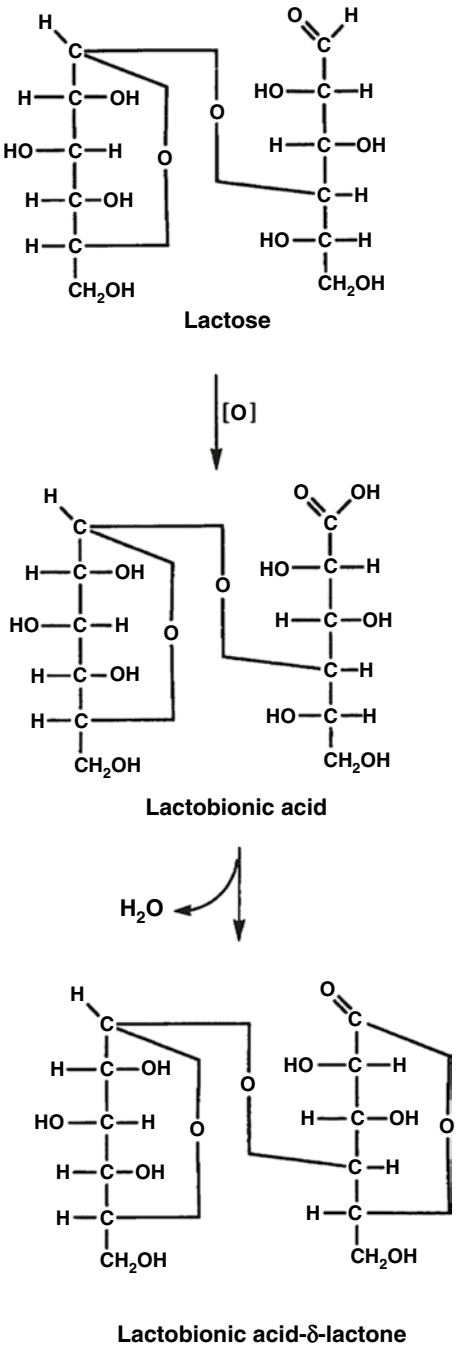


Fig. 2.24 Structure of lactobionic acid and its δ-lactone

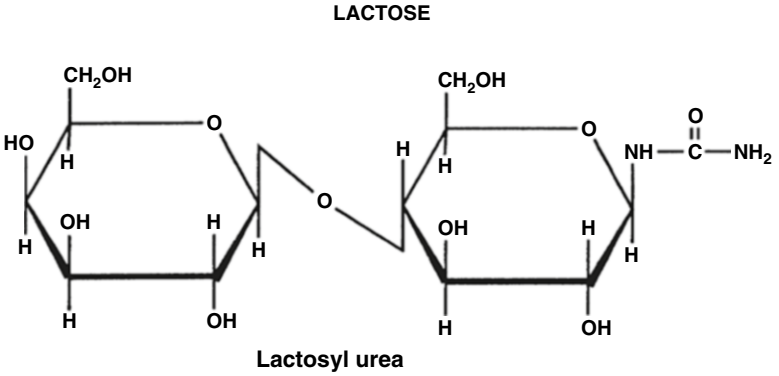


Fig. 2.25 Structure of lactosyl urea

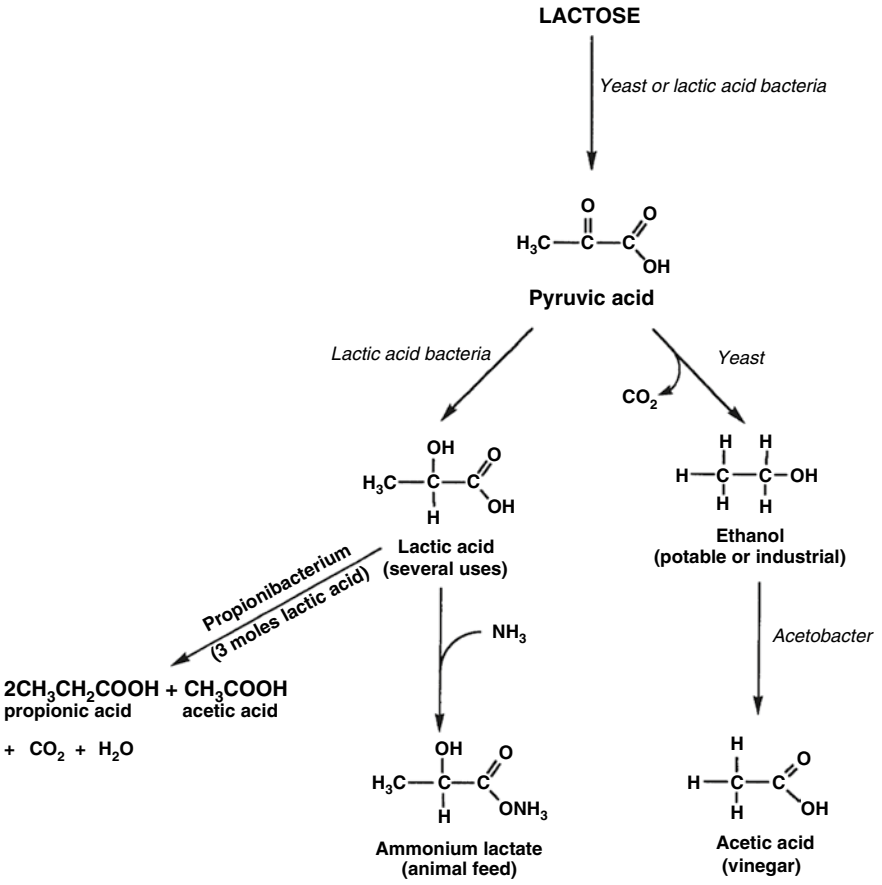


Fig. 2.26 Fermentation products from lactose

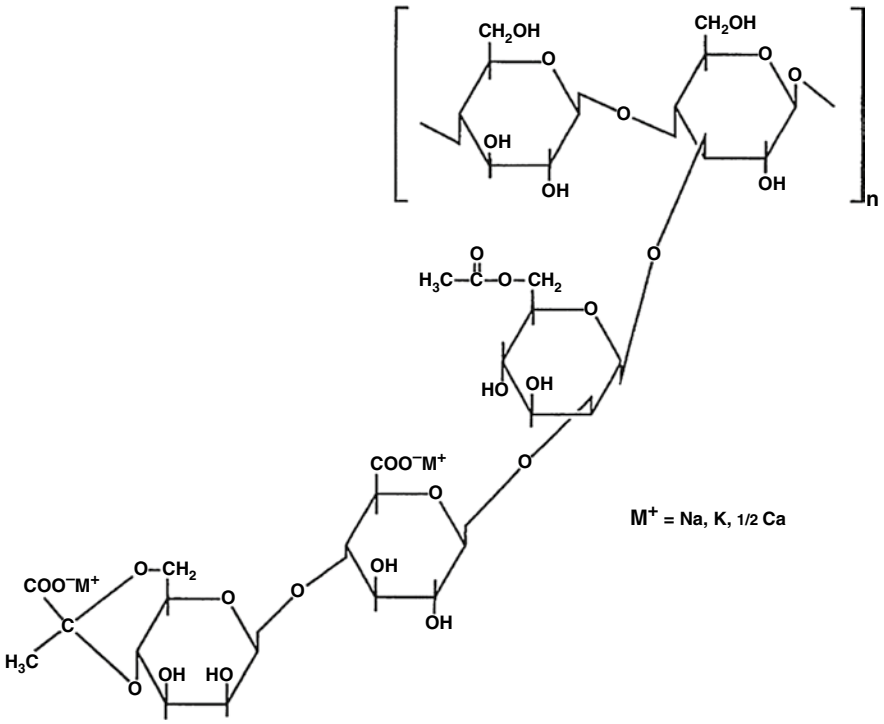


Fig. 2.27 Repeating unit of xanthan gum

in the manufacture of plastics, or converted to ammonium lactate as a source of nitrogen for animal nutrition. It can be converted to propionic acid, which has many food applications, by *Propionibacterium* spp. Potable ethanol is being produced commercially from lactose in whey or UF permeate. The ethanol may also be used for industrial purposes or as a fuel but in most cases is probably not cost-competitive with ethanol produced by fermentation of sucrose or chemically. The ethanol may also be oxidized to acetic acid. The mother liquor remaining from the production of lactic acid or ethanol may be subjected to anaerobic digestion with the production of methane (CH_4) for use as a fuel; several such plants are in commercial use.

Lactose can also be used as a substrate for *Xanthomonas campestris* in the production of xanthan gum (Fig. 2.27) which has several food and industrial applications.

All the fermentation-based modifications of lactose are probably not economical because lactose is not cost-competitive with alternative fermentation substrates, especially sucrose in molasses or glucose produced from starch. Except in special circumstances, the processes can be regarded as the cheapest method of whey disposal.

2.5 Lactose and the Maillard Reaction

As a reducing sugar, lactose can participate in the Maillard reaction, leading to non-enzymatic browning (see O'Brien 1997, 2009; Nursten 2011). The Maillard reaction involves interaction between a carbonyl (in this case, lactose) and an amino group (in foods, principally the ε-NH_2 group of lysine in proteins) to form a glycosamine (lactosamine) (Fig. 2.28). The glycosamine may undergo an Amadori rearrangement to form a 1-amino-2-keto sugar (Amadori compound) (Fig. 2.29).

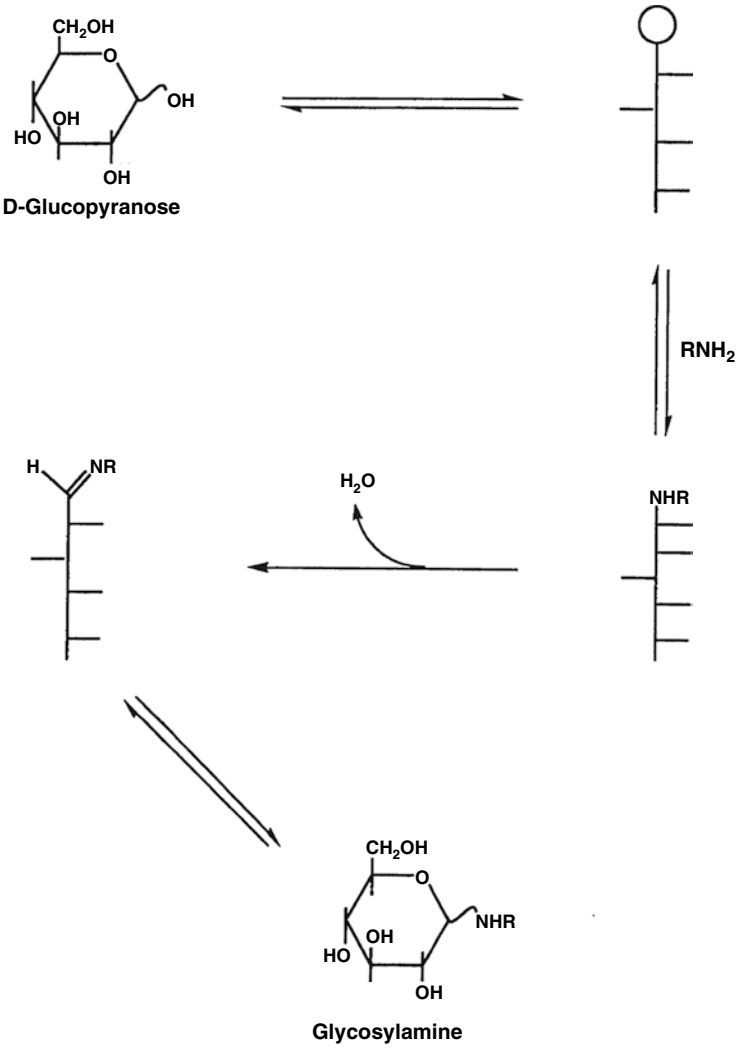


Fig. 2.28 Formation of glycosylamine, the initial step in Maillard browning

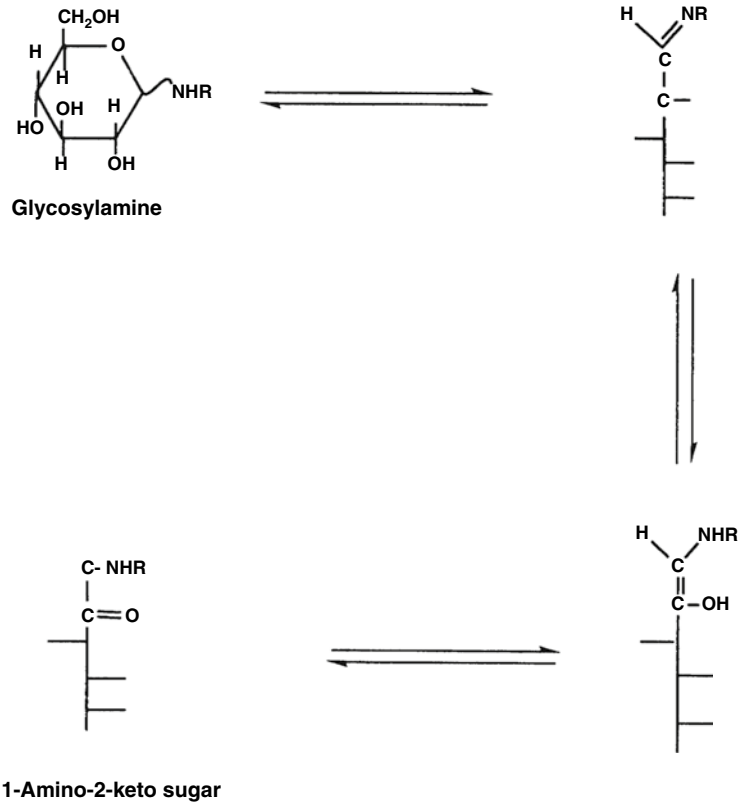


Fig. 2.29 Amadori rearrangement of a glycosylamine

The reaction is base-catalysed and is first order. The Amadori compound may be degraded via either of two pathways, depending on pH, to a variety of active alcohol, carbonyl and dicarbonyl compounds and ultimately to brown-coloured polymers called melanoidins (Fig. 2.30). Many of the intermediates are (off-) flavoured. The dicarbonyls can react with amino acids via the Strecker degradation pathway (Fig. 2.31) to yield another family of highly flavoured compounds While the Maillard reaction has desirable consequences in many foods, e.g., coffee, bread crust, toast, french fried potato products, its consequences in milk products are negative, e.g., brown colour, off-flavours, slight loss of nutritive value (lysine), loss of solubility in milk powders (although it appears to prevent or retard age-gelation in UHT milk products). Maillard reaction products (MRP) have antioxidant properties; the production of MRP may be a small-volume outlet for lactose.

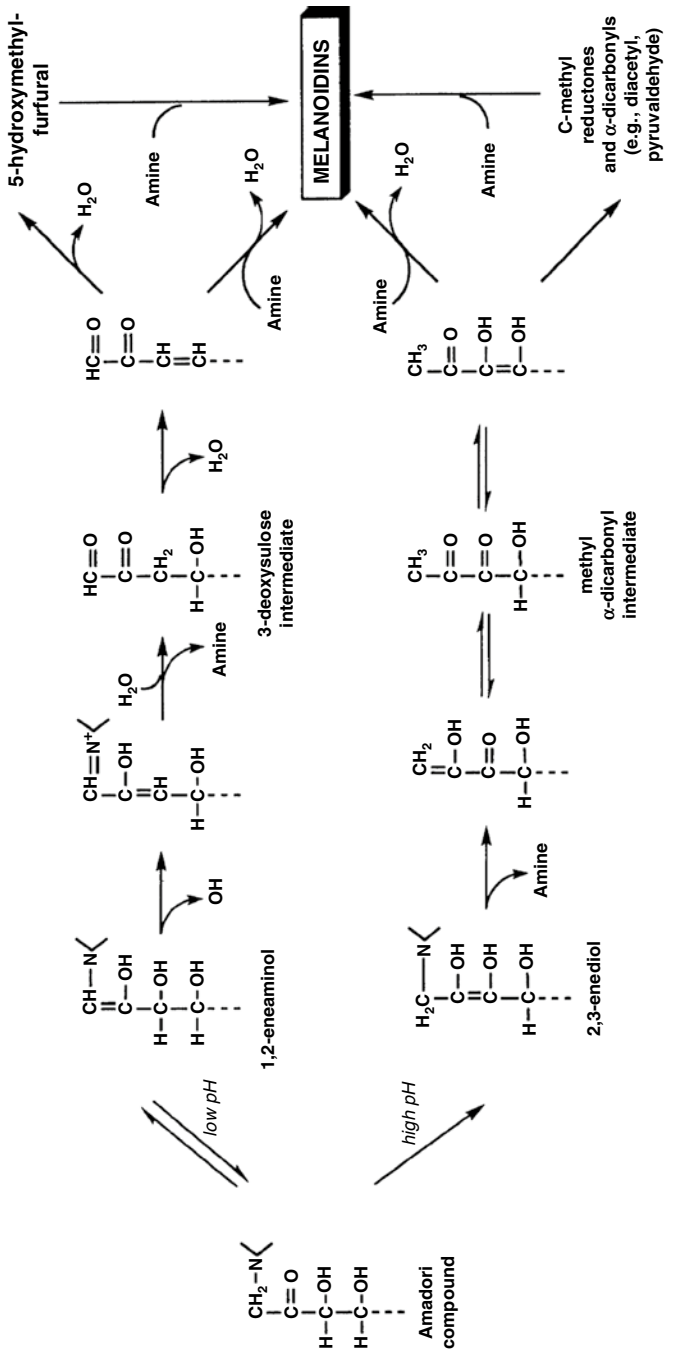


Fig. 2.30 Pathways for the Maillard browning reaction

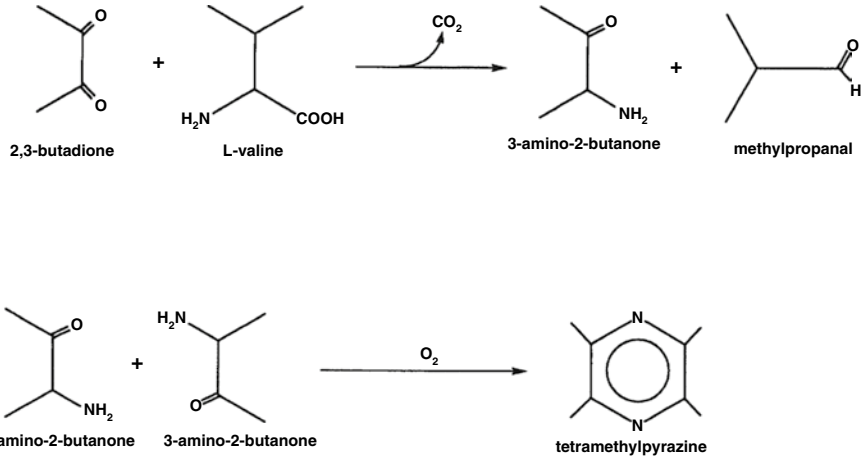


Fig. 2.31 Strecker degradation of L-valine by reaction with 2,3-butanedione

2.6 Nutritional Aspects of Lactose

Since the milk of most mammals contains lactose, it is reasonable to assume that it or its constituent monosaccharides have some nutritional significance. The secretion of a disaccharide rather than a monosaccharide in milk is advantageous since twice as much energy can be provided for a given osmotic pressure. Galactose may be important because it or its derivatives, e.g., galactosamine, are constituents of several glycoproteins and glycolipids, which are important constituents of cell membranes; young mammals have limited capacity to synthesize galactose.

Lactose appears to promote the absorption of calcium but this is probably due to a non-specific increase in intestinal osmotic pressure, an effect common to many sugars and other carbohydrates, rather than a specific effect of lactose.

However, lactose has two major nutritionally undesirable consequences—lactose intolerance and galactosemia. Lactose intolerance is caused by insufficient intestinal β-galactosidase—lactose is not completely hydrolysed, or not hydrolysed at all, in the small intestine and since disaccharides are not absorbed, it passes into the large intestine where it causes an influx of water, causing diarrhoea, and is fermented by intestinal microorganisms, causing cramping and flatulence.

2.6.1 Lactose Intolerance

A small proportion of babies are born with a deficiency of β-galactosidase (inborn error of metabolism) and are unable to digest lactose from birth. In normal infants (and other neonatal mammals), the specific activity of intestinal

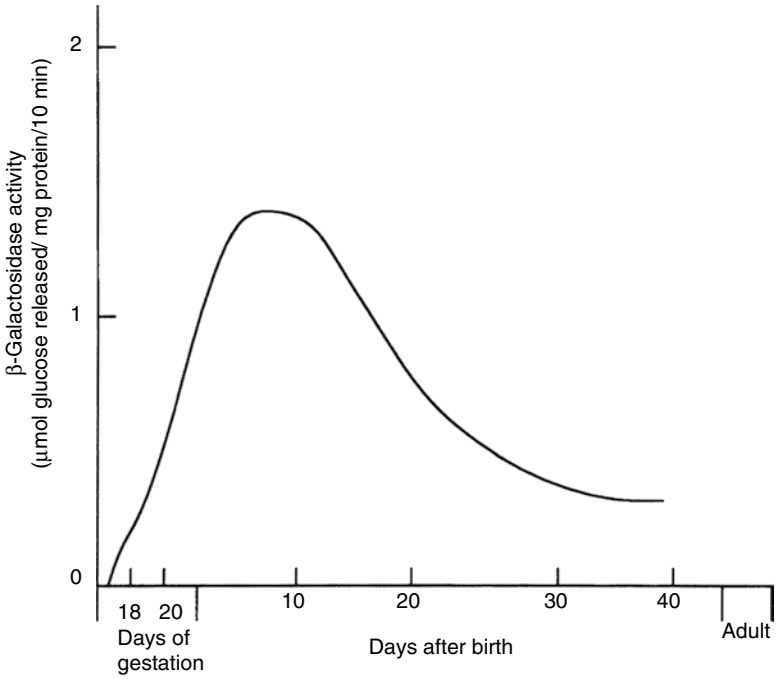


Fig. 2.32 β-Galactosidase activity in homogenates from the intestine of the developing rat

β-galactosidase increases to a maximum at parturition (Fig. 2.32), although total activity continues to increase for some time post-partum due to increasing intestinal area. However, in late childhood, total activity decreases and in an estimated 70 % of the world's population, decreases to a level which causes lactose intolerance among adults. Only northern Europeans and a few African tribes, e.g., Fulami, can consume milk with impunity; the inability to consume lactose appears to be the normal pattern in humans and other species and the ability of northern Europeans to do so presumably reflects positive selective pressure for the ability to consume milk as a source of calcium (better bone development) (see Ingram and Swallow 2009; Swallow 2011).

Lactose intolerance can be diagnosed by (1) jujunal biopsy, with assay for β-galactosidase or (2) administration of an oral dose of lactose followed by monitoring blood glucose level or pulmonary hydrogen level. A test dose of 50 g lactose in water (equivalent to 1 l of milk) is normally administered to a fasting patient; the dose is rather excessive and gastric emptying is faster for a fasted than a fed subject—the presence of other constituents in the meal will delay gastric emptying. Blood glucose level will increase in a lactose-tolerant subject shortly after consuming lactose or a lactose-containing product but not if the subject has a deficiency of

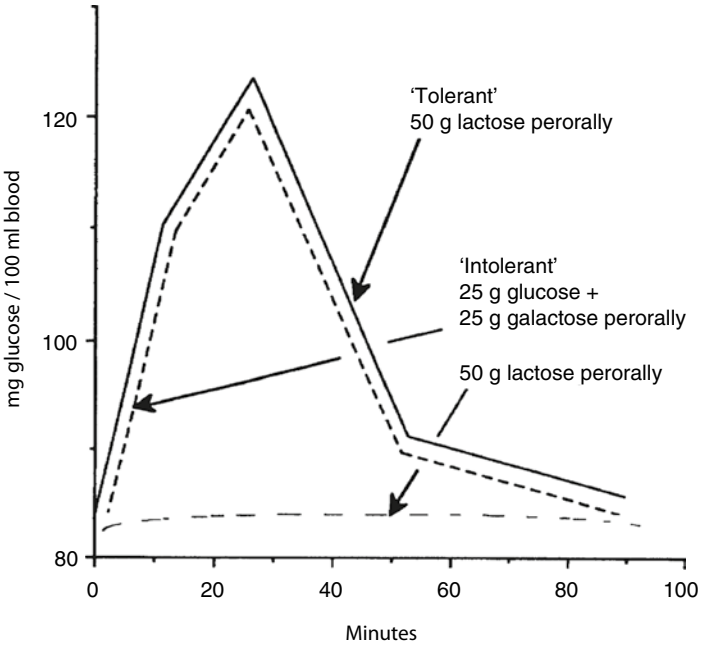


Fig. 2.33 Examples of the "lactose intolerance" test

β-galactosidase (Fig. 2.33). Pulmonary H_2 increases in lactose-intolerant subjects because lactose is metabolised by bacteria in the large intestine, with the production of H_2, which is absorbed and exhaled through the lungs.

Milk can be suitably modified for lactose-intolerant subjects by:

1. Ultrafiltration, which also removes valuable minerals and vitamins, and therefore the milk must be supplemented with these.
2. Fermentation to yoghurt or other fermented product in which ~25 % of the lactose is metabolised by lactic acid bacteria, and which contains bacterial β-galactosidase and is also discharged more slowly from the stomach due to its texture.
3. Conversion to cheese, which is essentially free of lactose.
4. Treatment with exogenous β-galactosidase, either domestically by the consumer or the dairy factory, using free or immobilized enzyme; several protocols for treatment have been developed (Fig. 2.34). Lactose-hydrolysed milks are technologically successful and commercially available but have not led to large increases in the consumption of milk in countries where lactose intolerance is widespread, presumably due to cultural and economic factors. However, there are niche markets for such products.

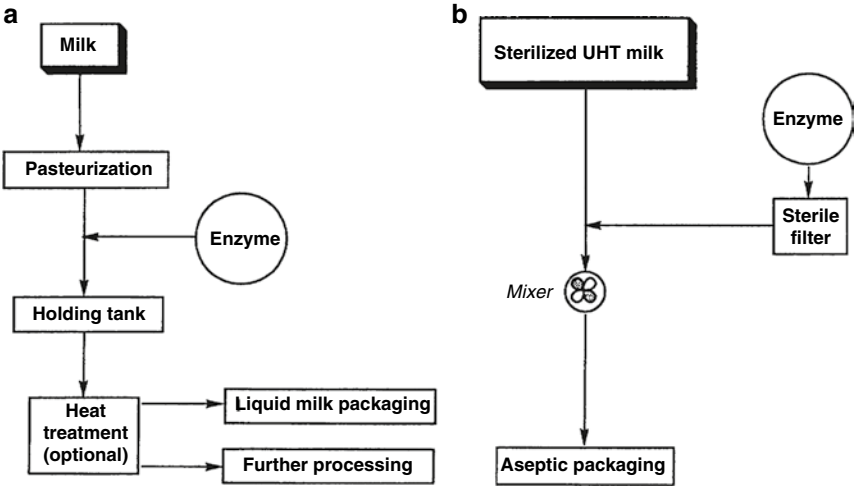


Fig. 2.34 (**a**) Scheme for manufacture of low-lactose milk using a "high" level soluble β-galactosidase. (**b**) Scheme for the manufacture of low-lactose milk by addition of a low level of soluble β-galactosidase to UHT-sterilized milk (redrawn from Mahoney 1997)

2.6.2 Galactosemia

Glactosemia is caused by the inability to metabolise galactose due to a hereditary deficiency of galactokinase or galactose-1-phosphate (Gal-1-P): uridyl transferase (Fig. 2.35). Lack of the former enzyme leads to the accumulation of galactose which is metabolised via other pathways, leading, among other products, to galactitol which accumulates in the lens of the eye, causing cataract in 10–20 years (in humans) if consumption of galactose-containing foods (milk, legumes) is continued. The incidence is about 1:40,000. The lack of Gal-1-P: uridyl transferase leads to the accumulation of Gal and Gal-1-P. The latter interferes with the synthesis of glycoproteins and glycolipids (important for membranes, e.g., in the brain) and results in irreversible mental retardation within 2–3 months if the consumption of galactose-containing foods is continued. The incidence of this disease, often called "classical galactosemia", is about 1 in 60,000.

The ability to metabolise galactose decreases on aging (after 70 years), leading to cataract; perhaps this, together with the fact that mammals normally encounter lactose only while suckling, explains why many people lose the ability to utilise lactose at the end of childhood.

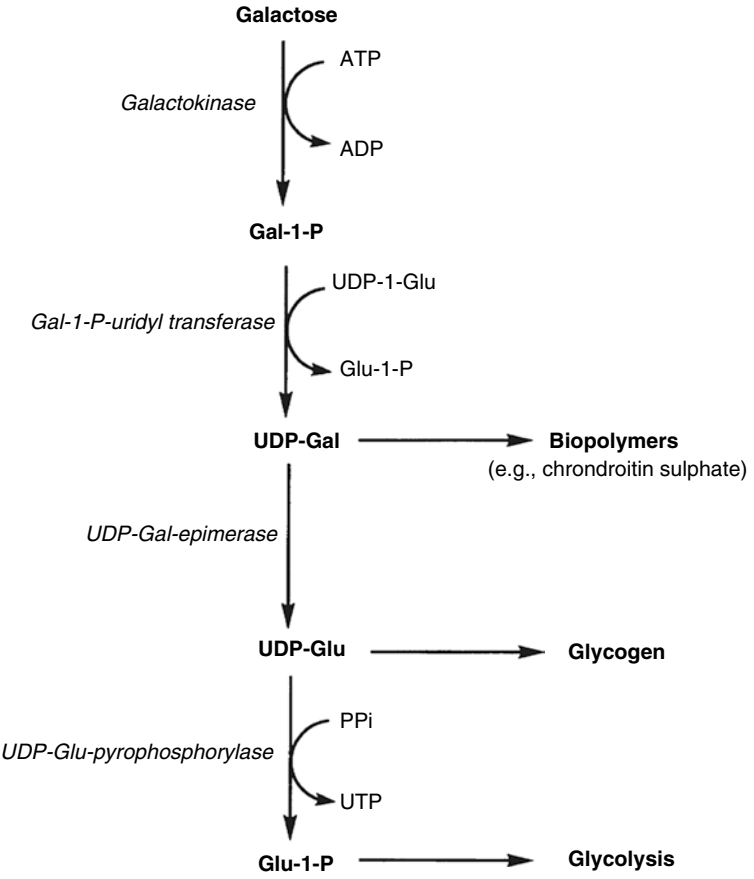


Fig. 2.35 Pathways for the metabolism of galactose

2.7 Determination of Lactose Concentration

Lactose may be quantified by methods based on one of five principles:

1. Polarimetry
2. Oxidation-reduction titration
3. Colorimetry
4. Chromatography
5. Enzymatically

2.7.1 Polarimetry

The specific rotation, $[\alpha]_D^{20}$, of lactose in solution at equilibrium is 55.4° expressed on an anhydrous basis (52.6° on a monohydrate basis). The specific rotation is defined as the optical rotation of a solution containing 1 g/ml in a 1 dm polarimeter tube; it is affected by temperature (20 °C is usually used; indicated by superscript) and wavelength [usually the sodium D line (589.3 nm) is used; indicated by a subscript].

$$[\alpha]_D^{20} = \mathrm{a/lc}$$

where: a is the measured optical rotation, l is the light path in dm and c is the concentration as g/ml

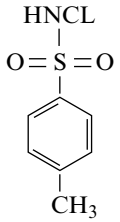
It is usually expressed as:

$$\begin{aligned} [\alpha]_D^{20} &= 100\,\mathrm{a/lc} \\ \text{where : c} &= \mathrm{g/100\,ml} \end{aligned}$$

The milk sample must first be defatted and de-proteinated, usually by treatment with mercuric nitrate [$Hg(NO_3)_2$]. In calculating the concentration of lactose, a correction should be used for the concentration of fat and protein in the precipitate, i.e., 0.92 for whole milk and 0.96 for skimmed milk.

2.7.2 Oxidation and Reduction Titration

Lactose is a reducing sugar, i.e., it is capable of reducing appropriate oxidising agents, two of which are usually used, i.e., alkaline copper sulphate ($CuSO_4$ in sodium potassium tartrate; Fehling's solution) or Chloroamine-T (2.1).



Chloroamine-T (2.1)

For analysis by titration with Fehling's solution, the sample is treated with lead acetate to precipitate protein and fat, filtered and the filtrate titrated with alkaline $CuSO_4$, while heating. The reactions involved are summarized in Fig. 2.36.

Cu_2O precipitates and may be recovered by filtration and weighed; the concentration of lactose can then be calculated since the oxidation of one mole of lactose (360 g) yields one mole of Cu_2O (143 g). However, it is more convenient to add an excess of a standard solution of $CuSO_4$ to the lactose-containing solution; the

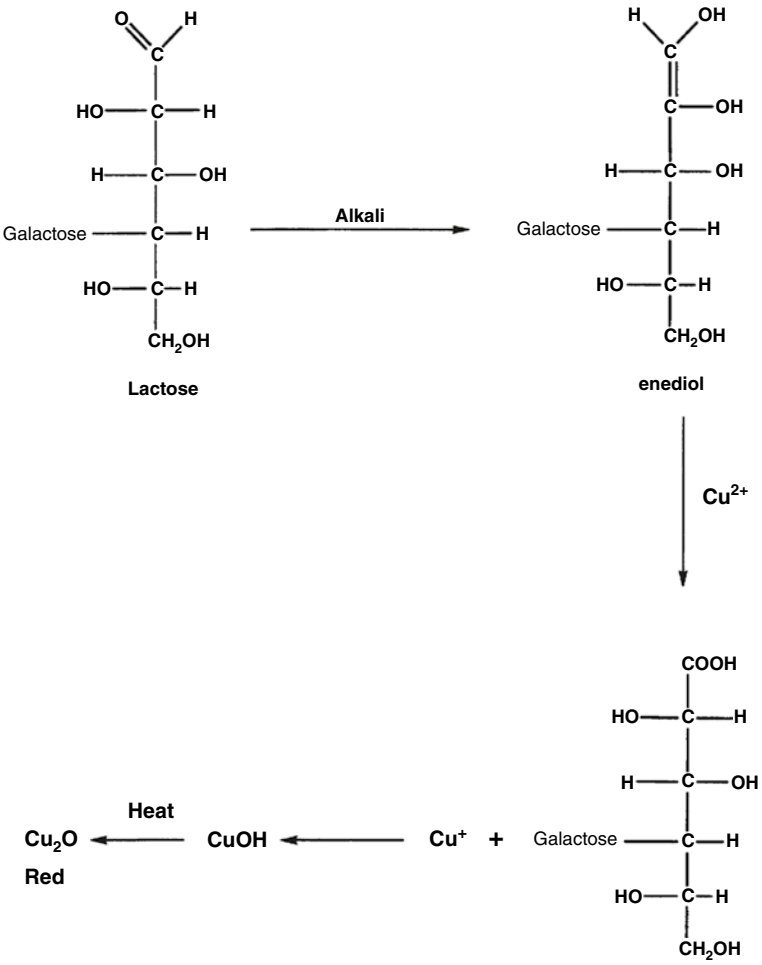


Fig. 2.36 Oxidation of lactose by alkaline copper sulfate (Fehling's reagent)

solution is cooled and the excess $CuSO_4$ determined by reaction with KI and titrating the liberated I_2 with standard sodium thiosulphate ($Na_2S_2O_3$) using starch as an indicator.

$$2CuSO_4 + 4KI \rightarrow CuI_2 + 2K_2SO_4 + I_2$$
$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_2O_6$$

The end point in the Fehling's is not sharp and the redox determination of lactose is now usually performed using Chloramine-T rather than $CuSO_4$ as oxidising agent.

The reactions involved are as follows:

$$CH_3C_6H_4SO_2NClH + H_2O + KI(\text{excess}) \leftrightarrow CH_3C_6H_4SO_2NH_2 + HCl + KIO(\text{K hypoiodate})$$

$$KIO + \text{lactose}\ (\text{-CHO}) \rightarrow KI + \text{lactobionic acid}(\text{-COOH})$$

$$KI + KIO \rightarrow 2KOH + I_2$$

The I_2 titrated with standard $Na_2S_2O_3$

$$I_2 + \underset{(\text{thiosulphate})}{2Na_2S_2O_3} \rightarrow 2NaI + Na_2S_4O_6$$

One ml of 0.04 N thiosulphate is equivalent to 0.0072 g lactose monohydrate or 0.0064 g anhydrous lactose.

The sample is deproteinized and defatted using phosphotungstic acid.

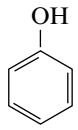
2.7.3 Infrared (IR) Spectroscopy

Stretching of the –O–H bond of lactose (and other sugars) by IR radiation of 9.5 μm, permits the quantitative determination of lactose. As discussed in Chaps. 3 and 4, respectively, the ester bond of triglycerides absorbs IR radiation at 5.7 μm and the peptide bond of proteins absorbs IR radiation at 6.46 μm. Thus, in a single scan, the concentrations of fat, protein and lactose in milk can be determined by IR spectroscopy using an Infra Red Milk Analyzer (IRMA).

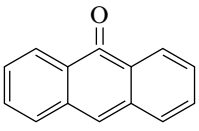
Such instruments are now widely used in the dairy industry.

2.7.4 Colorimetric Methods

Reducing sugars, including lactose, react on boiling with phenol (2.2) or anthrone (2.3) in strongly acidic solution (70 %, v/v, H_2SO_4) to give a coloured solution (2.1 and (2.3).



Phenol (2.2)



Anthrone (2.3)

The complex with anthrone absorbs maximally at 625 nm. The concentration of lactose is determined from a standard curve prepared using a range of lactose concentrations.

The method is very sensitive but must be performed under precisely controlled conditions.

2.7.5 Chromatographic Methods

While lactose may be determined by gas liquid chromatography, high performance liquid chromatography (HPLC), using an ion-exchange column and a refractive index detector, is now usually used.

2.7.6 Enzymatic Methods

Enzymatic methods are very sensitive but are rather expensive, especially for a small number of samples.

Lactose is first hydrolysed by β-galactosidase to glucose and galactose. The glucose may be quantified by reaction with:

1. Glucose oxidase using a platinum electrode or the H_2O_2 generated may be quantified by using a peroxidase and a suitable dye acceptor
   or
2. Glucose-6-phosphate dehydrogenase (G-6-P-DH):

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Gluconate-6-P} \xrightarrow{\text{G-6-DH, NADP}^+} \text{Gluconate-6-P} + \text{NADPH} + \text{H}^+$$

The concentration of NADPH produced may be quantified by measuring the increase in absorbance at 334, 340 or 365 nm.

Alternatively, the galactose produced may be quantified using galactose dehydrogenase (Gal-DH).

$$\text{D-galactose} + \text{NAD}^+ \xrightarrow{\text{Gal-DH}} \text{Galactonic acid} + \text{NADH} + \text{H}^+$$

The NADH produced may be quantified by measuring the increase in absorbance at 334, 340 or 365 nm.

2.8 Oligosaccharides

The milk of most, and probably all, species contains other free saccharides, mainly oligosaccharides (OSs), the concentration, proportions and types of which show large interspecies differences. The concentration of OSs is higher in colostrum than

in milk. General reviews on the OSs in milk include Newburg and Newbauer (1995), Mehra and Kelly (2006), and Urashima et al. (2001, 2009, 2011).

Almost all of the OSs have lactose at the non-reducing end, they contain three to eight monosaccharides, they may be linear or branched, and contain either or both of two unusual monosaccharides, fucose (a 6-deoxyhexose) and *N*-acetylneuraminic acid. Fucose occurs quite widely in tissues of mammals and other animals where it serves a wide array of functions (Becker and Lowe 2003). Its significance in the OSs in milk is not clear; perhaps it is to supply the neonate with preformed fucose.

The OSs are synthesized in the mammary gland, catalyzed by special transferases that transfer galactosyl, sialyl, *N*-acetylglucosaminyl, or fucosyl residues from nucleotide sugars to the core structures. These transferases are not affected by α-La and are probably similar to the transferases that catalyze the glycosylation of lipids and proteins.

The milk of all species examined contains OSs, but the concentration varies markedly. The highest levels are in the milk of monotremes, marsupials, marine mammals, humans, elephants, and bears. With the exception of humans and elephants, the milk of these species contains little or no lactose, and OSs are the principal carbohydrates.

The milk of the echidna contains mainly the trisaccharide, fucosyllactose, while that of the platypus contains mainly the tetrasaccharide, difucosyllactose. Among marsupials, the best studied is the Tammar wallaby; presumably, its lactation pattern and milk composition are typical of marsupials. A low level of lactose is produced at the start of lactation, but about 7 days after birth, a second galactosyltransferase appears and tri- to penta-saccharides are produced, which by ~180 days are the principal saccharides. During this period the content is high, ~50 % of total solids, and the level of lipids is low (~15 % of total solids). At about 180 days, the carbohydrate decreases to a very low level and consists mainly of monosaccharides, while the level of lipids increases to ~60 % of total solids (Sharp et al. 2011).

Human milk contains ~130 OSs, at a total concentration of ~15 g/L; these are considered to be important for neonatal brain development. Bear milk contains little lactose but a high level of total sugars (mainly OSs) −1.7 and 28.6 g/kg, respectively (Oftedal 2013). Elephant milk contains ~50 and 12 g/kg of lactose and OSs, respectively, a few days post- partum, but as lactation progresses, the concentration of lactose decreases while that of OSs increases (e.g., 12 and 18 g/kg, respectively), at 47 days (Osthoff et al. 2005). The milk of seals contains both lactose and OSs, but the milk of the Californian sea lion, Northern fur seal, and Australian fur seal contain neither, probably because they contain no α-La (Urashima et al. 2001).

Bovine, ovine, caprine, and equine milk contain relatively low levels of OSs, which have been characterized (see Urashima et al. 2001, 2009, 2011). Caprine milk contains about ten times as much OSs as bovine and ovine milk, and a process for their isolation by nanofiltration has been reported (Martinez-Ferez et al. 2006). Possible methods for producing OSs similar to those found in human milk, by fermentation or by transgenic animals or by recovering OSs from cow's milk whey or UF permeate were discussed by Mehra and Kelly (2006) and O'Mahony and Touhy (2013).

OSs with bactericidal properties were probably the saccharides present in the mammary secretions of early mammals; the high level of OSs in the milk of monotremes

and marsupials conforms with their secretion early in evolution. It is proposed that the primitive mammary glands of the first common ancestor of mammals produced lysozyme (a predecessor of α-La), and a number of glycosyltransferases but little or no α-La. This resulted in the production of a low level of lactose that was utilized in the synthesis of OSs and did not accumulate (Urashima et al. 2009). Initially, the OSs served mainly as bactericidal agents but later became a source of energy for the neonate. Both of these functions persist for monotremes, marsupials, and some eutherians such as bears, elephants, and marine mammals. However, most eutherians evolved to secrete predominantly lactose as an energy source, due to the synthesis of an increased level of α-La, while OSs continued to play a bactericidal role. Human and elephant milk, both of which contain high levels of lactose and OSs, seem to be anomalous. Work on the OSs of a wider range of species is needed to explain this situation.

The significance of OSs is not clear, but the following aspects may be significant: For any particular level of energy, they have a smaller impact on osmotic pressure than smaller saccharides, they are not hydrolyzed by β-galactosidase, and fucosidase or neuraminidase is not secreted in the intestine. Hence the OSs are not hydrolyzed and absorbed in the gastro-intestinal tract, and they function as soluble fiber and prebiotics that affect the microflora of the large intestine. It is claimed that they prevent the adhesion of pathogenic bacteria in the intestine; galactose, and especially *N*-acetylneuraminic acid, are important for the synthesis of glycolipids and glycoproteins, which are vital for brain development. It has therefore been suggested that the OSs are important for brain development (see Kunz and Rudloff 2006).

There is considerable interest in the development of OS-enriched ingredients from bovine milk (O'Mahony and Touhy 2013), primarily for infant formula applications. This interest has been spurred by the demonstrated bioactive functionality of these compounds in humans (Kunz and Rudloff 2006).

In addition to lactose and free OSs, the milk of all species examined contains small amounts of monosaccharides and some milk proteins, especially κ-casein, are glycosylated, and there are low levels of highly glycosylated glycoproteins, especially mucins, and glycolipids in the milk fat globule membrane.

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