2 Lactose

2.1 Introduction

Lactose is the principal carbohydrate in the milks of all mammals; non-mammalian sources are very rare. Milk contains only trace amounts of other sugars, including glucose ($50\,mg\,l^{-1}$), fructose, glucosamine, galactosamine, neuraminic acid and neutral and acidic 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, individuality factors, udder infection and especially stage of lactation. The concentration of lactose decreases progressively and significantly during lactation (Figure 2.1); this behaviour contrasts with the lactational trends for lipids and proteins, which, after decreasing during early lactation, increase strongly during the second half of lactation. 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) is compensated for by an increase or decrease in the soluble salt (excreted) constituents. 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 concentration of lactose and chloride, which is the basis of Koestler's chloride–lactose test

Table 2.1 Concentration (%) of lactose in the milks 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	Human	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				

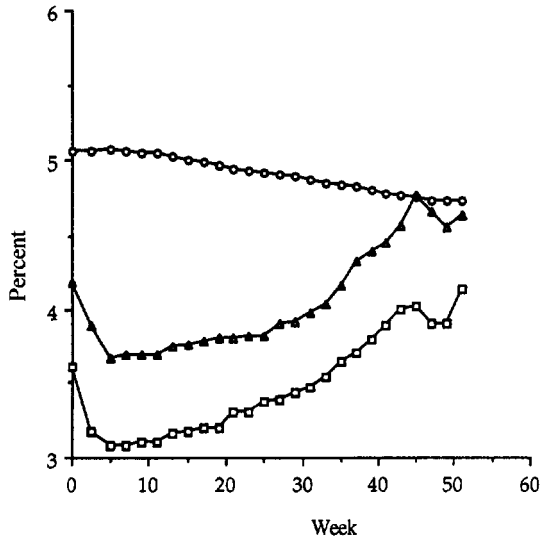


Figure 2.1 Changes in the concentrations of fat (△), protein (□) and lactose (○) in milk during lactation.

Table 2.2 Average concentration (%) of lactose and ash in the milks 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

for abnormal milk:

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

A Koestler number less than 2 indicates normal milk while a value greater than 3 is considered abnormal.

Lactose plays an important role in milk and milk products:

- it is an essential constituent in the production of fermented dairy products;

- 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 a syndrome known as **lactose intolerance**;
- 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.

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 (Figure 2.2). Its systematic name is β-*O*-D-galactopyranosyl-(1-4)-α-D-glucopyranose (α-lactose) or β-*O*-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).

2.2.2 Biosynthesis of lactose

Lactose is essentially unique to mammary secretions. It is synthesized from glucose absorbed from blood. One molecule of glucose is isomerized to UDP-galactose via the four-enzyme Leloir pathway (Figure 2.3). UDP-Gal is then linked to another molecule of glucose in a reaction catalysed by the enzyme, lactose synthetase, a two-component enzyme. Component A is a non-specific galactosyl transferase 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 decreases 1000-fold), leading to the synthesis of lactose. Thus, α-lactalbumin is an enzyme modifier and its concentration in the milk of several species is directly related to the concentration of lactose in those milks; the milks 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 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 the 'wastage' of the enzyme modifier.

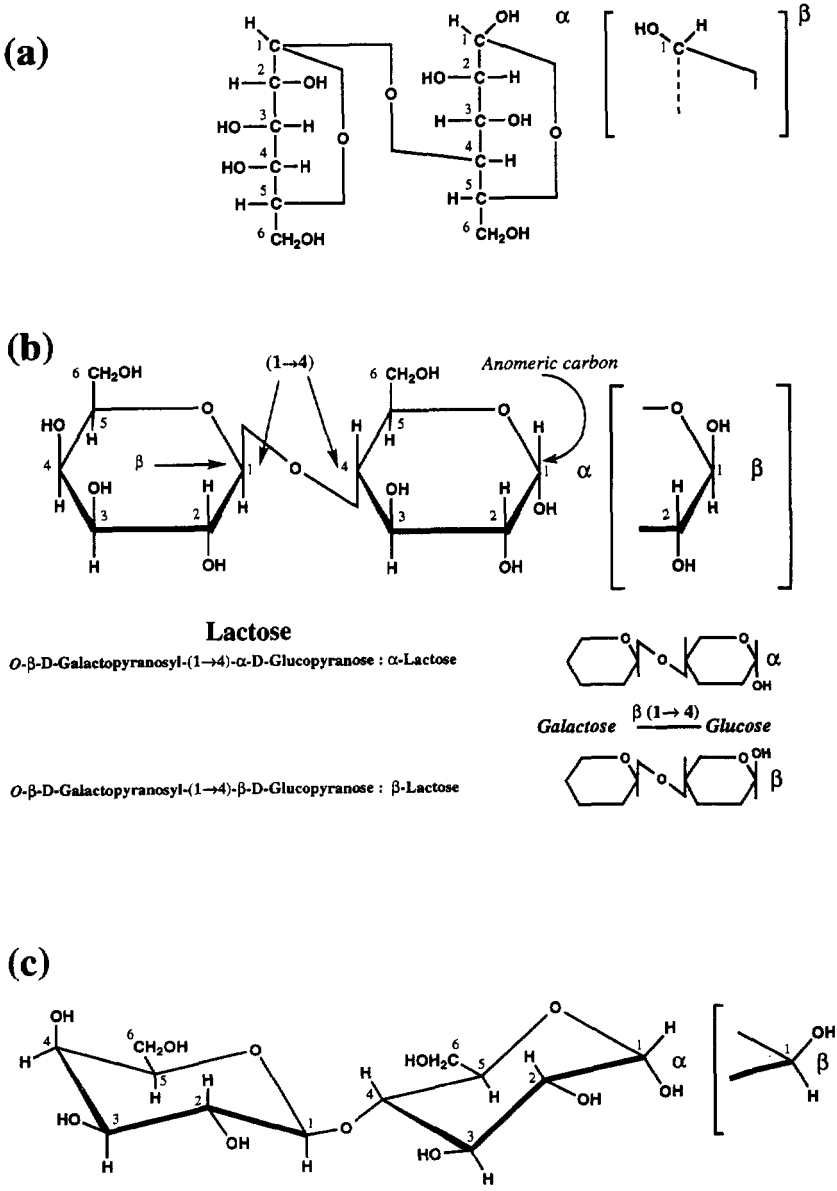


Figure 2.2 Structural formulae of α- and β-lactose. (a) Fischer projection, (b) Haworth projection and (c) conformational formula.

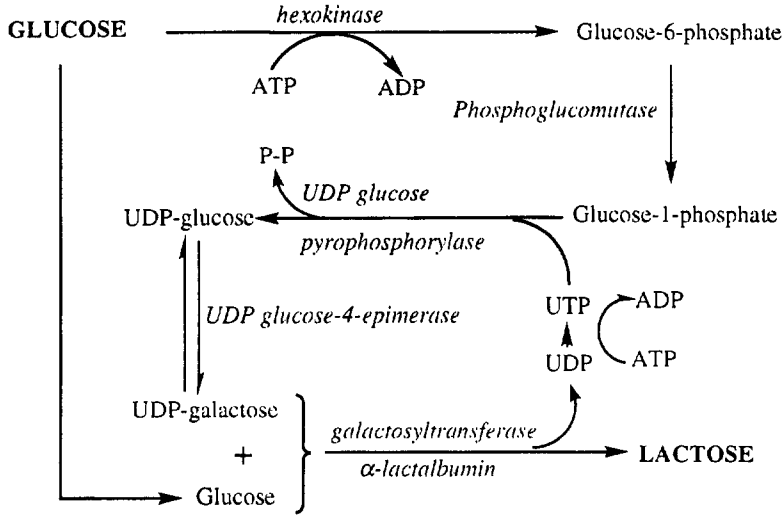


Figure 2.3 Pathway for lactose synthesis.

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 (Figure 2.2).

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. 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

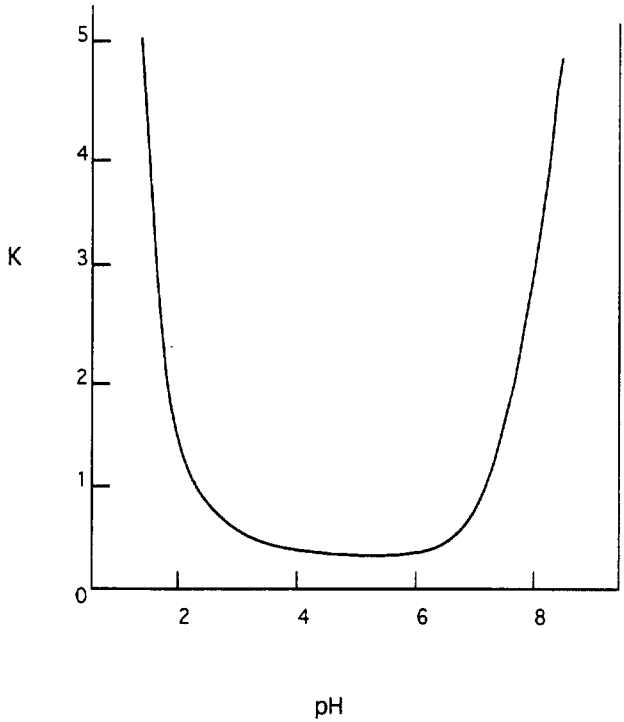


Figure 2.4 Effect of pH on the rate of mutarotation of lactose.

At equilibrium:

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

$$x = 37.3$$

$$100 - x = 62.7$$

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-lactose 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 (Figure 2.4).

2.2.4 *Significance of mutarotation*

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

- solubility;
- crystal shape and size;
- hydration of crystal form – 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. These two processes (mutarotation and solubilization of α-lactose) continue until two criteria are met: ~7 g α-lactose in solution and a β/α ratio of 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 × 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 $\beta \rightarrow \alpha$. 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 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 is the same.

The solubility of lactose as a function of temperature is summarized in Figure 2.5. 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 α-lactose or a total of 18.2 g 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.

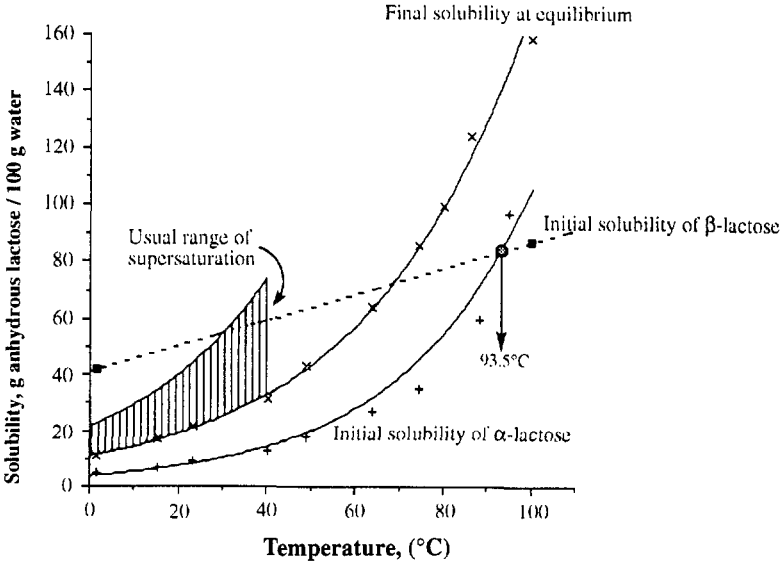


Figure 2.5 Solubility of lactose in water (modified from Jenness and Patton, 1959).

2.2.6 Crystallization of lactose

As discussed in section 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 Figure 2.6 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:

- Neither nucleation nor crystal growth occurs in the unsaturated region.
- Growth of crystals can occur in both the metastable and labile areas.
- Nucleation occurs in the metastable area only if seeds (centres for crystal growth) are added.

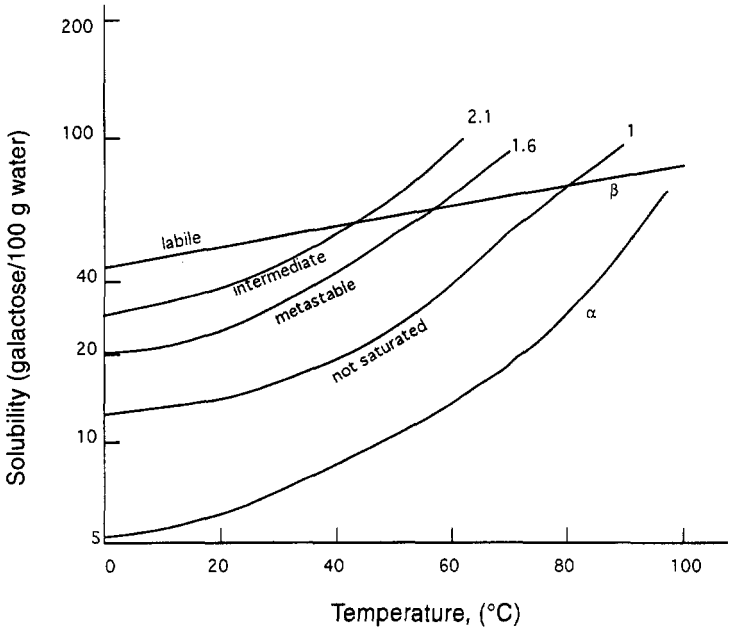


Figure 2.6 Initial solubility of α-lactose and β-lactose, final solubility at equilibrium (line 1), and supersaturation by a factor 1.6 and 2.1 (α-lactose excluding water of crystallization). (Modified from Walstra and Jenness, 1984.)

- 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' 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:

- degree of supersaturation;
- surface area available for deposition;
- viscosity;
- agitation;
- temperature;
- 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 aqueous lactose solutions to supersaturation and allowing crystallization to occur below

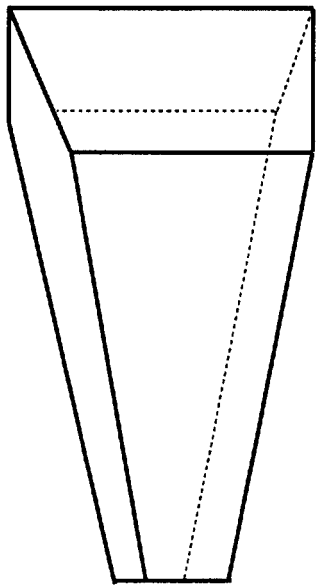


Figure 2.7 The most common crystal form of α-lactose hydrate.

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 (Figure 2.7). Crystals 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.

α-Anhydrous. Anhydrous α-lactose may be prepared by dehydrating α-hydrate *in vacuo* at temperatures 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 above 93.5°C, the crystals formed from aqueous solutions at temperatures above 93.5°C are β-lactose; these are anhydrous and have a specific rotation of 35°. β-Lactose is sweeter than α-lactose, but is not appreciably sweeter than the equilibrium mixture of α- and β-lactose normally found in solution.

Table 2.3 Some physical properties of the two common forms of lactose (modified from Jenness and Patton, 1959)

Property	α-Hydrate	β-Anhydride
Melting point[a] (°C)	202	252
Specific rotaltion[b] $[\alpha]_D^{20}$	+89.4°	+35°
Solubility in water (g 100 ml^{-1}) at 20°C	7	50
Specific gravity (20°C)	1.54	1.59
Specific heat	0.299	0.285
Heat of combustion (kJ mol^{-1})	5687	5946

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

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 Figure 2.8.

Lactose glass. When a lactose solution is dried rapidly, viscosity increases so quickly that crystallization is impossible. A noncrystalline 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 (Figure 2.9).

Dried milk and whey. Lactose is the major component of dried milk products: whole-milk powder, skim-milk powder and whey powder contain *c.* 30, 50 and 70% lactose, respectively. Protein, fat and air are dispersed in a continuous phase of amorphous solid lactose. Consequently, the behaviour of lactose has a major impact on the properties of dried milk products.

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 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

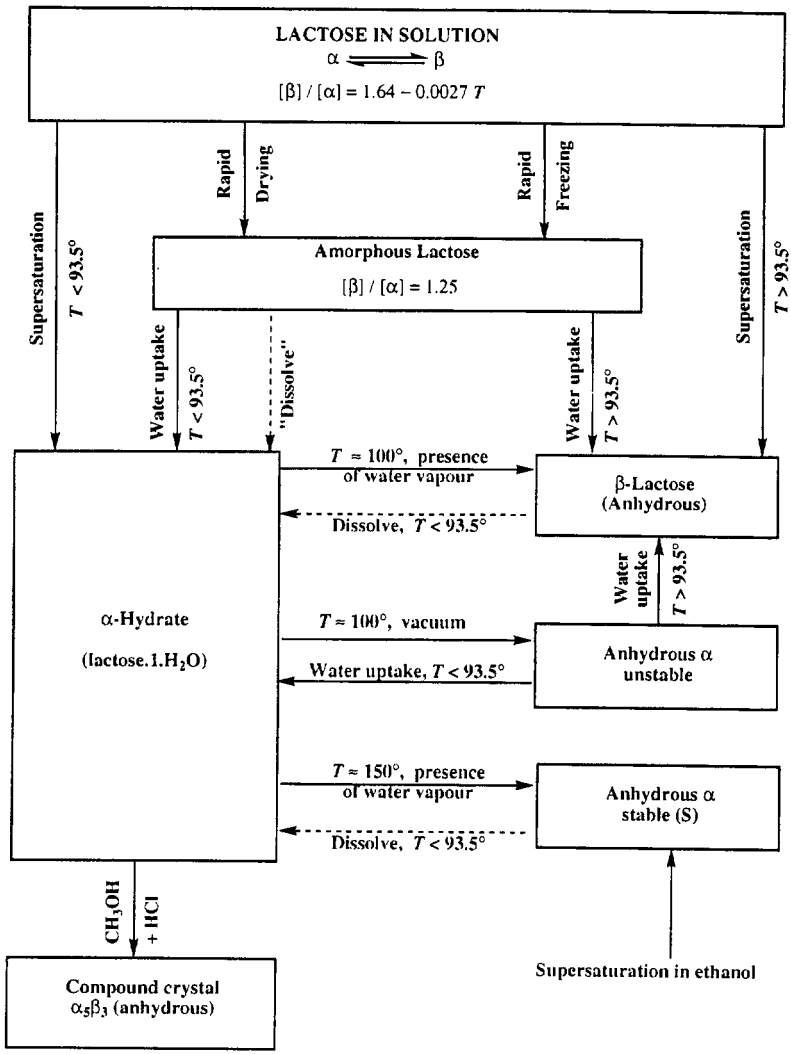


Figure 2.8 Modifications of lactose (T, temperature in °C) (from Walstra and Jenness, 1984).

monohydrate. These crystals are small, usually with dimensions of less than 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.

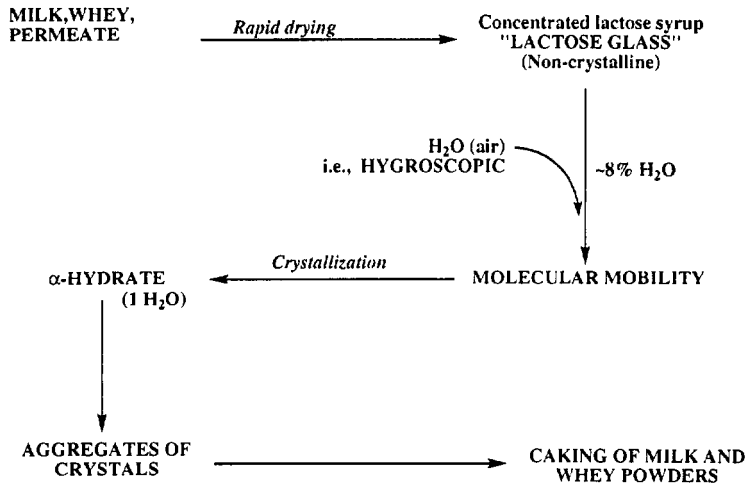


Figure 2.9 Formation and crystallization of lactose glass.

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 water is prevented, thereby improving the dispersibility of the powder. Lactose crystallization is achieved by rehydrating freshly dried powder to *c.* 10% water and redrying it, or by removing partly dried powder from the drier and completing drying in a fluidized bed dryer. 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 lumps. This problem is overcome by agglomeration and, in this respect, lactose crystallization is important since it facilitates the formation of large, sponge-like aggregates.

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 less than 4% water. 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 crystallizes. Addition of 0.5 kg of 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 c. 10^6 crystals ml^{-1}, about 95% of which will have dimensions less than 10 μm and 100% less than 15 μm, i.e. too small to cause textural defects.

Diagrams of spray dryers with instantizers are shown in Figures 2.10 and 2.11.

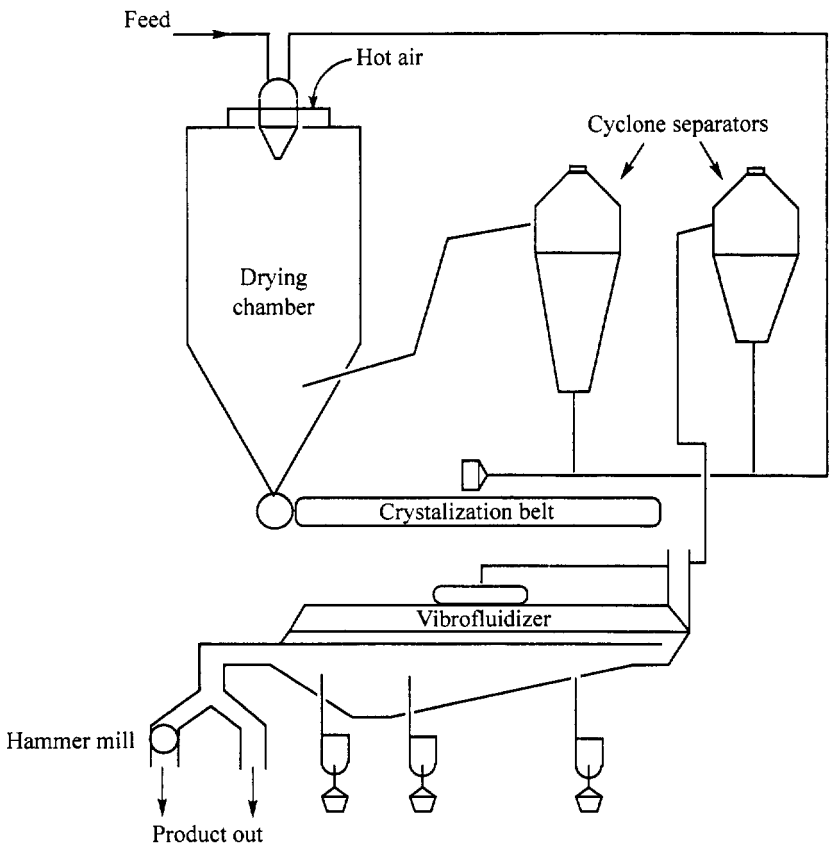


Figure 2.10 Schematic representation of a low temperature drying plant for whey (modified from Hynd, 1980).

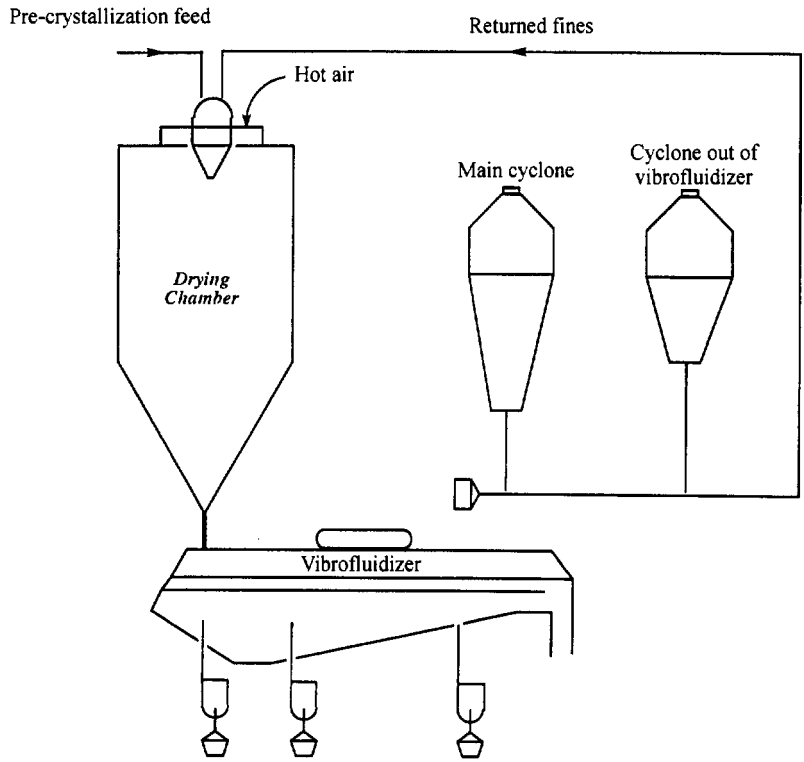


Figure 2.11 Schematic representation of a straight through drying plant for whey (modified from Hynd, 1980).

Thermoplasticity of lactose. Unless certain precautions are taken during the drying of whey or other solutions containing high concentrations of lactose, the hot, semi-dry powder may adhere to the metal surfaces of the dryer, forming deposits. This phenomenon is referred to as thermoplasticity. The principal factors influencing 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 (Figure 2.12). 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 (Figure 2.12). Pre-crystallization of the concentrate feed to the dryer thus permits considerably higher feed concentrations and drying temperatures.

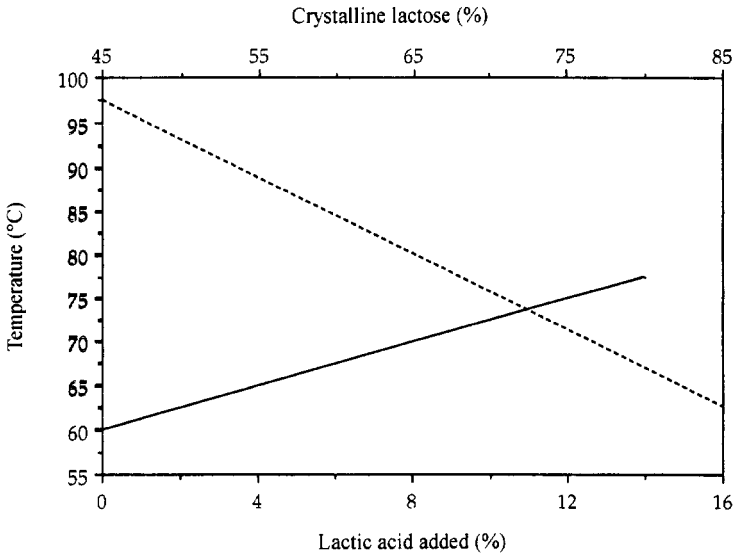


Figure 2.12 Effect of added lactic acid (----) and degree of lactose crystallization (_) on the sticking temperature of whey powder (1.5–3.5% moisture).

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, Figure 2.13). However, as a result of evaporative cooling, the temperature of the particles in the dryer is lower than the outlet temperature (t_p, Figure 2.13) 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, Figure 2.13) and where the two curves (t_s and t_p) intersect (point TPC, Figure 2.13) 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.

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 eventually crystallizes 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-lactose (ex-evaporator). To obtain a smooth texture, crystals with dimensions of less than 10 μm are desirable. The optimum temperature

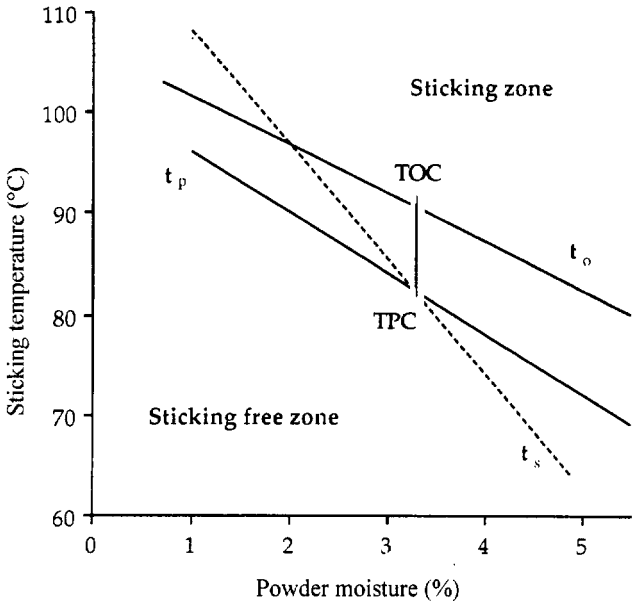


Figure 2.13 Influence of moisture content on the temperature of powder in a spray dryer (t_p), dryer outlet temperature (t_o) and sticking temperature (t_s). The minimum product temperatured required to avoid problems with sticking is at TPC with the corresponding dryer outlet temperature TOC. (Modified from Hynd, 1980.)

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.

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 limited 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 pressure 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.

Other frozen dairy products. Although milk may become frozen inadvertently, freezing is not a common commercial practice. However, concentrated or unconcentrated milks are 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 Chapter 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^+ (Chapter 5); precipitation of $Ca_3(PO_4)_2$ occurs on freezing because pure water crystallizes, 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 contains 80 g lactose per 100 g, whereas the solubility of lactose at $-8°C$ is only about 7%. During storage at low temperatures, 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).

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

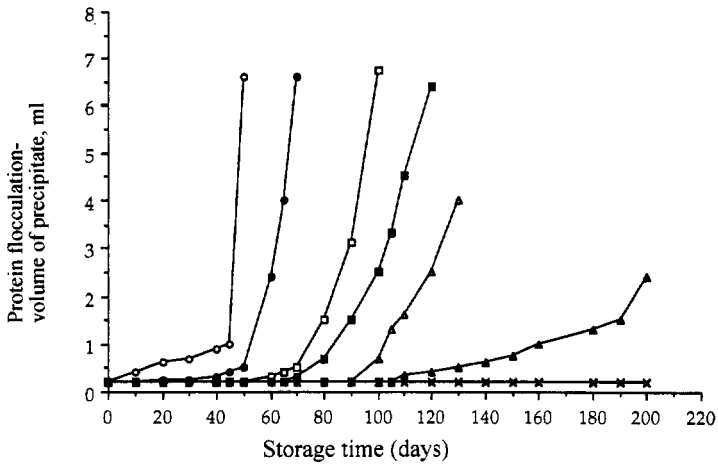


Figure 2.14 Effect of lactose hydrolysis (0, ○; 5, ●; 10, □; 15, ■; 20, △; 30, ▲; 50–85, ×, %) on the stability of milk to freezing (modified from Tumerman, Fram and Cornely, 1954).

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

$$3Ca^{2+} + 2H_2PO_4^- \rightleftharpoons Ca_3(PO_4)_2 + 4H^+$$

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

Any factor that accelerates the crystallization of lactose shortens the storage life of the product. At very low temperatures (below −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 (Figure 2.14).

2.3 Production of lactose

In comparison with sucrose (the annual production of which is 93×10^6 tonnes) 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 *c.* 1.4×10^7 tonnes, the whey from which contains *c.* 6×10^6 tonnes of lactose; *c.* 0.3×10^6 tonnes of lactose are contained in the whey produced during casein manufacture. According to Horton (1993),

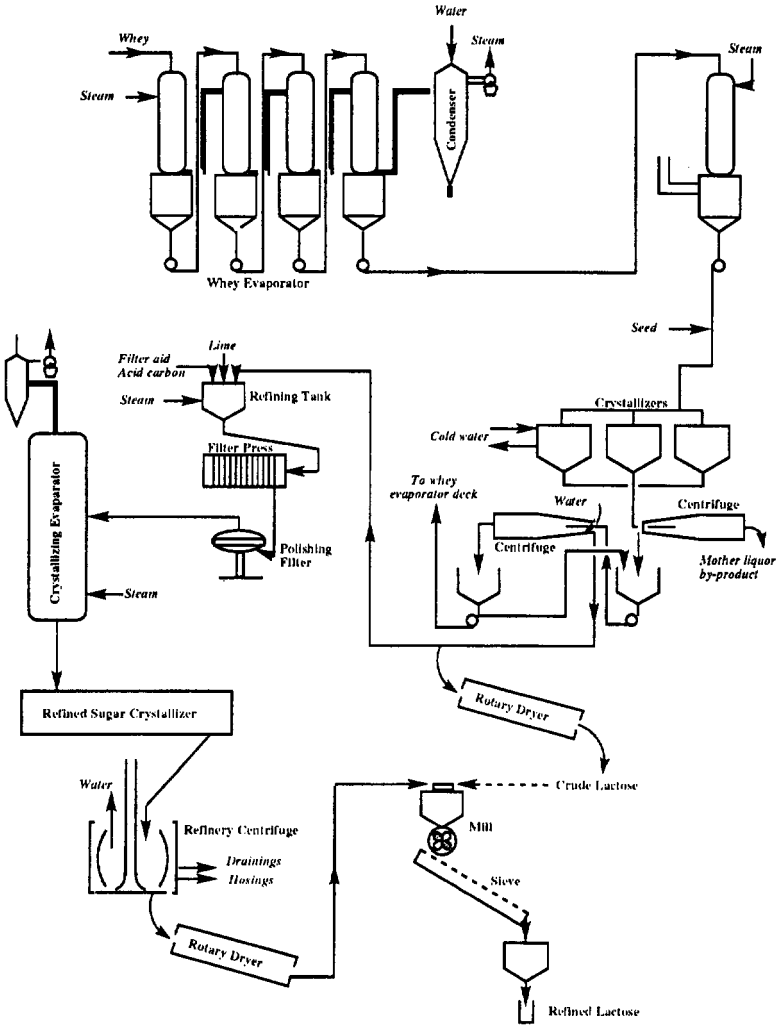


Figure 2.15 Schematic representation of plant for the manufacture of crude and refined lactose, from sweet whey.

only about 420 000 tonnes of lactose are produced annually, i.e. only about 7% of that potentially available.

Production of lactose essentially involves concentrating whey or ultrafiltration permeate by vacuum concentration, crystallization of lactose from the concentrate, recovery of the crystals by centrifugation and drying of the crystals (Figure 2.15). The first-crop crystals are usually contaminated with riboflavin and are therefore yellowish; a higher grade, and hence more

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

Analysis	Fermentation	Crude	Edible	USP[a]
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 $[\alpha]_D^{25}$	[b]	[b]	52.4°	52.4°

[a]USP, US Pharmacopoeia grade.
[b]Not normally determined.

Table 2.6 Food applications of lactose

Humanized baby foods
Determineralized 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

Table 2.7 Relative sweetness of sugars (approx. 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		
	60%		100%
	1 h	9 days	25 days
Sugar	Humectancy		
Lactose	0.54	1.23	1.38
Glucose	0.29	9.00	47.14
Sucrose	0.04	0.03	18.35

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.

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 high-quality, expensive lactose) 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.

2.4 Derivatives of lactose

Although the demand for lactose has been high 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, profitable, or at least inexpensive, 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* spp.) which produce enzymes with neutral pH optima. β-Galactosidases were considered to have

considerable commercial potential as a solution to the 'whey problem' and for the treatment of lactose intolerance (section 2.6.1). 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). Technological problems in the production of glucose–galactose syrups have been overcome but the process is not commercially successful. 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 section 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 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 not been commercially successful.

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 (galacto-oligosaccharides, Figure 2.16) which are later hydrolysed (Figure 2.17). 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* spp. in the lower intestine; a product (oligonate, 6′-galactosyl lactose) is produced commercially by the Yokult Company in Japan for addition to infant formulae. Some galacto-oligosaccharides have interesting functional properties and may find commercial applications.

2.4.2 Chemical modifications

Lactulose. Lactulose is an epimer of lactose in which the glucose moiety is isomerized to fructose (Figure 2.18). The sugar does not occur naturally and

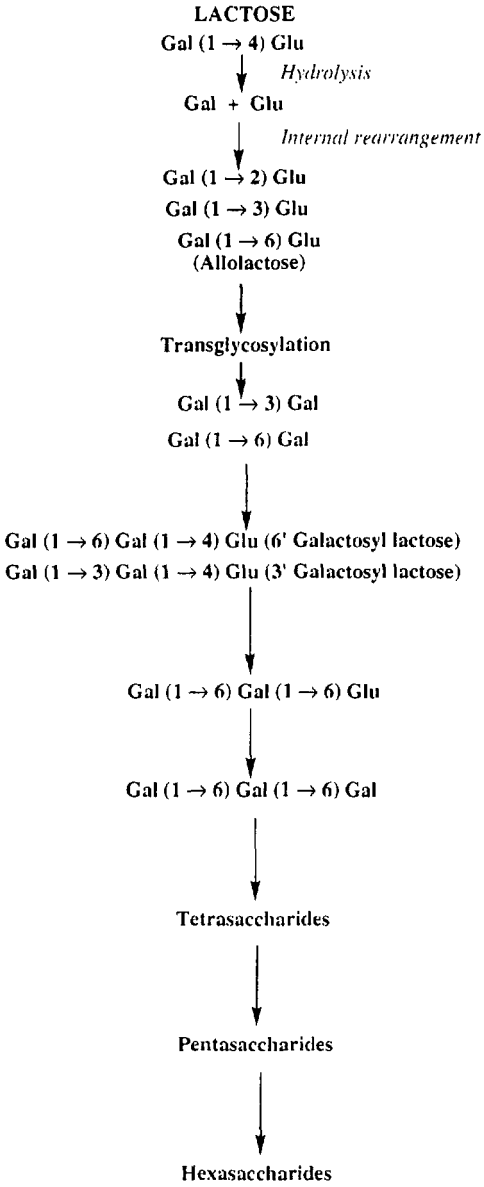


Figure 2.16 Possible reaction products from the action of β-galactosidase on lactose (from Smart, 1993).

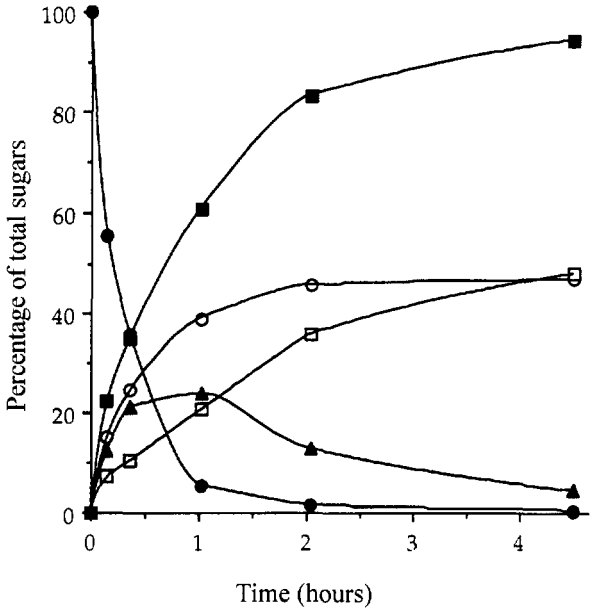


Figure 2.17 Production of oligosaccharides during the hydrolysis of lactose by β-galactosidase; ●, lactose; ■, monosaccharides; ○, glucose; ▲, oligosaccharides; □, galactose (modified from Mahoney, 1997).

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 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 (ultra-high temperature) milk (Figure 2.19); it is not present in raw or HTST (high temperature short time) pasteurized milk.

Lactulose is sweeter than lactose and 48–62% 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 metabolized 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 (Figures 2.20–2.22). It is now commonly added to infant formulae to simulate the bifidogenic properties of human milk – apparently, 20 000 tonnes annum^{-1} are now used for this and similar applications. Lactulose is also reported to suppress the growth of certain tumour cells (Figure 2.23).

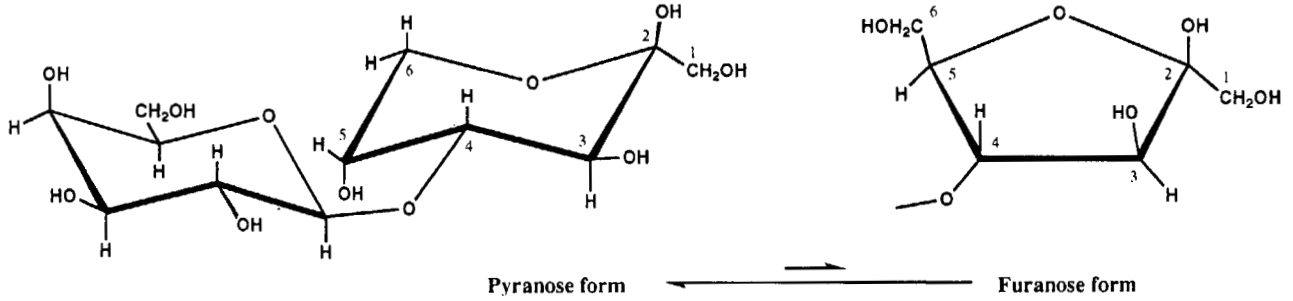


Figure 2.18 Chemical structure of lactulose.

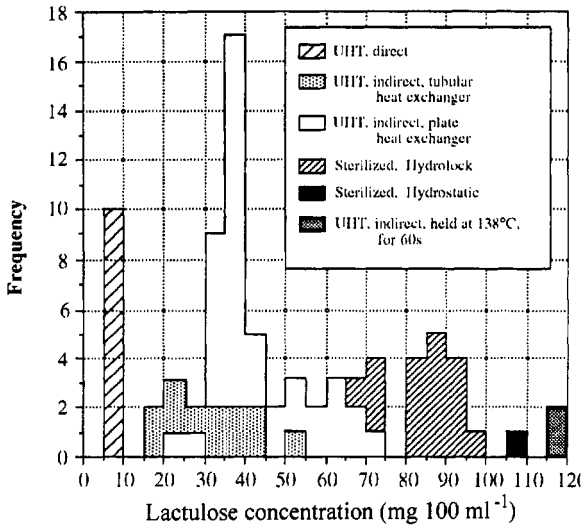


Figure 2.19 Concentration of lactulose in heated milk products (modified from Andrews, 1989).

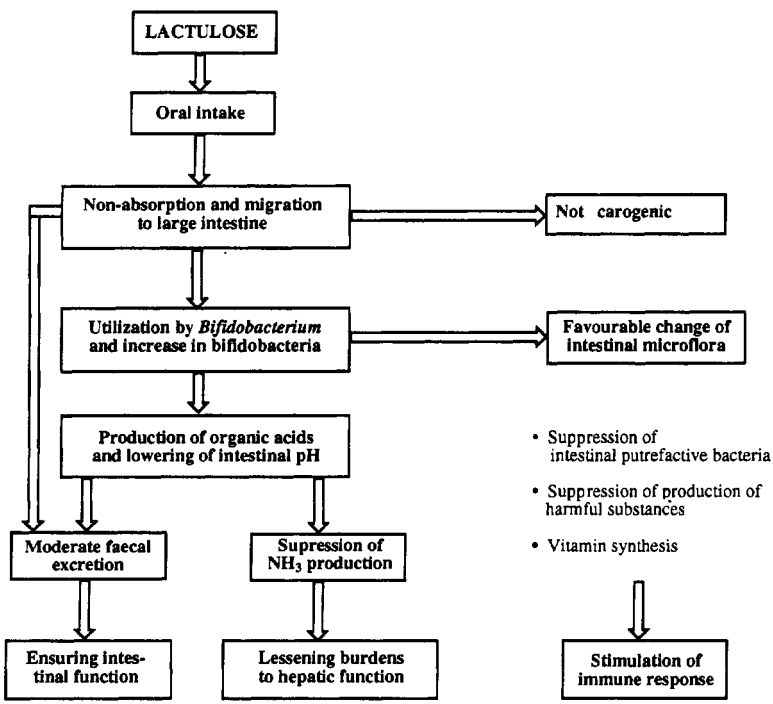


Figure 2.20 Significance of lactulose in health (modified from Tamura *et al.*, 1993).

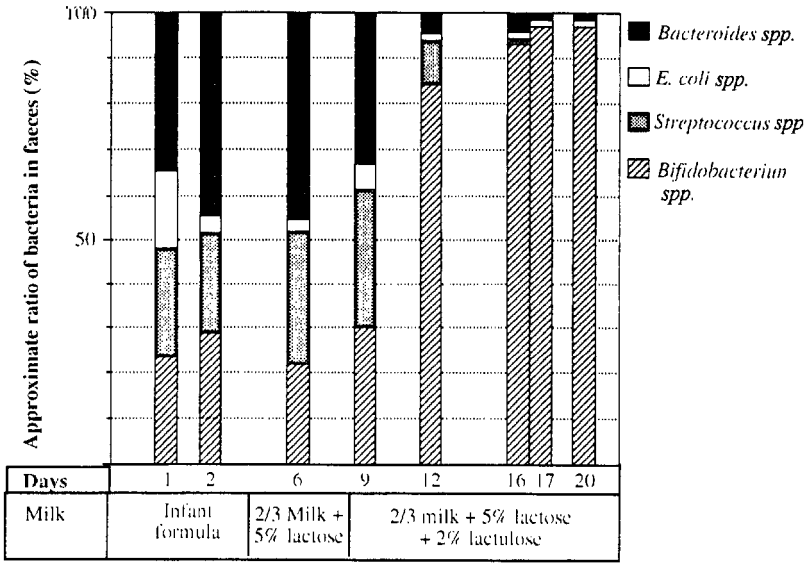


Figure 2.21 Effect of lactulose on the intestinal microflora of 2-month-old infants (modified from Tamura *et al.*, 1993).

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

Lactitol. Lactitol (4-O-β-D-galactopyranosyl-D-sorbitol), is a synthetic sugar alcohol produced on reduction of lactose, usually using Raney nickel. 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, blood and liver cholesterol levels and to be anticariogenic. 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. sweets.

It can be esterified with one or more fatty acids (Figure 2.24) to yield a family of food emulsifiers, analogous to the sorbitans produced from sorbitol.

Lactobionic acid. This derivative is produced by oxidation of the free carbonyl group of lactose (Figure 2.25), chemically (Pt, Pd or Bi), electrolytically, enzymatically or by fermentation. 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 prior to transplants.

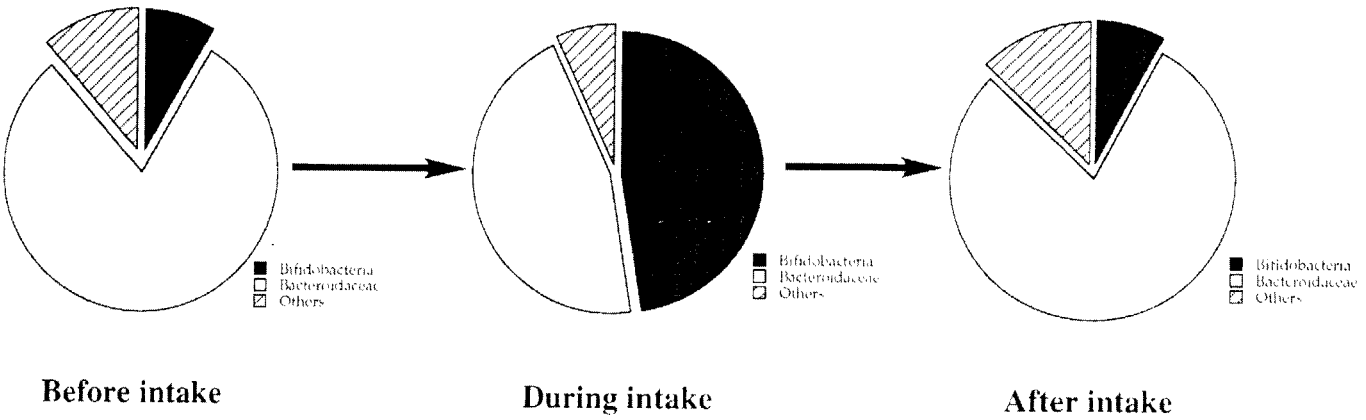


Figure 2.22 Increase in *Bifidobacterium* spp. by administration of lactulose to healthy adults (modified from Tamura *et al.*, 1993).

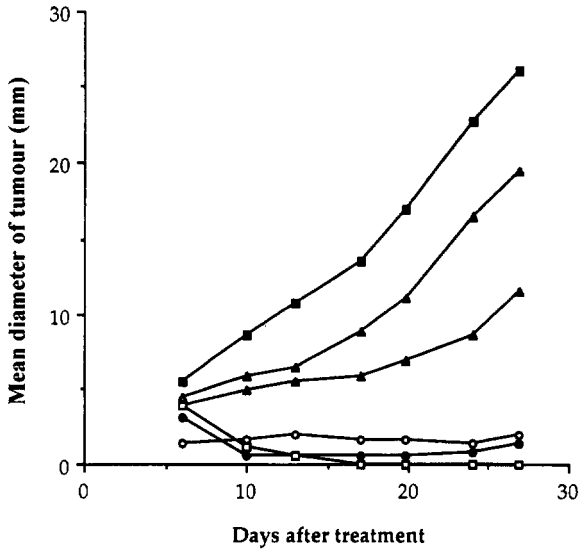
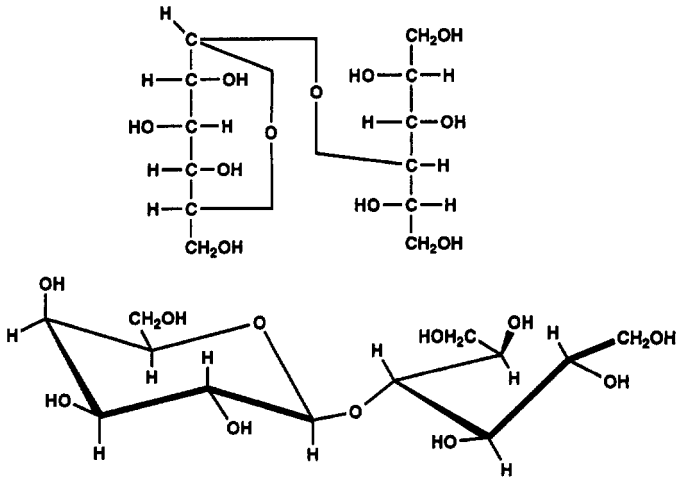


Figure 2.23 Effect of different doses of whole peptidoglycan (WPG) from *Bifidobacterium infantis* on the growth rate of Meth A tumour. Mice were inoculated subcutaneously with a mixture of 10^5 Meth A cells and 0 (■), 10 (△), 20 (▲), 25 (○), 50 (●) or 100 (□) μg of WPG. (Modified from Tamura *et al.*, 1993.)

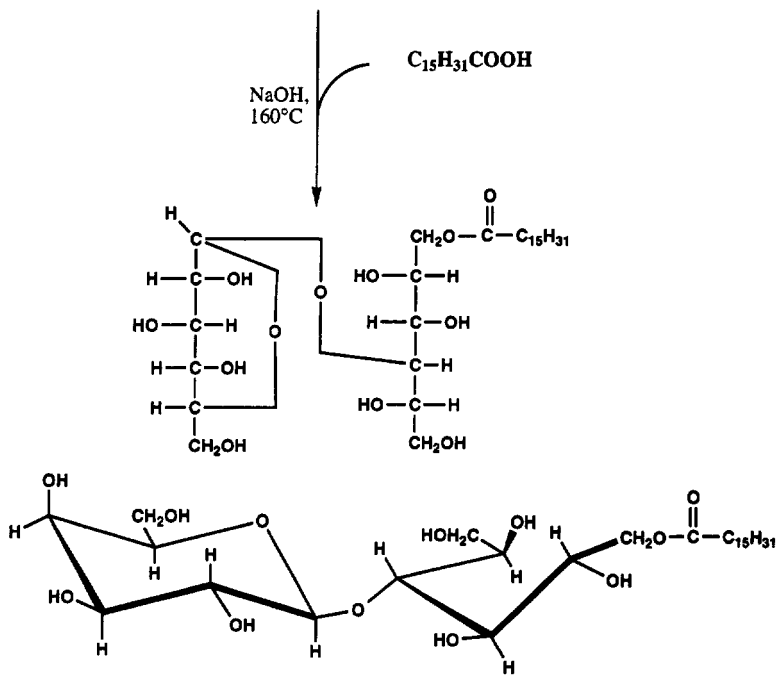
Lactosyl urea. Urea can serve as a cheap source of nitrogen for cattle but its use is limited because NH_3 is released too quickly, leading to toxic levels of NH_3 in the blood. Reaction of urea with lactose yields lactosyl urea (Figure 2.26), from which NH_3 is released more slowly.

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* spp., to ethanol (Figure 2.27). Lactic acid may be used as a food acidulant, as a component 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 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.



Lactitol, 4-O-β-D-galactopyranosyl-D-sorbitol



Lactitol monoester

Figure 2.24 Structure of lactitol and its conversion to lactyl palmitate.

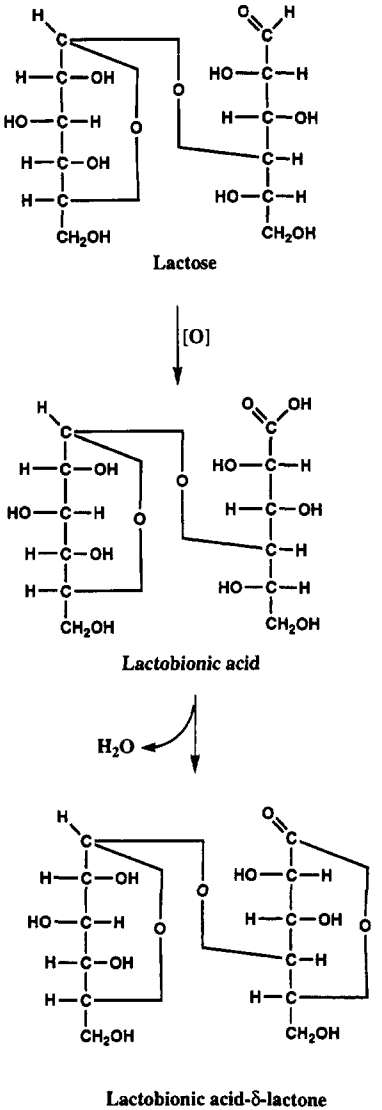


Figure 2.25 Structure of lactobionic acid and its δ-lactone.

Lactose can also be used as a substrate for *Xanthomonas campestris* in the production of xanthan gum (Figure 2.28) which has several food and industrial applications.

All the fermentation-based modifications of lactose are probably not really economical because lactose is not cost-competitive with alternative

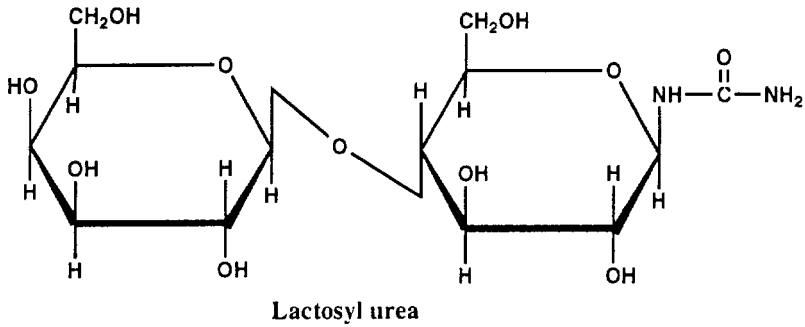


Figure 2.26 Structure of lactosyl urea.

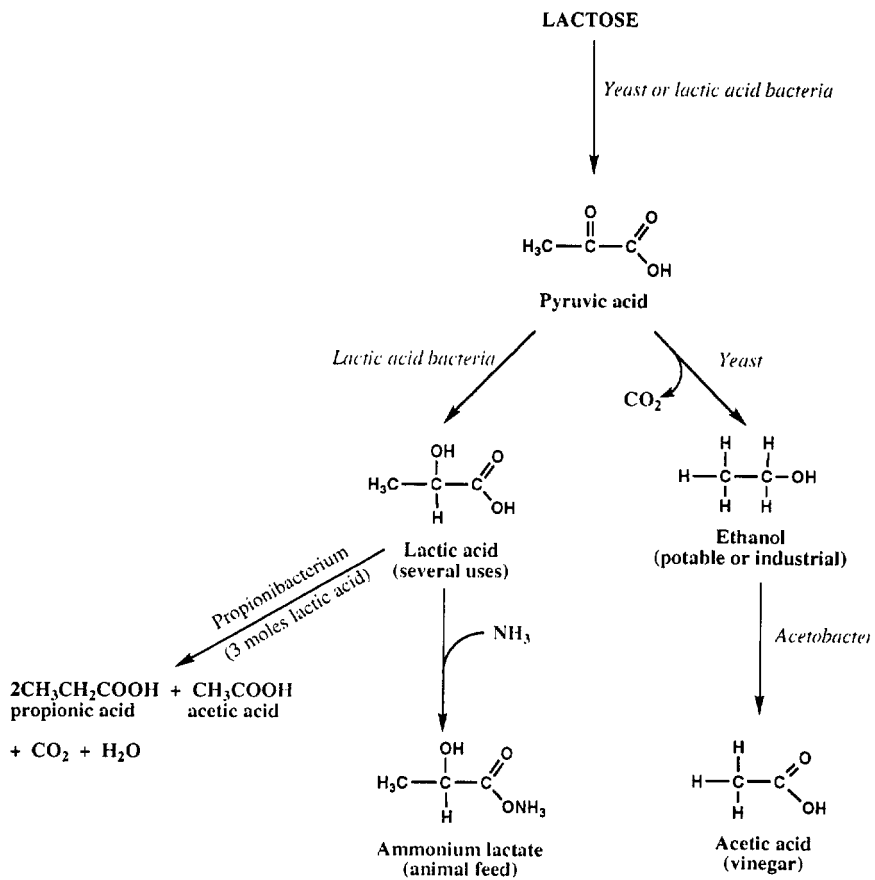


Figure 2.27 Fermentation products from lactose.

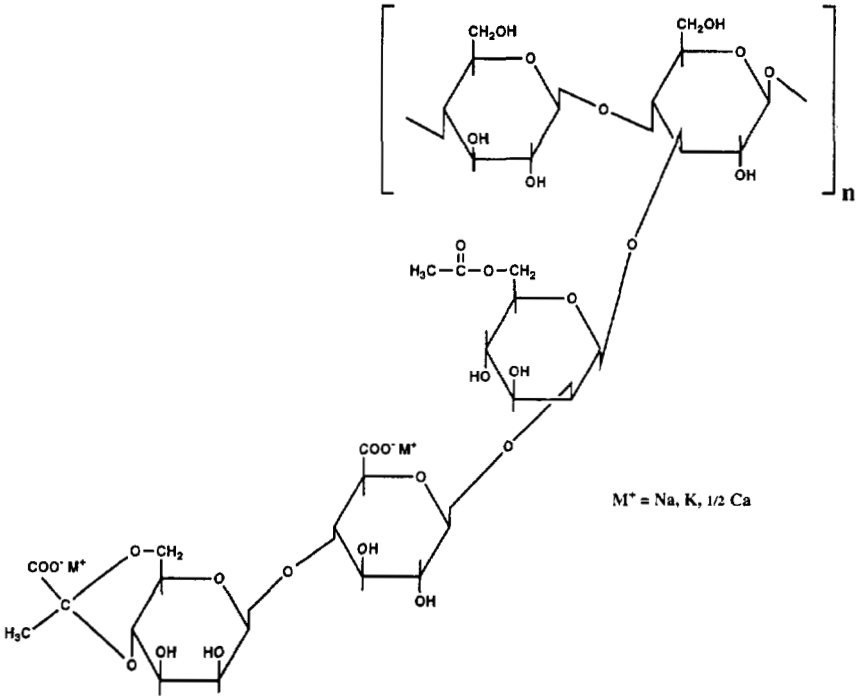


Figure 2.28 Repeating unit of xanthan gum.

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. 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) (Figure 2.29). The glycosamine may undergo an Amadori rearrangement to form a 1-amino-2-keto sugar (Amadori compound) (Figure 2.30). The reaction is base-catalysed and is probably first order. 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

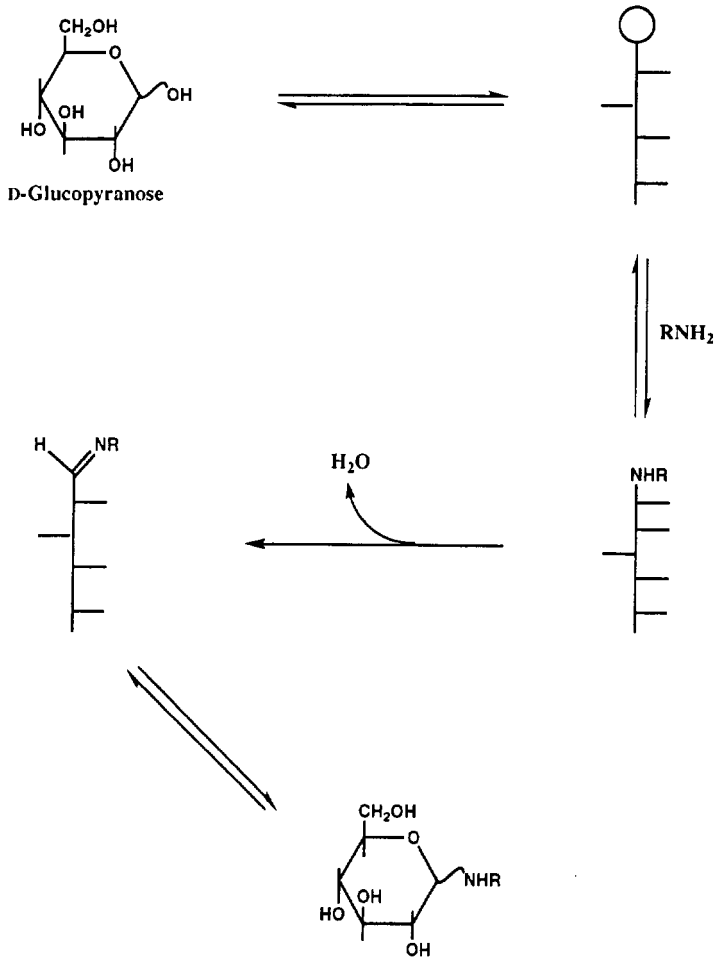


Figure 2.29 Formation of glycosylamine, the initial step in Maillard browning.

prevent or retard age-gelation in UHT milk products). Maillard reaction products (MRPs) have antioxidant properties; the production of MRPs may be a small-volume outlet for lactose.

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 (Figure 2.31). Many of the intermediates are (off-) flavoured. The dicarbonyls can react with amino acids via the Strecker degradation pathway (Figure 2.32) to yield another family of highly flavoured compounds.

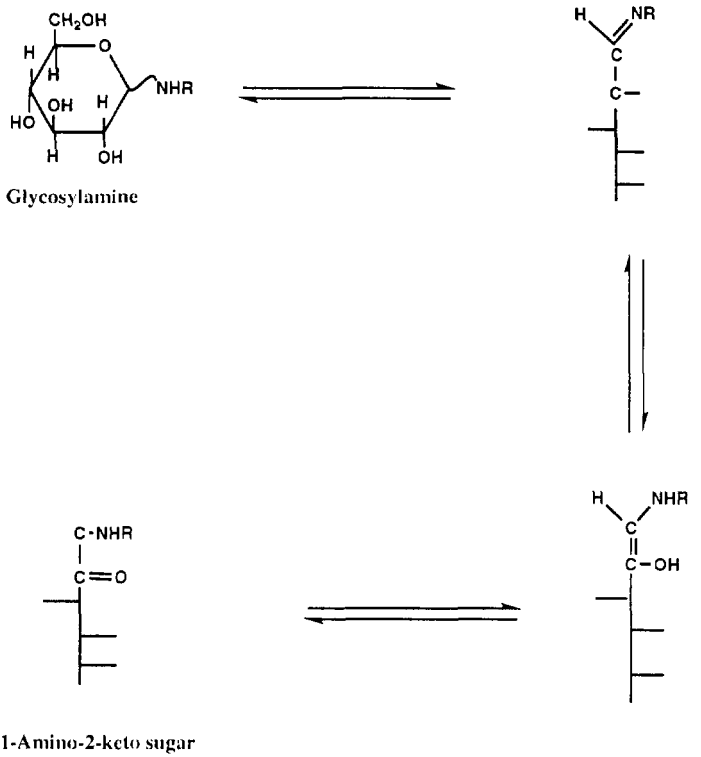


Figure 2.30 Amadori rearrangement of a glycosylamine.

2.6 Nutritional aspects of lactose

Since the milks of most mammals contain 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 a limited capacity to synthesize galactose.

Lactose appears to promote the absorption of calcium but this is probably due to a nonspecific 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 galactosaemia. Lactose intolerance is caused by an insufficiency of intestinal β-galactosidase – lactose is not completely

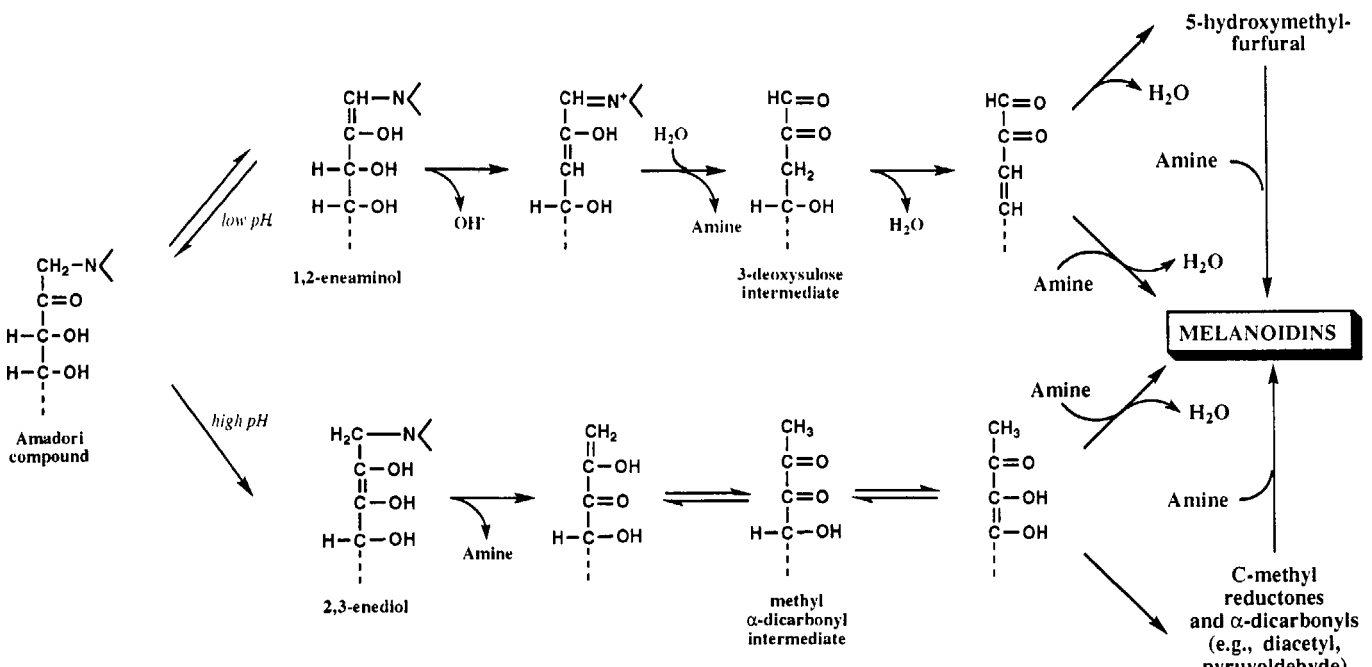


Figure 2.31 Pathways for the Maillard browning reaction.

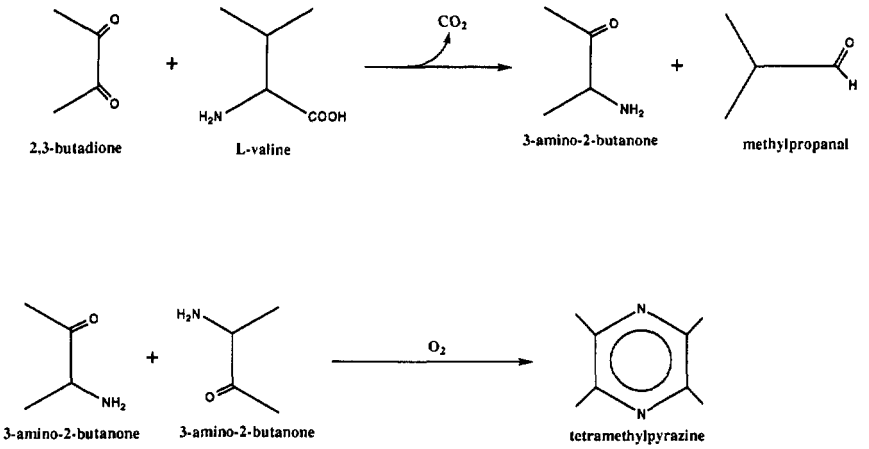


Figure 2.32 Strecker degradation of L-valine by reaction with 2,3-butadione.

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 micro-organisms, 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 β-galactosidase increases to a maximum at parturition (Figure 2.33), although total activity continues to increase for some time postpartum 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).

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 levels or pulmonary hydrogen levels. A test dose of 50 g lactose in water (equivalent to 1 litre of milk) is normally administered to a fasting patient; the dose is rather excessive and gastric

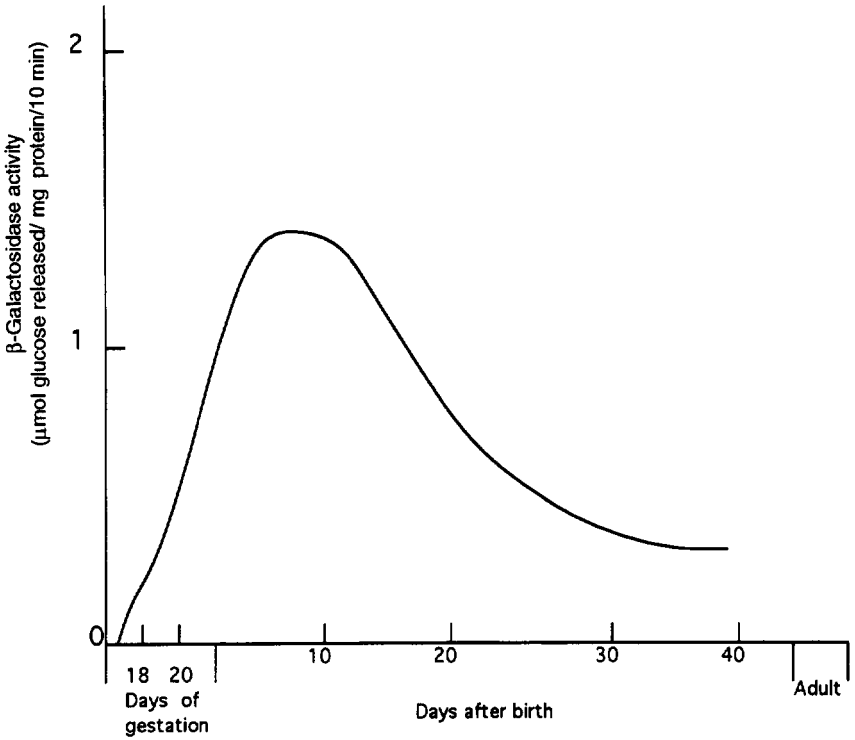


Figure 2.33 β-Galactosidase activity in homogenates from the intestine of the developing rat.

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 β-galactosidase (Figure 2.34). Pulmonary H_2 increases in lactose-intolerant subjects because lactose is metabolized 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:

- ultrafiltration, which also removes valuable minerals and vitamins, and is therefore not recommended;
- fermentation to yoghurt or other fermented products in which *c.* 25% of the lactose is metabolized, and which contains bacterial β-galactosidase and is also discharged more slowly from the stomach due to its texture;
- conversion to cheese, which is essentially free of lactose;

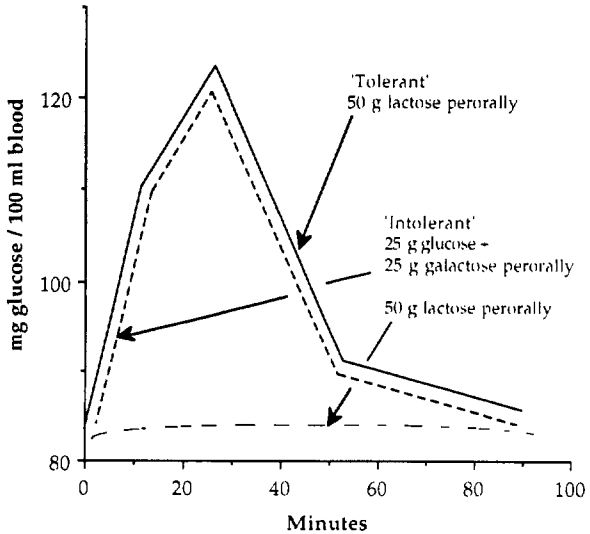


Figure 2.34 Examples of the 'lactose intolerance' test.

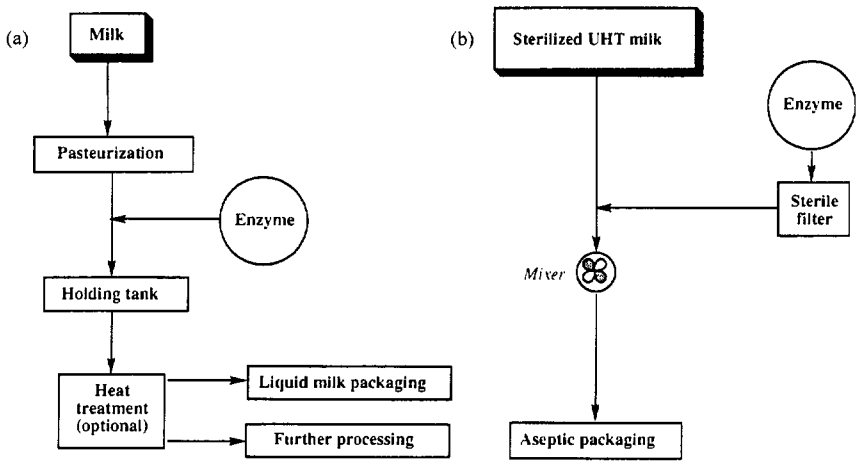


Figure 2.35 (a) Scheme for manufacture of low-lactose milk using a 'high' level of soluble β-galactosidase. (b) Scheme for the manufacture of low-lactose milk by addition of a low level of soluble β-galactosidase to UHT-sterilized milk. (Redrawn from Mahoney, 1997.)

- 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 (Figure 2.35).

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.

2.6.2 *Galactosaemia*

This is caused by the inability to metabolize galactose due to a hereditary deficiency of galactokinase or galactose-1-phosphate (Gal-1-P) : uridyl transferase (Figure 2.36). Lack of the former enzyme leads to the accumulation of galactose which is metabolized 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

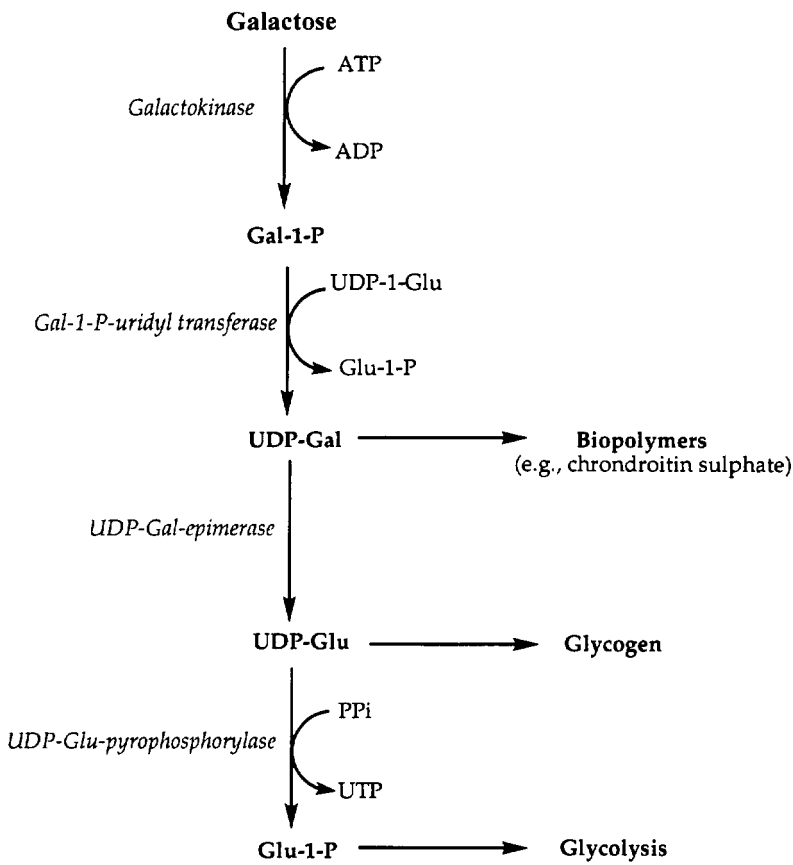


Figure 2.36 Pathways for the metabolism of galactose.

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 galactosaemia', is about 1 in 60 000.

The ability to metabolize galactose decreases on ageing (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 utilize lactose at the end of childhood.

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^{-1} 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 subscript).

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

where a is the measured optical rotation; l, the light path in dm; and c, the concentration as g ml^{-1}. It is usually expressed as:

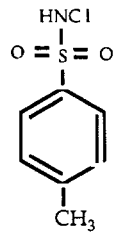
$$[\alpha]_D^{20} = 100\,a/lc$$

where c is in g per100 ml.

The milk sample must first be defatted and deproteinated, 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.

2.7.2 *Oxidation and reduction titration*

Lactose is a reducing sugar, i.e. it is capable of reducing appropriate oxidizing 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 Figure 2.37.

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 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 oxidizing agent.

The reactions involved are as follows:

$$CH_3C_6H_4SO_2NClH + H_2O + KI \text{ (excess)}$$

$$\rightleftarrows CH_3H_6H_4SO_2NH_2 + HCl + KIO \text{ (K hypoiodate)}$$

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

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

The I_2 is titrated with standard $Na_2S_4O_6$ (sodium thiosulphate):

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

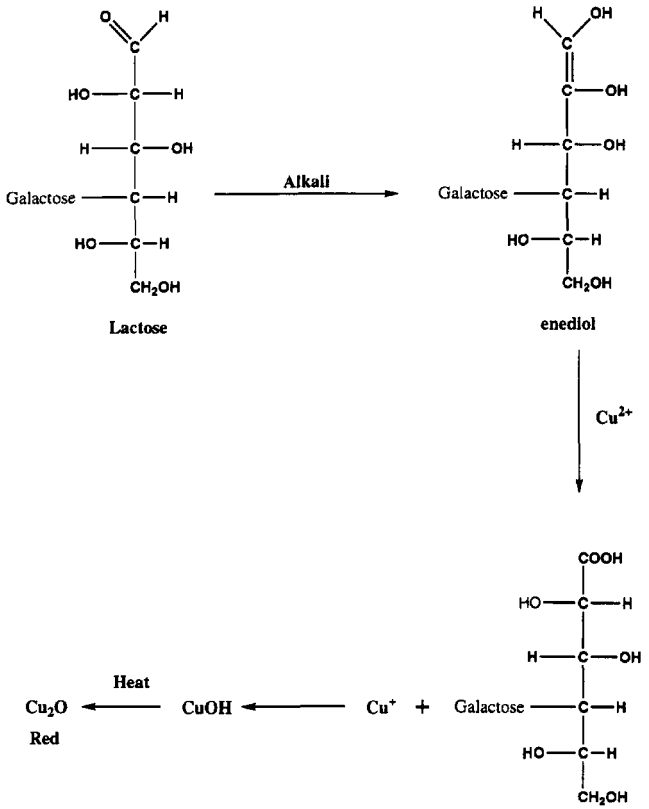


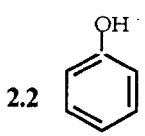
Figure 2.37 Oxidation of lactose by alkaline copper sulphate (Fehling's reagent).

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

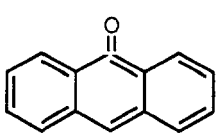
The sample is deproteinized and defatted using phosphotungstic acid.

2.7.3 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.



2.2 Phenol



2.3 Anthrone

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.

2.7.4 Chromatographic methods

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

2.7.5 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 using:

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)

$$\text{D-Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-P} + \text{ADP}$$

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

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.

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