Review of literature

CHAPTER 2

REVIEW OF LITERATURE

Lactose is a disaccharide present in milk and milk products. Certain individuals are unable to digest lactose, leading to a condition called lactose intolerance (LI). It is characterised by gastrointestinal symptoms such as abdominal pain, vomiting, diarrhoea, borborygmi etc.

Milk is a highly nutritious drink; rich in protein, calcium and other nutrients. Devoid of milk in the diets of LI subjects can lead to nutritional deficiencies. In view of this, the present study was conducted with the primary objective to determine the presence of lactose intolerance in different age groups using Hydrogen Breath Analyser test (HBT) and also detect the effect of supplementation of lactose hydrolysed milk on dietary intake, nutritional status, quality of life and its acceptability.

This chapter highlights the available literature for the study under the following heads.

2.1 Lactose intolerance and its prevalence, symptoms, pathophysiology, types and its genetic predisposition.

- 2.1.1 Global and national prevalence of Lactose intolerance
- 2.1.2 Symptoms of Lactose intolerance
- 2.1.3 Pathophysiology of Lactose intolerance
- 2.1.4 Types of Lactose intolerance
- 2.1.5 Genetic predisposition of Lactose intolerance

2.2 Diagnosis of lactose intolerance.

- 2.2.1 Lactose tolerance test
- 2.2.2 Breath analyser test
- 2.2.3 Biopsy
- 2.2.4 Genetic analysis

2.1 Lactose intolerance and its prevalence, symptoms, pathophysiology, types and its genetic predisposition.

2.1.1 Global and national prevalence of Lactose intolerance

Various ethnic groups have varying degrees of lactose intolerance. In adults, white northern Europeans, North Americans, and Australasians have the lowest rates, ranging from 5% in the British population to 17% in Finland and northern France. Lactase non-persistence affects more than half of the population in South America, Africa, and Asia, and it is nearly 100% in some Asian countries (Silanikove N et al 2015; Lomer MCE et al 2007; Solomon NW et al 2002; Swallow DM et al 2003; Olds LC and Sibley E 2003).

Table 2.1 depicts (Storhaug CL et al 2017) various countries' lactose intolerance prevalence. This table demonstrates the variation in data worldwide. European countries such as Denmark and Ireland have prevalence rates as low as 4% lactose intolerance, whereas countries like the Czech Republic have 81% lactose intolerance. Asian countries have comparatively higher percent of prevalence than European countries. Asian countries such as Pakistan and India have 58% and 61% of lactose intolerance respectively whereas Yemen and South Korea have 100% of lactose intolerance. African countries such as Niger showed 13% lactose intolerance, whereas Malawi showed 100% lactose intolerance. Lactose intolerance was found to be 44% in Australia. North American countries showed 42%, whereas southern American countries showed 62.5% lactose intolerance.

At times, the decline in lactase synthesis occurs in childhood, whereas in others it might happen during adolescence. Ethnicity, physiological factors, diseases, and injury are some of the factors determining it.

Kanth N in the year 2019 reported in their study- 31% prevalence of lactose intolerance among 220 school-going children aged 5 to 15 years in Pakistan. The majority of lactose intolerant people (36.4%) were over the age of 11 years. The proportions of tolerant and intolerant children in the normal height group were 73% and 27%, respectively. The stunted group had 42% lactose intolerant and 58% lactose tolerant children. Twenty five percent of children were underweight, among whom 37% were lactose intolerant and 63% lactose tolerant. It is possible that lactose intolerance might be associated with malnutrition.

In a study from Indonesia, 98 subjects aged over 60 years tested for lactose intolerance using HBT revealed prevalence of lactose intolerance amounting to 66%, 54%, and 73% in the total population, dairy users and non-dairy users, respectively (Dewiasty E et al 2021). Figure 2.1 illustrates the global prevalence of lactose intolerance.

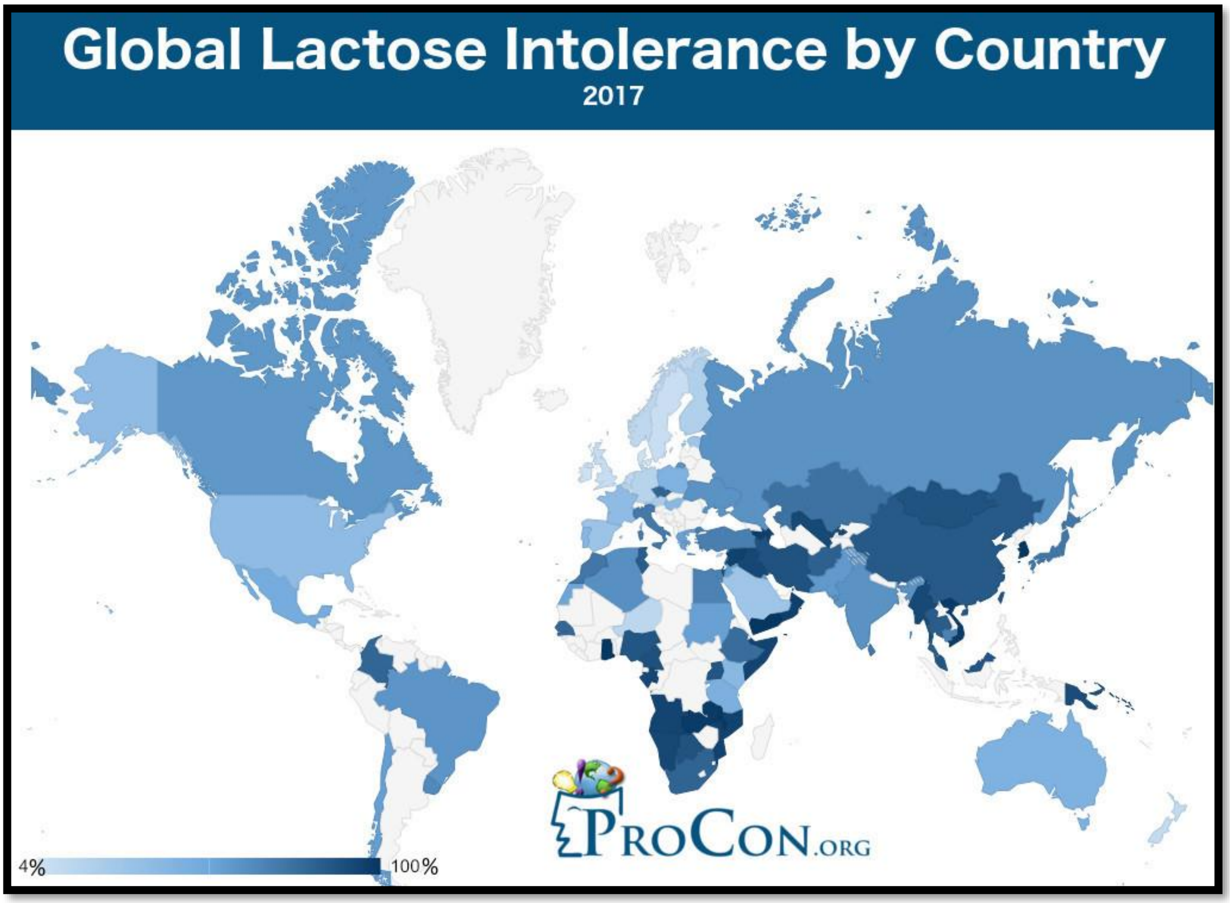

Figure 2.1- Global prevalence of lactose intolerance

Table 2.1- Prevalence of lactose intolerance in various countries

Country	Prevalence	Country	Prevalence
Afghanistan	82%	India	61%
Algeria	62%	Iran	88%
Angola	94%	Iraq	93%
Armenia	98%	Ireland	4%
Australia	44%	Israel	89%
Austria	22%	Italy	72%
Azerbaijan	96%	Japan	73%
Belgium	15%	Jordan	56%
Botswana	88%	Kazakhstan	75%
Brazil s. America	60%	Kenya	39%
Cambodia	68%	Kuwait	56%
Cameroon	89%	Lebanon	78%
Canada	59%	Malawi	100%
Chile	56%	Malaysia	87%
China	85%	Mexico	48%
Colombia	80%	Mongolia	88%
Cyprus	16%	Morocco	73%
Czech Republic	81%	Mozambique	95%
Democratic Republic of the Congo	95%	Myanmar	92%
Denmark	4%	Namibia	93%
Egypt	68%	Netherlands	12%
Estonia	28%	New Zealand	10%
Ethiopia	77%	Niger	13%
Finland	19%	Nigeria	87%
France	36%	Norway	12%
Gabon	93%	Oman	96%
Germany	16%	Pakistan	58%
Ghana	100%	Papua New Guinea	91%
Greece	55%	Poland	43%

Hungary	39%	Portugal	40%
Republic of Congo	93%	Russia	61%
Rwanda	49%	Taiwan	88%
Saudi Arabia	28%	Tanzania	45%
Senegal	79%	Thailand	84%
Solomon Islands	99%	Tunisia	84%
Somalia	94%	Turkey	69%
South	81%	Uganda	87%
South Korea	100%	Ukraine	61%
Spain	29%	United Kingdom	8%
Sri Lanka	73%	United States	36%
Sudan	55%	Uruguay	65%
Sweden	7%	Uzbekistan	92%
Syria	95%	Vietnam	98%
Western Sahara	53%	Yemen	100%
Zambia	98%		

2.1.2 Symptoms of lactose intolerance

The undigested lactose leads to increased excretion of electrolytes and fluids. Lactose intolerance is clinically characterised by abdominal pain and bloating. Fermentation of unabsorbed lactose by gut microflora leads to the production of short chain fatty acids (SCFA) and gases such as hydrogen, methane, etc. Lactose intolerance causes diarrhoea, borborygmi, vomiting, and, at times, lethargy and headaches (Matthews SB et al 2005; Lomer MCE et al 2007; Rienzo T D et al 2013; Szilagyi A et al 2018).

2.1.3 Pathophysiology

2.1.3.a Lactose source and synthesis

In mammalian milk, the main source of energy (carbohydrate) is lactose. Lactose is a disaccharide which consists of 2 monosaccharides: glucose and galactose (Figure 2). Lactase-phlorizin hydrolase, more commonly known as lactase, is the enzyme responsible for hydrolysing lactose into glucose and galactose (Adam A C et al 2005).

Human milk contains approximately 70 g/L of lactose, and in exclusively breast-fed infants, lactose constitutes about 40% of the daily energy consumption. For comparison, bovine milk contains approximately 46 g/L of lactose (Schaafsma G et al 2008; Wijesinha-Bettoni R et al 2013; Grenov B et al 2016; Harvey L et al 2018; Szilagyi A et al 2018).

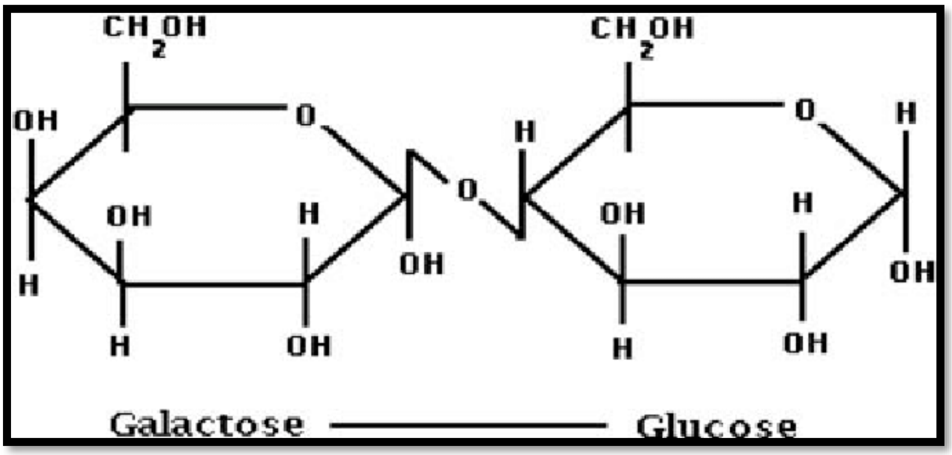

Figure 2.2 – Structure of glucose and galactose

2.1.3.b Metabolism

Lactase is present in the brush borders of the small intestine, with the maximum of its expression being in the mid-jejunum region. Initially, this enzyme is produced as a 220 KDa precursor polypeptide, which goes through significant changes after post-transcriptional modification and becomes a matured protein of 150 kDa. After hydrolysis of lactose, glucose is utilised as a source of energy, whereas galactose becomes a component of glycolipids or glycoproteins (Rienzo TD et al 2013) (figure 2.2, 2.3).

If the lactase phlorizin hydrolase enzyme is absent (lactasia) or deficient (hypolactasia), unabsorbed lactose molecules osmotically attract fluid into the bowel lumen, leading to an increased volume and fluidity of the intestinal content. In addition, the unabsorbed lactose passes into the colon, where it is fermented by bacteria, producing short-chain fatty acids and gases (CO2, CH4, and H2), possibly leading to various gastrointestinal symptoms (Sharikadze O 2022; Szilagyi A et al 2018 Bayless TM et al 2017). Figure 2.4 demonstrates fermentation of lactose by colonic microbiota (modified based on Reily KJ and Rombeau JL, 1993).

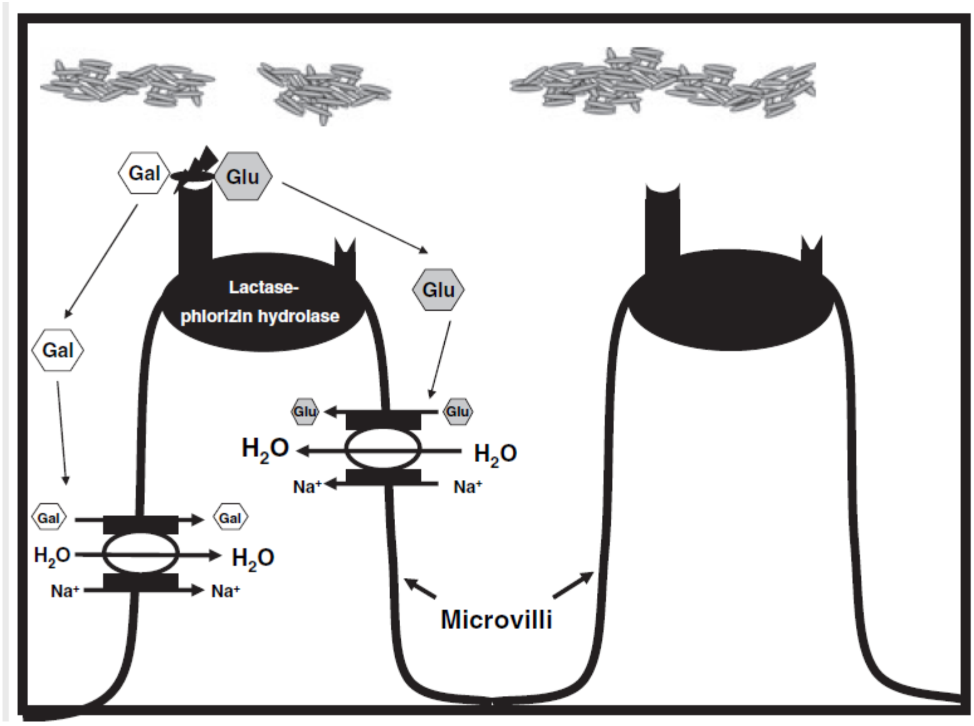

Figure 2.3 – Intestinal absorption of lactose intolerance
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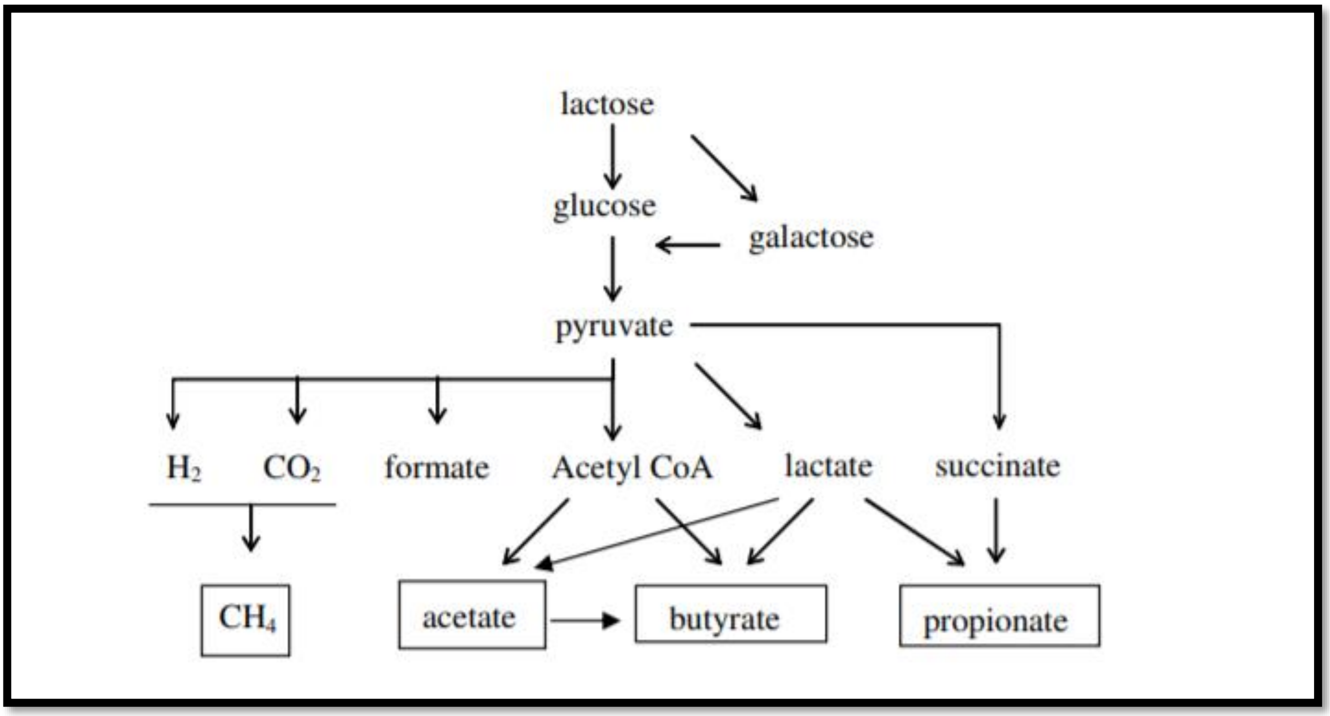

Figure 2.4- Breakdown of lactose

2.1.4 Types of lactose intolerance

Lactose intolerance can be classified into **primary lactose intolerance, secondary lactose intolerance, developmental lactose intolerance, and congenital lactose intolerance** (Szilagyi A et al 2018; Rusynyk RA and Still CD, 2001).

2.1.4.1 Primary LI is the most common cause of lactase deficiency. It is also known adult-type hypolactasia. It is genetic, irreversible and usually develops during childhood.

2.1.4.2 Secondary LI is caused due to any existing intestinal injury or surgery that might lead to low lactase synthesis and lactose malabsorption. This can occur at any age. Disease or toxins which affects the proximal small intestine can lead to loss of intestinal surface which will lead to lactase deficiency. After recovery the surface can improve, if there's no genetic predisposition then symptoms also improve. Example of such diseases are- coeliac disease, rota virus in children, upper gastrointestinal surgery etc (Harvey L et al 2018; Matthews SB et al 2005).

2.1.4.3 Developmental lactose intolerance might occur in infants born prematurely- this usually last for a short period of time after a child is born.

2.1.4.4 Congenital lactose intolerance is extremely rare, genetic disorder (genes inherited from parents causes disorder). This condition is most common in Finland, where it affects an estimated 1 in 60,000 new borns. It is associated with diarrhoea, acidosis and hypercalcemia.

2.1.5 Genetic predisposition

Continued production of lactase throughout adult life (lactase persistence, LP) is a genetically determined trait and is found at moderate to high frequencies in Europeans and some African and Middle Eastern populations.

There is a close correlation between lactase persistence and two polymorphisms, C/T13910 and G/A22018, upstream from the lactase gene1 8 9, CC/GG being associated with lactase non-persistence and lactose intolerance. The loss of the lactase enzyme differs from one ethnic group to another. Chinese and Japanese people are depleted of 80%–90% lactase enzyme within three to four years after weaning, whereas Asians and Jews can retain some 20%–30% of lactase enzyme, taking several years to reach the lowest level. Studies have also reported that heterozygotes can still have severe lactose intolerance. Studies suggest that C ⁄ T13 910 is the dominant polymorphism with the C allele linked to a decline in lactase mRNA expression. However, the exact mechanism of this decline after weaning is uncertain (Lomer MCE et al 2007; Anguita-Ruiz A et al 2020; Catanzaro R et al 2021).

2.2 Diagnosis of lactose intolerance

There is various technique of detecting lactose intolerance such as Lactose tolerance test, breath analyser test, biopsy and genetic analysis (Robles L and Preifer R, 2020). Table 2.2 summarises the various technique to diagnose lactose intolerance.

2.2.1 Lactose tolerance test

One of the earliest methods of determining lactose intolerance is the lactose tolerance test. In this test, a standard dose of lactose is given to the patients. It is the measurement of blood glucose levels following the ingestion of 50 gm of lactose. If the blood glucose increases significantly after 30 min, it means an incidence of lactose intolerance. Lactose intolerance is determined when there is a maximal plasma glucose increase of 1.1 mmol/L or less. However, diabetes or other pre-existing diseases that may influence post-prandial blood glucose level (Robles L and Priefer R, 2020; Rienzo TD et al 2013; Szilagyi A et al 2018) could have an impact on this report.

2.2.2 Breath analyser test

The second method of determining lactose intolerance is by using a breath analyser (Robles L and Priefer R, 2020). A H2 breath test is considered golden standard for the diagnosis, as it is safe, cost-effective, and non-invasive investigative tool with a high sensitivity (70-100%) and high specificity (69-100%) (Di Camillo M et al 2006) (Romagnuolo J et al 2002). In this test, a standard dose of 25 gm is administered orally to subjects after an overnight fast. After oral consumption of lactose, the individual is required to exhale out into the mouth piece of the breath analyser six times in a period of 30 minutes each. If the value of hydrogen emitted is greater than 20 ppm, then lactose intolerance is detected (Rienzo TD et al 2013; Szilagyi A et al 2018; Pretto FM et al 2002). Geyter CD et al 2021 mentioned in their study a majority of hydrogen producers showed symptoms, whereas this was only the case in half of the H2-negative CH4 producers. These results indicate that CH4 measurements may possibly be of additional value for the diagnosis of LI, since 5.7% of patients were negative for H2 and positive for CH4, and half of them experienced symptoms during the breath test. Thereby indicating methane analyser is more specific in detecting lactose intolerance than hydrogen breath analyser (Beyerlein L et al 2008).

2.2.3 Biopsy

A biopsy means a medical test in which a sample of cells is extracted and studied for the presence of disease. This test is based on a colorimetric reaction that develops when the endoscopic biopsy from the post-bulbar duodenum is incubated with lactose on a test plate. The colour reaction develops within 20 min after hydrolysis of lactose in patients with normolactasia (positive result), while no reaction develops in patients with severe hypolactasia (negative result) (Szilagyi A et al 2018; Rienzo TD et al 2013).

2.2.4 Genetic analysis

The genetic analysis identified a single nucleotide polymorphism that shows complete association with the lactase non-persistence/persistence39 and is characterized by a C to T change in the position 13910 of the lactase phlorizin hydrolase (LPH) gene. In particular, it has been shown that the genotype C/C -13910 is associated with adult type hypo lactasia, whereas genotypes C/T -13910 and T/T -13910 are associated with lactase persistence (Rienzo TD et al 2013). A tabular representation of various methods to detect lactose intolerance is listed in table 3 (Misselwitz B et al 2013).

Table 2.2- Summary of tests for lactose intolerance

	H_2-Breath Test (Scarpellini, E et al 2009; Corazza, GR et al 1994)	Lactose tolerance test (Arola H et al 1994)	Genetic test (Enattah, N.S et al 2002; Tishkoff, S.A et al 2007)	Lactase activity at jejunal brush border (Newcomer, AD et al 1975; Maiuri, L et al 1991)
Test principle	Increase of H2 in respiratory air after lactose challenge.	Increase of blood sugar after lactose challenge.	Genetic-13910C/T polymorphism	Enzymatic activity of lactase enzyme in biopsy sample
Cut off	>20 ppm within 3 h	<1.1 mmol/l within 3 h	C:C13910 Lactase non persistence	<17-20 IU/g
Availability	Good	Excellent	Variable	Rare
False positive (incorrect diagnosis)	Rapid gi-transit, small-intestinal bacterial overgrowth	Rapid GI-transit, impaired glucose tolerance	Rare (<5%) in Caucasians	Probably rare
False negatives malabsorption wrongly excluded	Non H2 producers, full colonic adaptation	Fluctuations in blood sugar	All cases of secondary lactose malabsorption	Patchy enzyme expression
Secondary causes	Cannot be excluded, kinetic of H2 increase can be suggestive	Cannot be excluded	Cannot be excluded	Can be excluded (histopathology at same procedure)
Symptom assessment	Possible	Possible	Not possible	Not possible
Comment	Method of choice for assessment of lactose intolerance	Rarely performed due to inferior sensitivity and specificity	Definitive in Caucasians, less in other populations. Not suitable in secondary lactase deficiency.	Reference standard for detection of lactase deficiency (primary or secondary)
Cost	Low	Lowest	High	Highest

2.3 Treatment of lactose intolerance

There are various modes of treatment for combating lactose intolerance. The first approach is **Avoidance of dairy products.** The second method is **Enzyme replacement therapy** and third is **Inclusion of probiotic in diet** and **fourth is introduction of lactose hydrolysed milk in diet.**

2.3.1 Avoidance of dairy products

The usual approach to treating lactose intolerance is to avoid dairy products from the diet. Restriction of lactose intake is recommended because in blinded studies patients with self-reported lactose intolerance, even those with IBS, can ingest at least 12 g lactose without experiencing symptoms (Szilagyi A et al 2018; Shaukat A et al 2010; Savaiano DA et al 2006). However, in IBS patients, lactose intolerance tends to be part of a wider intolerance to poorly absorbed, fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) (Szilagyi A et al 2018; Yang JF et al 2015; Bohn L et al 2013).

Restriction of milk and milk products leads to a deficiency of nutrients such as calcium, phosphorus, vitamins etc. Sawsan MA Abuhamdah et al 2013 stated that symptoms disappear with the avoidance of lactose in the diet. The most common piece of advice that physicians give patients with LI is to avoid dairy foods (Savaiano DA et al 2013).

2.3.2 Enzyme replacement therapy

An external enzyme (β-galactosidase) is introduced into the diet of lactose intolerant patients in this type of treatment. Lactase produced largely from fungi or yeasts can be used prior to or added to dairy meals to aid in digestion of lactose. Enzymes come as gels, liquids, capsules or tablets. (Szilagyi A et al 2018). In a more recent study two lactase tablets containing 7500 IU were used to test outcome for 25 g lactose in water (lactose equivalent to 500 mL milk). The results were variable in 96 participants all of whom were genetically lactase deficient. About 22% became negative, in about 18% there was a significant reduction in tested breath hydrogen but in the rest, there was no significant difference from baseline (Ibba I et al 2014). Combinations of lactase enzyme with freeze dried yogurt was reported to improve variability of hydrogen and symptoms. In a randomized controlled trial in 24 patients freeze dried yogurt combined with lactase, hydrolysis of lactose was more complete than with lactase alone, however, only 12.5 g of lactose was tried (de Vrese M et al 2015). In their study, (Lomer MCE et al 2007) recommended avoiding lactose in the diet until symptoms subside, that typically takes 4 weeks. Exogen lactase is derived from *Aspergillus oryzae* or *Kluyveromyces lactis* and is capable of breaking lactose into glucose and galactose for improved absorption. Rosada et al 1984 found that adding 1 gm of β -galactosidase produced from K. lactis to 360 ml of cow's milk at the time of intake removes considerable imperfect sugar absorption in 60–77% of malabsorbers. According to (Barrilas C et al 1987), when 27 of 48 children who could not adequately digest whole milk, where subjected to whole milk along with lactase derived from k. lactis, then this enzyme was 82% effective.

Exogenous β -galactosidase is helpful and has no side effects when used in lactose malabsorbers, even when taken at lunch. More recently, probiotics containing β -galactosidase activity have been found to be effective in the treatment of lactose intolerance.

2.3.3 Inclusion of probiotic in diet

Probiotics are live bacteria or yeast that supplements the gastrointestinal flora. Studies have shown that probiotics exhibit various health beneficial properties such as improvement of intestinal health, enhancement of the immune responses, and reduction of serum cholesterol (Leis R et al 2020; Oak J Sophia and Jha R, 2018).

Probiotic strains with high hydrolytic capacity can be used to lower the amount of lactose in a product, such as yoghurt. It can also be administered to boost the small intestine's overall hydrolytic capability (HS Kim et al 1983). However, following 7 days of milk intake supplemented with Lactobacillus acidophilus (Vonk RJ et al 2012) discovered a decrease in hydrogen generation, but not all patients had alleviation from their symptoms. Although 5 days of Bifidobacterium breve did not help lactose intolerance symptoms, it did result in a reduction in breath hydrogen (Park MR et al 1999; Vonk RJ et al 2012).

Overall, these inconsistencies have prevented probiotics from being widely accepted as an effective treatment for lactose intolerance (Levri KM et al 2005; Shaukat A et al 2010; Wilt TJ et al 2010; Vonk RJ et al 2012). Saltzman JR et al 1999 found that 7 days of Lactobacillus acidophilus supplementation had no effect on hydrogen generation or symptoms.

2.3.4 Introduction of lactose hydrolysed milk in diet

Significant improvement in absorption with 90% lactose hydrolysed milk is seen in low lactase subjects. Lactose hydrolysed milk may serve as an important supplement for lactose intolerant subjects (DM Paige et al 2015; Reasnor J et al 1981). Lactose hydrolysed milk are beneficial for lactose intolerant subjects as they are easily digestible and do not produce any gastrointestinal symptoms. These are also healthy for lactose intolerant subjects. In lactose hydrolysed milk lactose is in its hydrolysed from- glucose and galactose. Thus, making it sweeter compared to standard dairy milk, thus addition of sugar to milk is also decreased by 10-15g/kg of the dairy products (Saji R and Balakrishnan S 2021; McCain HR et al 2018). Currently the lactose free milk market is growingly rapidly among the dairy industries. Lactose-free dairy is expected to reach a €9 billion turnover by 2022 and continues to outgrow overall dairy (7.3% vs. 2.3%) (data extracted from a Euromonitor analysis (Euromonitor Database). The lactose-free sector is generating sales growth in the dairy industry, as mentioned by the high increase in new product releases between 2012 and 2016 [Based on an analysis of the Mintel database (Mintel Global New Product Database).

2.4. Lactose intolerance and nutritional status

A healthy diet helps to protect against malnutrition in all its forms, as well as NCDs. Healthy dietary practices start early in life- breastfeeding fosters healthy growth and improves cognitive development, and may have longer term health benefits such as reducing the risk of becoming overweight or obese and develop diseases later in life. Adequate intake of protein is one of the key nutritional factors to maintain independence, predominantly by preventing loss of muscle mass and strength (sarcopenia), frailty and associated comorbidities in later life (Wolfe RR 2012; Bauer J et al 2013; Moore LL et al 2017; Lonnie M et al 2018).

Calcium is a mineral involved in a large number of vital functions. Calcium intake is usually associated with the intake of dairy products such as milk, yogurt and cheese, as they are rich sources of calcium. Calcium-rich foods are dairy products, especially hard cheese that can provide 1 g of calcium per 100 g, whereas milk and yogurt can provide between 100 mg to 180 mg per 100 g. Cereals usually have around 30 mg per 100 g, however if they are fortified, the amount can reach 180 mg per 100 g. Nuts and seeds are also rich in calcium, especially almonds, sesame and chia that can provide between 250 to 600 mg per 100 g. Vegetables rich in calcium are kale, broccoli and watercress, which provide between 100 and 150 mg per 100 g (US Department of Agriculture, Agricultural Research Service, 2019). However, the impact that these foods have on total calcium intake depends on the population food consumption patterns.

Whereas dairy products represent around 14% of total dietary energy intake in developed countries, they represent only around 4% of total energy intake in developing countries. In this way, some

Asian countries have higher proportion of total calcium intake from non-animal foods such as vegetables, legumes and grains than from dairy products, though they also have a much lower calcium intake (Silanikove N 2015).

In the United States and in Holland, 72 and 58% of calcium supply come from dairy products, respectively, whereas in China, only around 7% of total calcium intake comes from dairy products, while most comes from vegetables (30.2%) and legumes (16.7%) (Huang F, et al 2018; Willemse, J.P.M.M et al 2019). Fortified foods such as cereals and juices can additionally become important sources of calcium (Cormick G and Belizan JM 2019).

In most low- and middle-income countries, the daily calcium intake is well below recommendations; however, low intakes are also observed in special age groups such as adolescents of high-income countries. a recent systematic review of diets in 195 countries based on nationally or sub nationally representative nutrition surveys jointly with many dietary data sources of the Global Health Data Exchange for nationally or sub nationally representative nutrition surveys provides information about age-standardised intake of dietary factors among adults aged 25 years or older at the global and regional level in 2017. These data show an average global calcium daily intake around 400 mg/day. Lower values were seen in Sub-Sharan African countries and in Southeast Asia, with figures around 200 mg/day. In high-income-countries (HICs), calcium daily intake was around 600–800 mg/day (Afshin A et al 2019).

Calcium has various benefits for body. Blood pressure is regulated by intracellular calcium in vascular smooth muscle cells, through vasoconstriction and variations of the vascular volume (Yim HE and Yoo KH 2008). An adequate dietary calcium intake has been associated not only with the prevention of hypertensive disorders of pregnancy and blood pressure reduction but also with low-density lipoprotein (LDL) cholesterol levels and prevention of osteoporosis and colorectal adenomas (Heaney RP 2006). The main complication of lactose intolerance is nutrient deficiency. Milk and milk products form an important part of a healthy diet. These foods provide vitamins A, B12 and D as well as milk proteins and calcium.

2.5. Lactose intolerance and Quality of life

Physical, mental, and social health, degree of independence, social contact with the environment, and other elements all influence quality of life. A person's contentment with his or her life dimensions in comparison to his or her ideal life can be defined as quality of life. The term "health-related quality of life" (HRQOL) refers to a multi-dimensional concept that examines the impact of one's health on one's quality of life (WHO). As previously noted, one of the most common treatments for lactose intolerance is to avoid milk and milk products, which has a negative impact on quality of life owing to food restrictions.

In their study, Cassellas F et al 2016 found that self-perception of intolerance was linked to worse HRQOL scores. Physical life quality scores declined as diarrhoea complaints grew, and physical and environmental life quality reduced as abdominal pain increased, according to a study in Turkey involving individuals aged 18 to 60. Due to gastrointestinal discomfort, lactose intolerant people restrict their consumption of milk and milk products. As a result, it's critical to educate lactose intolerant people on the consequences of lactose intolerance, because the discomfort induced by intolerance can reduce nutrient intake and diminish quality of life (Kutuk MO et al 2021).

2.6. Dairy milk-its benefits, production in India and dairy milk alternatives

2.6.1 Milk and its benefits

Milk is a whitish liquid comprising milk proteins, lipids, lactose, and a variety of vitamins and minerals that is produced by the mammary glands of all adult female mammals after childbirth and used to feed their young.

The nutritional value of dairy milk is particularly high due to the balance of the nutrients that compose it. Its rich in energy, protein and a range of micronutrients such as calcium, magnesium, zinc and phosphorus (Bailey RL et al 2010; Black RE et al 2002; Bechthold, A et al 2019; Rizzoli R 2014; Burgess K. 2014). The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet.

Lactose is the main carbohydrate of milk. It is formed by the union of one molecule of D-galactose (engaged by its semi-acetyl function) and one molecule of D-glucose (committed by its hydroxyl 4 position). All of the essential amino acids that our body cannot generate are found in milk protein. The four primary caseins present in milk are s1 caseins, s2, B, and k caseins. Other milk proteins are present in the whey serum, and whey proteins are defined as soluble proteins in the whey after precipitation of caseins at pH 4.6 and at 20°C. Fat is the primary source of energy in milk. Milk contains fat in the form of an emulsion of fat cells; the fat content of milk is concentrated in small cells suspended in water, which varies greatly depending on race and feed composition. (Junes, R et al 1988).

2.6.2 Benefits of milk for children

In 2009, a total of 630 middle and high school students, aged 15-16 years, were surveyed. Students with higher milk and milk product consumption showed significantly higher scores (Kim SH et al 2016). In American male and female students aged 11-13 years, intake of more than 600 ml of milk/day was shown to be related to significantly higher math scores compared to intake of less than 600 ml, and students showed higher reading scores. However, students that consumed over 360 ml of sweetened beverages/day showed significantly lower math and reading scores compared to those who consumed less than 360 ml, suggesting that milk rather than sweetened beverage consumption can positively affect math and reading scores (Edwards JU et al 2011).

Johansson I, 2002; Petti S et al 1997; Petridou E et al 1996 mentioned in their studies that milk intake has been negatively correlated with the development of dental caries in children and adolescents. (Grenby TH et al 2001; Johansson I 2002) stated removal of casein, fat or lactose from the milk does not affect its protective capacity on demineralization. Free calcium and phosphate have some effect, but after removal of all these components milk still contains powerful protective factors identified as water-soluble proteose-peptones, amounting to 1 g l-1 milk.

Children on milk-free diets had significantly lower intake of energy, fat, protein, calcium, riboflavin and niacin. Use of milk substitutes improved the nutritional content of the cow's milk-free diets; however, the recommendations for riboflavin and calcium were still not met (Henriksen C et al 2000).

Children who avoided milk had low dietary calcium intakes and low bone mineral density values. All of the fractures occurred before puberty, the majority (18 of 22) being associated with only slight trauma. Forearm fractures were especially common (12 fractures). Significantly more of the children who avoided milk reported fractures (16 observed vs 6 expected, chi (2) =31.0, P<.001, df=5). They also experienced more total fractures than the birth cohort population (22 observed vs 8 expected, chi (2) =33.6, P<.001, df=5). We conclude that young children avoiding milk are prone to fracture (Goulding A et al 2004).

Herber C et al 2020 mentioned in their study the final sample observations for wasting, underweight and stunting include 668.463, 693.376, and 673.177 of children aged 6 to 59 months, respectively. There results suggested that milk consumption is associated with a reduced probability of being underweight of 1.4 percentage points (95% confidence interval −0.02, −0.01) and a reduced probability of being stunted of 1.9 percentage points (95% confidence interval −0.02, −0.01). The association is stronger for children from wealthier households, which might indicate that milk consumption is important for better overall nutrition or socio-economic status.

2.6.3 Benefits of milk for adults

Pereira MA et al 2002 and Tremblay A 2009 mentioned in their study that milk is inversely associated with abnormal homeostasis, dyslipidemia, insulin resistance syndrome in young adults. Fumeron F et al 2011; Wang, Y et al 2008 and Azadbakht L et al 2005 stated in their study that milk is associated with decrease in metabolic syndrome.

Dose-response analyses indicated that an increment of 200 ml of milk intake per day was associated with a lower risk of cardiovascular disease, stroke, hypertension, colorectal cancer, metabolic syndrome, obesity and osteoporosis. De Goede J et al 2016 mentioned in their study a decrease in risk of stroke by 7% (Guo Y et al 2021). A decrease in risk of CVD by 6% and decrease in risk of hypertension by 4% was observed by Soedamah-Muthu SS et al 2011 and Soedamah-Muthu SS et al 2012 respectively. An increment of milk by 200gm/day lead to 13% and 16% lower risk of metabolic syndrome and obesity respectively as mentioned by Malmir H et al 2020 correspondingly. Major amount of observations from various observational, cross-sectional and prospective studies showed a negative relation between milk and milk products and body weight and central obesity (Dougkas A et al 2011; Guo Y et al 2021).

Hypertension is one of the major risk factors for CVD and for stroke in particular. There is now good evidence that the proteins in milk and milk-derived products, particularly whey proteins, have beneficial hypotensive effects. This has been shown in vitro (Giromini C et al 2020; Guo Y et al 2021) and in vivo.

Increase in milk intake was also associated with a decrease in risk of Alzheimer’s disease (0.63; 0.44-0.90) as stated by (Lee J et al 2018; Guo Y et al 2021) in their study.

2.6.4 Benefits of milk for elderly

Sarcopenia is a disorder that is characterised by a gradual loss of muscular mass and strength as one gets older (Cruz-Jentoft et al 2019). As a result, it is a condition that is particularly important among the geriatric population (though not exclusively), with an increasing prevalence mainly associated with the increasing age of populations worldwide. Sarcopenia can have far-reaching consequences because, for example, it reduces bone protection, thus increasing the risk of breakage in a fall and leading to reduced mobility, disability, and lower quality of life.

Shafiee G et al 2017 conducted a meta-analysis on the prevalence of sarcopenia worldwide based on general population research, encompassing 58,404 typically healthy adults >60 years of age. This study showed a mean prevalence of 10% in both men and women, with a higher value in non-Asian than Asian populations. Protein consumption and resistance exercise are both recognised to offer an anabolic stimulus for skeletal muscle protein synthesis, but the amounts and timing of protein consumption and exercise required to increase muscle protein synthesis in the elderly have been a source of controversy.

Dairy products contain significant amounts of protein as well as a variety of minerals and vitamins that are important for a healthy ageing (Buchowski M S et al 2002). In their systematic study, Federico CT et al 2019 underlined the benefit of dairy products in avoiding sarcopenia. Older persons who consume dairy products may have a lower risk of sarcopenia.

2.6.5 Milk Production in India

In fiscal year 2020, milk production was at 198 million metric tonnes, up from 187 million metric tonnes the previous year. The country's milk production increased by 5.6 percent over the previous year (Published by Statista Research Department, Oct 29, 2021).

2.6.5.a. Overview of bovine breeding sector in India

The livestock sector has emerged as a vital sector for ensuring a more inclusive and sustainable agriculture system. Evidence from the National Sample Survey Office’s (NSSO) 70th round survey showed that more than one-fifth (23 per cent) of agricultural households with very small parcels of land (less than 0.01 hectare) reported livestock as their principal source of income. Farming households with some cattle head are better able to withstand distress due to extreme weather conditions.

Growing population, changing lifestyles, expanding urbanization and accelerated climate changes are creating new challenges in Bovine breeding systems. In the past, the challenge was to ample feed, but now it is to provide essential nutrients to promote health especially reproductive health; and in the future, the challenge would be to provide optimal nutrients based on an animal's genetic profile and productivity. Fortunately, along with challenges, the developments in science are creating new avenues for tackling the challenges.

Further, biodiversity of livestock, which is so crucial for sustaining long-term productivity, is also under jeopardy. The genetically uniform systems are vulnerable to external shocks under extreme weather conditions, emerging diseases and pathogens. In livestock sector, due to continued focus on exotic germplasm based cross breeding, the number of indigenous breeds with better adaptability, disease-resistance and feed efficiency ratio is declining. The situation is made worse by unregulated blood levels in the crossbred progeny, in attempts to increase milk yield indiscriminately. Hence it is the need of the hour to conserve and improve the productivity of Indian indigenous breeds. For accomplishing this task, the department is now therefore focusing on 100 percent Artificial Insemination coverage along with the application of advanced cutting-edge reproductive technology developments.

In this context, India is blessed with a huge biodiversity of 43 indigenous cattle breeds and 13 Buffalo breeds which have survived over last hundreds of years in respect of their suitability for specific purposes in the concerned local environment. The Department's strategy is thus to enhance the average productivity of milk of select breeds from the overall available breed types (e.g. Gir for high milk productivity) from the present level of 4.85 kg per day to 6.77 kg per day per indigenous animal.

As per 19th Livestock census, there are 88 million In-Milk animals whose records are unavailable on an annual basis even. Records of those in breeding stage, their productivity, treatment and vaccination are also not properly maintained by State Animal Husbandry Departments. This is because the system for maintaining records on the above aspects has not yet evolved in complete shape due to lack of prioritisation. Impediments like lack of animal identification and traceability, inability to meet sanitary and phyto sanitary conditions also need to be addressed in this connection.

In this context, an initiative has been taken namely, "E- PashuHaat", the e-market portal for bovine germplasm which provides real time data on availability of high-quality germplasm along with identification and traceability of germplasm sold through e-market, connecting breeders, State agencies and stake holders.

The sex- sorted semen technology will be standardized for indigenous breeds like Sahiwal, Hariana, Red Sindhi, Rathi and Gir during initial phases in the near future.

Further, an initiative has been taken up for establishment of a National Bovine Genomics Centre for Indigenous Breeds (NBGC-IB). The NBGC-IB will pave way for systematic and fast pace improvement of the precious indigenous animal resources using highly precise gene-based technology. All these steps promise to give a long-term sustainable solution to both livelihood and security of about 70 million farming community of India as well as provide nutritional security to the country (DAHD, GoI).

2.6.5.b Dairy development in India

India ranks first among the world's milk producing Nations since 1998 and has the largest bovine population in the World. Milk production in India during the period 1950-51 to 2017-18, has increased from 17 million tonnes to 176.4 million tonnes as compared to 165.4 million tonnes during 2016-17 recording a growth of 6.65 %. FAO reported 1.46% increase in world milk Production from 800.2 million tonnes in 2016 to 811.9(estim) million tonnes in 2017. The per capita availability of milk in the country which was 130 gram per day during 1950-51 has increased to 374 gram per day in 2017-18 as against the world estimated average consumption of 294 grams per day during 2017. This represents sustained growth in the availability of milk and milk products for our growing population.

Dairying has become an important secondary source of income for millions of rural families and has assumed the most important role in providing employment and income generating opportunities particularly for marginal and women farmers. Most of the milk is produced by animals reared by small, marginal farmers and landless labours. Of the total milk production in India, about 48 % milk is either consumed at the producer level or sold to non-producers in the rural area. The balance 52 % of the milk is marketable surplus available for sale to consumers in urban areas. Out of marketable surplus it is estimated that about 40 % of the milk sold is handled by the organized sector (i.e., 20% each by C-operative & Private Dairies) and the remaining 60 % by the unorganized sector.

About 16.6 million farmers have been brought under the ambit of about 1,85,903 village level Dairy Corporative Societies (DCS) up to March 2018. Despite the slump in world market and better procurement prices by dairy cooperatives along with decrease in procurement volume by major private players led to increase in milk collection by the dairy cooperatives by about 11%. The dairy cooperatives have procured daily average of milk about 475.6 Lakh Kg per day (LKgPD) during 2017-18 as compared to 428.7 lakh kg procured during 2016-17. The sale of liquid milk reached to

349.6 Lakh Litre per day (LLPD) during 2017-18 recording a growth of 6% as compared to 331 LLPD marketed during 2016-17. Women members of the DCS are also being encouraged to assume leadership roles. As on 31.03.2018, the total number of women in dairy cooperatives across the country was 4.9 million in 32,092 women DCS which is 29.5% of total farmers.

Government of India is making efforts for strengthening infrastructure for production of quality milk, procurement, processing and marketing of milk and milk products through following Dairy Development Schemes:

- National Programme for Dairy Development (NPDD)
- National Dairy Plan (Phase-I)
- Dairy Entrepreneurship Development Scheme (DEDS)
- Support to Dairy Cooperatives

Dairy Processing and Infrastructure Development Fund (DIDF) (DAHD, GoI)

2.6.5.c. Milk production

Milk production has increased tremendously in India in past twenty years. White revolution was a dairy movement started in the year 1970 with the intend to make India a self-independent nation in milk production. Dr Verghese Kurien, is known as the 'Father of White Revolution in India' for his contribution to milk production. Table 2.3 illustrates Milk production and per capita availability of milk in in India.

In India, Uttar Pradesh is the highest milk producing state in India contributing around 18% to the total milk production followed by Rajasthan, Andhra Pradesh, Gujarat and Punjab contributing 11%, 10%, 8% and 7% respectively. Figure 2.5 and table 2.4 depicts milk production of various states in India.

Table 2.3- Milk production and per capita availability of milk in in India

Milk production and per capita availability of milk in India		
Year	**Production (million tonnes)**	**Per capita availability (gms/day)**
1991-92	55.6	178
1992-93	58.0	182
1993-94	60.6	186
1994-95	63.8	192
1995-96	66.2	195
1996-97	69.1	200
1997-98	72.1	205
1998-99	75.4	210
1999-2000	78.3	214
2000-01	80.6	217
2001-02	84.4	222
2002-03	86.2	224
2003-04	88.1	225
2004-05	92.5	233
2005-06	97.1	241
2006-07	102.6	251
2007-08	107.9	260
2008-09	112.2	266

2009-10	**116.4**	**273**
2010-11	121.8	281
2011-12	127.9	290
2012-13	132.4	299
2013-14	137.7	307
2014-15	146.3	322
2015-16	155.5	337
2016-17	165.4	355
2017-18	176.3	375
2018-19	187.7	394
Source: Basic Animal Husbandry Statistics, DAHD&F, GoI		

Table 2.4- State wise estimates of milk production ('000 tonnes)

State	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19
All India	84,406	86,159	88,082	92,484	97,066	102,580	107,934	112,183	116,425	121,848	127,904	132,431	137,685	146,314	155,491	165,404	176,347	187,749
Andhra Pradesh	5,814	6,584	6,959	7,257	7,624	7,938	8,925	9,570	10,429	11,203	12,088	12,762	13,007	9,656	10,817	12,178	13,725	15,044
Arunachal Pradesh	42	46	46	48	48	49	32	24	26	28	22	23	43	46	50	53	54	55
Assam	682	705	727	739	747	750	752	753	756	790	796	800	815	829	843	861	872	882
Bihar	2,664	2,869	3,180	4,743	5,060	5,451	5,783	5,934	6,124	6,517	6,643	6,845	7,197	7,775	8,288	8,711	9,242	9,818
Chhattisgarh	795	804	812	831	839	849	866	908	956	1,029	1,119	1,164	1,209	1,232	1,277	1,374	1,469	1,567
Goa	45	46	48	57	56	57	58	59	59	60	60	61	68	67	54	51	55	57
Gujarat	5,862	6,089	6,421	6,745	6,960	7,533	7,911	8,386	8,844	9,321	9,817	10,315	11,112	11,691	12,262	12,784	13,569	14,493
Haryana	4,978	5,124	5,221	5,222	5,299	5,366	5,442	5,745	6,006	6,267	6,661	7,040	7,442	7,901	8,381	8,975	9,809	10,726
Himachal Pradesh	756	773	786	870	869	933	1,007	1,026	971	1,102	1,120	1,139	1,151	1,172	1,283	1,329	1,392	1,460
J & K	1,360	1,389	1,414	1,422	1,400	1,400	1,519	1,565	1,592	1,609	1,614	1,631	1,615	1,951	2,273	2,376	2,460	2,540
Jharkhand	940	952	954	1,330	1,335	1,401	1,442	1,466	1,463	1,555	1,745	1,679	1,700	1,734	1,812	1,894	2,016	2,183
Karnataka	4,797	4,539	3,857	3,917	4,022	4,124	4,244	4,538	4,822	5,114	5,447	5,718	5,997	6,121	6,344	6,562	7,137	7,901
Kerala	2,718	2,419	2,111	2,025	2,063	2,119	2,253	2,441	2,509	2,645	2,716	2,791	2,655	2,711	2,650	2,520	2,576	2,548
Madhya Pradesh	5,283	5,343	5,388	5,506	6,283	6,374	6,572	6,855	7,167	7,514	8,149	8,838	9,599	10,779	12,148	13,445	14,713	15,911
Maharashtra	6,094	6,238	6,379	6,567	6,769	6,978	7,210	7,455	7,679	8,044	8,469	8,734	9,089	9,542	10,153	10,402	11,102	11,655
Manipur	68	69	71	75	77	77	78	78	78	78	79	80	82	82	79	79	82	86
Meghalaya	66	68	69	71	73	74	77	77	78	79	80	81	82	83	84	84	85	87
Mizoram	14	15	15	16	15	16	17	17	11	11	14	14	15	20	22	24	25	26
Nagaland	57	58	63	69	74	67	45	53	78	76	78	79	81	76	77	79	74	73
Orissa	929	941	997	1,283	1,342	1,431	1,625	1,598	1,651	1,671	1,721	1,724	1,861	1,903	1,930	2,003	2,088	2,311
Punjab	7,932	8,173	8,391	8,554	8,909	9,168	9,282	9,387	9,389	9,423	9,551	9,724	10,011	10,351	10,774	11,282	11,855	12,599
Rajasthan	7,758	7,789	8,054	8,310	8,713	10,309	11,377	11,931	12,330	13,234	13,512	13,946	14,573	16,934	18,500	20,850	22,427	23,668

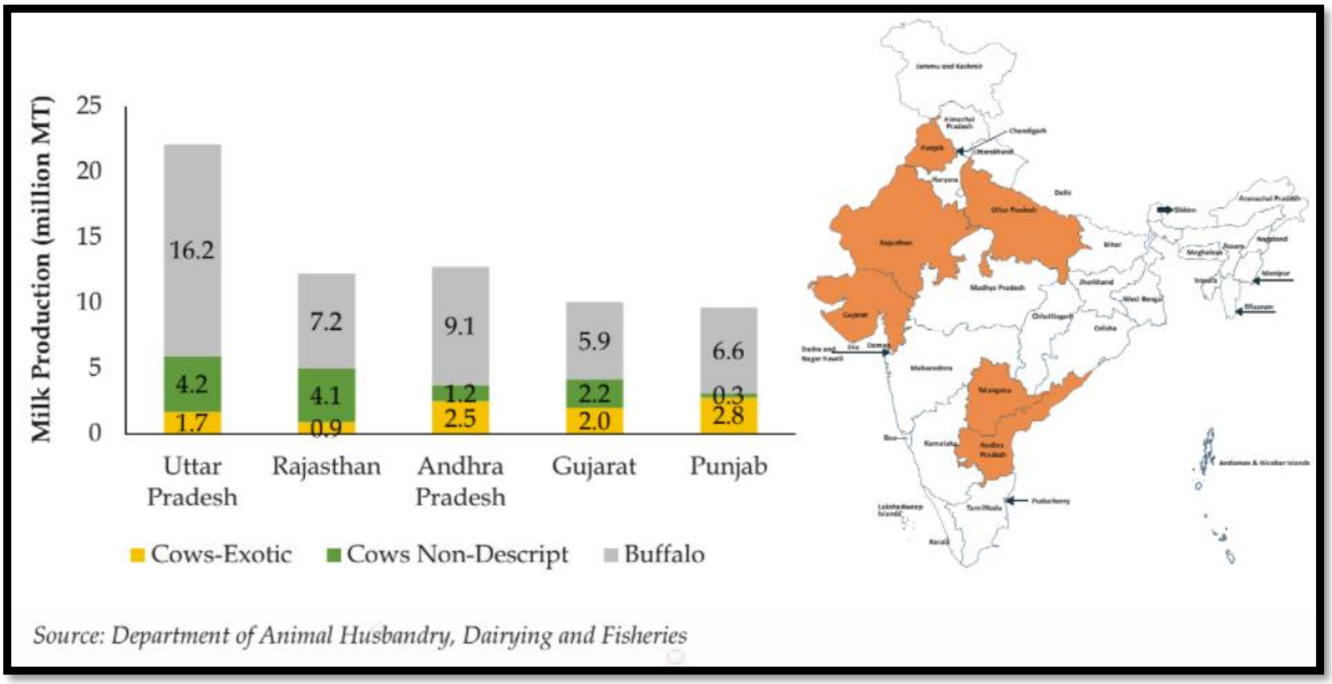

Figure 2.5-Milk production of various states in India

2.6.5.d. Dairy milk alternative

Current food market is flooded with plant-based milk which is attributed to various factors namely lactose intolerance, milk allergy, cultural reason or preference in diet choice such as veganism. Commonly available plant-based milk are soy milk, almond milk, oat milk etc (table 2.5).

The dietary energy of bovine milk varies based on the fat content of the milk. This value ranged from 34 kcal 100ml^{-1} for skim/fat-free milk to 61 kcal 100mL^{-1} for whole (3.25%) milk. Research by (Jeske S et al 2017) reported the energy content and GI of commercial plant-based milk alternatives were much greater than that of bovine milk. Thus, there would be little effect on total dietary energy consumption of consumers purchasing bovine milk of the same fat content across different brands of milk whereas purchasing different brands or types of plant-based milk alternatives is likely to impact the energy intake of the consumer as the energy content is less consistent in plant-based milk alternatives. It is also worth noting that a larger proportion of energy is made up of carbohydrates and sugars in milk alternatives, which relatively elevates the GI of these beverages. The link between GI with natural lactose versus refined sugars present in some plant-based milk alternatives is important to consider. A study by (Jeske S et al 2017) determined the GI of many plant-based milk alternatives and bovine milk, and found that most milk alternatives possess a higher GI than bovine milk; milk possessed a GI of 46.93 whereas milk alternatives ranged from 47.53 to 99.96. Bovine milk meets the criteria for a 236 low GI food and would be suitable for consumption in individuals aiming to keep blood sugar low, while it is possible that some plant-based milk alternatives may not meet criteria for low GI food, as previously reported by (Jeske S et al 2017). Many plant-based milk alternatives contained less carbohydrates than that of milk carbohydrates than that of milk (4.78 g 100 mL-1 for whole milk and 4.96 g 100 mL-1 241 for skim milk). The variation in plant-based alternative milk beverages can be attributed to differences in product formulations as well as different dilutions of the vegetable extract used.

Protein is essential as it provides the body with amino acids that act as an important structural component of every cell in the human body, functioning as enzymes, hormones, transport carriers, and more (National Academy of Sciences 2005). Deficiencies in protein intake can lead to a wide number of ailments including kwashiorkor, stunted growth, muscle wasting, week immunity, increased risk of cardiovascular disease, diabetes, cancer, osteoporosis, obesity and other health related challenges (Wu G 2016). The plant-based milk alternatives typically had a lower protein content than bovine milk, which had a range of 3.15 to 3.37 g 100 mL^{-1}. The plant-based milk alternatives with the highest overall protein content were soy-based, which had values that ranged from 2.50 to 3.16 g $100mL^{-1}$.
The coconut-based milk alternatives had protein contents of 0.59, 1.25, and 2.00 g 100 mL-1 263. The rice-based milk alternative had the lowest protein content of 0.28 g 100 mL-1, and the hemp-based milk alternative had a protein content of 0.83 g 100 mL^{-1}. There was significant variation in the protein contents of the plant-based milk alternatives, even when they utilised the same plant base.

Overall, the lower protein content in plant-based milk alternatives and the variability between products can potentially lessen the protein intake of consumers. The 1.98 g of fat 100 g-1 of 2% bovine milk is made up of 1.26 g of saturated fat, 0.56 g of monounsaturated fat and 0.07 g of polyunsaturated fat. Information was not available for the full breakdown of saturated and unsaturated fats for all the plant-based milk alternatives; however, the data available generally expressed a trend of having less saturated fats and more monounsaturated and polyunsaturated fats. The soy-based milk alternatives that had their fatty acid composition listed had higher levels of polyunsaturated fatty acids. Which was expected as most plant-based sources (including soybean oil) typically contain high levels of polyunsaturated fatty acids (Dornbos DL and Mullen RE 1992). The exceptions to the trend were the coconut-derived milk alternatives, which contained much higher proportions of saturated fat when compared with bovine milk and the other plant-based milk alternatives.

Even though consumed, nutritive value of plant-based milk is less than dairy milk.

Table 2.5- Plant categories that serve as basic foodstuff for plant drinks or milk substitutes

Categories	Species
Legumes	Cowpea, mug bean, peanut, soy bean
Nuts	Almond, Hazelnut
Oil seeds	Hemp, sesame
Oil plants of other families	Coconut
Cereals	Corn, kamut, millet, oat, rice, spelt, wheat
Pseudo cereals	Amarnath, buckwheat, quinoa
Sedges	Earth almond

2.7. Sensory evaluation of food products

When the quality of a food product is assessed by means of human sensory organs, the evaluation is said to be sensory or subjective or organoleptic. Sensory quality is a combination of different senses of perception combing into play in choosing and eating food. The effective characteristic is not the property of the food, but the subject 's reaction to the sensory qualities of foods. This reaction is highly conditioned by a variety of psychological and social factors and in the final analysis, plays a vital role in the acceptance and preference of foods (Srilakshmi B 2003).

2.7.1. Attributes of a food product

Various attributes of a food product are briefly described below:

2.7.1.1) Appearance: Appearance is the first characteristics perceived by the human senses and play an important role in the identification and final selection of food. This is the visual perception of food comprised of colour, shape, size, gloss, dullness and transparency. The appearances of a meal have shown impact on appetite stimulation or depression resulting in pleasure or total depression. The look of a food or beverage impacts craving ability and acceptance, before the product touches the lips. This is because we eat with our eyes before we ever smell or taste (Sharif MK et al 2017).

2.7.1.2) Flavour: It is sensory phenomenon which is used to denote the sensations of odour, taste and mouthfeel. Flavouring substances are aromatic compounds which are conceived by the combination of taste and odour and perceived by the mouth and nose. Odour improves the delight of eating e.g., aroma of freshly cooked rice and most of the baked products. Taste helps in identification, acceptance and appreciation of food.

2.7.1.3) Taste: It is perceived by the taste buds on the tongue. There are four types of taste perception: sweet, salty, sour and bitter. Sour and bitter are often confused. Lemon juice has a sour taste whereas coffee has a bitter taste. In case of mouthfeel, nerves present inside the mouth are enthused by chemical or thermal responses e.g. coldness of ice cream or the fiery impression of pepper (Sharif MK et al 2017).

2.7.1.4) Aroma: Aroma is the first cousin of taste. These are volatile compounds which are perceived by the odour receptors of olfactory tissues of the nasal cavity. Aromatic compounds are released during the mastication process. Smell appraises the aroma of food that is important in the gratitude of flavour. A pleasant smell makes food delicious. To provoke a sensation of smell, the stuff must be in a gassy state. Furthermore, aroma is valuable in perceiving fresh, rancid or intermittently poisonous food (Sharif MK et al 2017).

2.7.1.5) Texture: Texture is perceived by a combination of senses i.e., touch, mouthfeel, sight and hearing. It is one of the most imperative features of a food. If a customer bites a soggy biscuit or eats ice cream with sandy texture, it is improbable they will be back. Texture is prerequisite in the acceptance of numerous foodstuffs e.g., tenderness of meat and softness of bread. It also includes the consistency, thickness, fragility, chewiness and the size and shape of particles in food. Texture analyzer is helpful to guarantee the target texture from the laboratory to the user 's kitchen (Sharif MK et al 2017).

2.7.1.6) Sound: Hearing deliberates the sounds made by food during preparation and ingesting e.g., the crackle of fried food, the effervescence of drinks, the cracking of hard biscuits. So, in sensory analysis, the senses are used to measure, analyse and interpret the organoleptic or sensory properties of food (Sharif MK et al 2017).

2.7.2 Types of tests

Different sensory tests are employed for food evaluation. The tests are grouped into four types.

a) **Difference tests:** a.1) Triangle test a.2) Paired comparison test a.3) Duo- trio test
b) **Rating tests:** b.1) Ranking test b.2) Single sample test b.3) Two sample difference test b.4) Multiple sample difference test b.5) Hedonic rating test b.6) Numerical scoring test b.7) Composite scoring test
c) **Sensitivity tests:** c.1) Sensitivity threshold test c.2) Dilution test
d) **Descriptive tests** (Srilakshmi B 2003)

a) **Difference Tests:** These tests are used in food industries to perceive minor differences in the samples but not the size of the difference. These tests can be accomplished by skilled as well as unskilled panelists. These are carried out to find out differences among the samples and how people notice and describe the difference. These are frequently used for screening and training of taste assessors. Difference testing is further classified into triangle test, paired comparison test, duo-trio test, multiple comparison test and ranking. The brief description of each is given below (Srilakshmi B 2003)

b.3) Two sample difference test: This test is a variation of the paired test and measures the amount of difference. Each taster is provided four pairs of samples. Each pair consists of an identified reference and coded test sample. The test sample is a duplicate of the reference sample in two pairs. In other two pairs, the test sample is the test variable. The panellist is asked to judge each pair independently as to the degree of difference between the test sample and standard on a scale of 0 representing no difference to _3 representing extreme difference (Srilakshmi B 2003).

b.2) Multiple sample difference test: Each panellist is served with 3-6 samples. One sample is a known standard; panellist compares each coded sample with the known standard. One coded sample is a duplicate of the sample. Direction and degree of difference is also to be judged (Srilakshmi B 2003).

b.5) Hedonic test: Hedonic rating depicts pleasurable or unpleasurable experiences. It asks each panelist to taste each sample and check a box from _1 Dislike very much to _5. Like very much to indicate their preference. This is a 5- point scale and sometimes even a 9-point scale is also used (Sri Lakshmi B 2003).

b.6) Numerical scoring test: Each panellist is provided with one or more samples. The panellist evaluates each sample on a specific scale for particular characteristics indicating the rating of sample. The panellists are trained to follow the sensory characteristics corresponding to the agreed quality descriptions and scores (Srilakshmi B 2003).

b.7) Composite scoring test: In this test, specific characteristic of a product are rated separately. It is useful in grading products and comparison of quality attributes by indicating which characteristic is at fault in a poor product (Srilakshmi B 2003).

c. **Sensitivity tests:** Sensitivity tests are done to assess the ability of individual to detect different tastes, odour and feel the specific factors like hotness. These tests are used to select and train panel members for evaluating the quality of products containing spices, salt and sugar (Srilakshmi B 2003).

   c.1) Sensitivity threshold test: It is defined as a statistically determined point on the stimulus scale at which a transition in a series of sensations or judgements occurs. There are mainly three types of threshold tests: Stimulus detection threshold, recognition identification threshold and terminal saturation threshold. These tests are also used where a minimum detectable difference of an additive or of an off flavour needs to be established (Srilakshmi B 2003).

c.2) Dilution test: This test is designated to establish the smallest amount of an unknown material developed as a substitute for a standard product. The quality of the test material is represented by the dilution number. The bigger the dilution numbers the better the quality of the test material (Srilakshmi B 2003).

d) Descriptive tests: This is a qualitative and quantitative description method for flavour analysis in product containing different tastes and odour (Srilakshmi B 2003).

a.1) Triangle test: Triangle test can also be used for screening panelists who are able to perceive a difference. This test is valuable in quality control to detect ingredient substitution results and odd product from various manufacturing lots. These tests do not specify degree of amount of difference. For the purpose, the assessor should be requested to postulate dissimilar attribute. In triangle testing, each assessor gets three coded samples, one is different and two are identical. The task is to pick out the unusual sample. (Sharif MK et al 2017).

a.2) Paired Comparison Test: This test is also recognized as the 2-AFC test (2 samples, alternate forced choice test). Each evaluator is provided with two coded samples and the task is to select the sample with the highest concentration of a pre-defined descriptor such as sweetness. This test is only meant for a detectable difference and does not specify the degree of difference. The likelihood of choosing the right sample by chance is 50%; hence, paired comparison test is more authoritative in finding differences than triangle test. A paired comparison test is suitable for use in quality control; nevertheless, the exact characteristic evaluated is clearly stated must be known earlier (Sharif MK et al 2017).

a.3) Duo-Trio Test: In this test, three samples are given to the judge; one is reference (labelled as R) and other two are coded. One coded sample is a duplicate of reference and other one is not similar. The assessor is asked to isolate the odd sample. It is mostly used with strong flavour products because less tasting is required. It is less effective than triangle test because the probability of selecting the correct answer by chance is 50%. It is less sensitive compared with triangle test as it is easier to conjecture the right one (Sharif MK et al 2017).

b.1) Ranking: Ranking is a quick technique for evaluating numerous samples at once and is frequently used for screening of one or two of the best samples in a group. The assessor is provided with three or more coded samples and is asked to rank them for a specific trait. This test is similar to ranking for a primary taste but uses food samples rather than pure solutions (Sharif MK et al 2017).

b.2) Single sample test: In this test, the panelist is asked to indicate the presence or absence and/or intensity of a particular quality characteristic. The completed analyses of two or more samples evaluated at different times can be compared with trained panelists. In market and consumer analysis, the results of different samples evaluated at different times by a different set untrained panelist can be compared (Srilakshmi B 2003) (table 2.6).

Table 2.6- Number of Panelists and samples required for sensory test

Sl. No.	**Method**	**Panelists**		**No. of sample tests**
		Type	Number	
A.	**Difference**			
1.	***Paired comparison***	Trained Untrained	5-12 72-80	**2**
2.	***Duo-Trio***	Trained	5-12	**3 (2 identical and 1 different)**
3.	***Triangle***	Trained	5-12	**3 (2 identical and 1 different)**
B.	**Rating**			
1.	***Ranking***	Trained Semi-trained Untrained	5-12 10-15 72-80	**2-7**
2.	***Single sample***	Trained Untrained	6-25 72-80	**1**
3.	***Two sample Difference***	Trained	6-25	**4 pairs of unknown and control sample**
4.	***Multiple sample difference***	Trained	6-25	**3-6**
5.	***Hedonic***	Semi-trained	10-25	**5-10**
6.	***Numerical scoring***	Untrained Trained	72-80 5-12	**1-4** **1-6** **1-10**
7.	***Composite***	Trained	5-12	**1-4**
C.	**Sensitivity**			
1.	***Threshold***	Untrained	-	**5-10**
2.	***Dilution***	Trained	12-24	**5-10**
D.	**Descriptive**			
	Flavour Profile	**Trained**	**3-6**	**1-5**

2.7.3. Selection and screening of Panel Members.

Assessors need to be screened by sensory expert regarding sensory perception. A variety of tests regarding the products under investigation and some general tasks required by the panellist are carried out by the sensory specialist. Screening test is suggested to be simple and not to over-test judges before performing true product assessments. A large number of screening tests could reduce the eagerness and motivation of the assessors at the time of actual evaluation. Medical screening is required before participation in the study, in certain situations (Sharif MK et al 2017).

2.7.4. Training of panellists

In depth training is required for descriptive tests whereas only minimum training is prerequisite for discrimination tests which depend upon the level of sensory evaluation. The panelists must realize that sensory evaluation is a difficult task which requires full concentration and attention. Sometimes fresh assessors need to work with experienced judges who have been trained for other product categories for training purpose. The panelists get motivated and encouraged when they get appreciation from the top management (Sharif MK et al 2017).

2.8 Future scope of lactose hydrolysed milk production and challenges

Due to the sheer enormous number of people who suffer from lactose intolerance, the lactose-free dairy market has developed drastically over the years. Because of the superior amino acid profile, lactose-free dairy products are nutritionally more appealing than lactose-free non-dairy alternatives.

Lactose hydrolysis and filtration are the two methods for making a lactose-free product. Lactose hydrolyzed dairy products yield more product than filtering since they do not produce any waste products. Lactose hydrolysis also makes the result sweeter; lactose has a sweetness of 20% that of sucrose, whereas glucose has a relative sweetness of 75% and galactose has a relative sweetness of 60%. (Ganzle MG et al 2008; Schulz P and Rizvi SS 2021).

In nano filtered lactose-free products, lactose is filtered out, leaving the rest of the dairy product constituents. This technique results in a 52 percent decreased yield of milk solids when lactose is eliminated. The generation of GOS is another advantage of lactose hydrolysis. GOS are non-digestible prebiotics that are comprised of long chain of monomers containing one glucose molecule and anywhere from 2– 20 galactose molecules. GOS enhances the growth of Bifidobacteria and

Lactobacilli in the human gut.

Currently, pre-process hydrolysis using soluble β-galactosidase is the most common way to produce lactose hydrolyzed milk industrially.

2.8.1 Hydrolysis

Lactase (β-galactosidase) is an enzyme that hydrolyzes lactose and converts it to glucose and galactose. Almost all industrially produced β-galactosidase is in the form of a soluble enzyme. Table 2.7 depicts a few of the most common sources of β-galactosidase and the most optimal reaction environment (Harju M et al 2012). Kluveromyces lactis and Kluveromyces fragilis, both sources of β- galactosidase are often used to hydrolyze lactose in milk because their relative activity is best at pH 6.5–7.3, which is the pH range of milk. Furthermore, their optimum temperatures are lower than those of other sources of β-galactosidase reducing the risk of liquid milk spoilage.

Acidified dairy products such as acid whey commonly utilize Aspergillus niger and Aspergillus oryzae in lactose hydrolysis due to their peak relative activity at lower pH. Although Escherichia coli’s optimum conditions are similar to Kluveromyces lactis and Kluveromyces fragilis, it is not commonly used unless as a host for the production of enzymes by recombinant DNA technology.

Table 2.7- Various sources of β- galactosidase including their optimum ph and temperature for hydrolyzing lactose

Enzyme source	Optimum Ph	Optimum temperature (°C)
Aspergillus niger	3.0–4.0	55–60
Aspergillus oryzae	5.0–6.2	50–55
Kluveromyces lactis	6.5–7.3	35
Kluveromyces fragilis	6.6	37
Escherichia coli	7.2	40
Bacillus circulans	6.0	60
Bacillus subtilis	6.5–7.0	50
Bacillus sterarothermophilis	5.8–6.4	65
Lactobacillus acidophilus	6.2–6.6	55
Streptococcus thermophilus	7.1	55

2.8.2. Comparison to filtration

In the market, there is a large selection of lactose-reduced and lactose-free milk. Lactose-reduced or lactose-free milk is sweeter and has a lower freezing point than conventional milk after being treated with -galactosidase. The β-galactosidase treatment increases the cost of fluid milk by $0.06–$0.08/L (Rehman SU et al 2009).

Reducing lactose in milk by chromatographic or membrane technology followed by converting residual lactose by β-galactosidase to its constituent monosaccharides has helped manufacturers provide milk with a genuine taste of milk; however, this process is costly. The chromatographic method of lactose removal, previously used by Valio Ltd., is very expensive and has not found widespread use in industry. Typically, removing lactose from milk by membrane technology increases the price by about 20–30%, making enzymatic hydrolysis of milk a more economically attractive approach (Schulz P and Rizvi SS 2021; Rehman SU et al 2009).

2.8.3. Potential future products and challenges

Lactose hydrolyzed skim milk powder- Skim milk powder (SMP) has a high amount of lactose, approximately 52% by weight, and is available at a lower cost than whole milk powder, dry whey, buttermilk powder, and whey protein concentrate. Additionally, large amounts of skim milk powder enter into commerce in many parts of the world every year. In 2018, 665,124 metric tons of skim milk powder was exported by the US (Schulz P and Rizvi S. S 2021; Levitt A et al 2019). This, combined with a large fraction of the world population being lactose intolerant, proves there is high potential to produce lactose hydrolyzed skim milk powder.

Production of lactose hydrolyzed SMP would be tremendously beneficial since it would allow individuals with lactose intolerance to enjoy milk or be a sweeter alternative in confectionaries without adding calories. Milk powders also can be very profitable when used as a protein source in a product. The falling price of milk powders makes them even more attractive when added as a protein source. The average price per gram of milk protein is 5 cents, where the price per gram of protein of a nutritional bar is 20 cents. Milk protein can easily be made into a 400% markup if it is transformed into a nutritional bar (Schulz P and Rizvi SS 2021; Nielson 2019). Due to the large population who exhibit lactose intolerance, lactose hydrolyzed skim milk powder (LHSMP) has a great potential to be utilized more than regular SMP. The lactose hydrolysis products, galactose, and glucose are highly susceptible to Maillard browning, significantly more than lactose.

Additionally, lactose hydrolysis increases its hygroscopicity, causing problems when drying. Therefore, the product causes many drying issues, such as fowling to drying components and Maillard browning when spray-dried (Torres J K et al 2017; Schulz P and Rizvi SS 2021).

LHSMP has been successfully freeze-dried, but the high cost of freeze-drying does not make a suitable industrial product (Jouppila K, 1994). A possible application to lower the high browning potential of LHSMP is to increase the production of GOS, produced by the transgalactosylation during enzymatic hydrolysis of lactose. When galactose, GOS, galactose plus glucose, and GOS plus galactose and glucose, all within a casein protein model, were analyzed for Maillard reaction during heating; GOS and GOS plus galactose and glucose were found to have a lower degree of browning when compared to galactose and glucose (Zhang W 2018).

2.8.4. GOS enriched lactose free UHT milk

It has been stated previously that GOS synthesis by transgalactosylation increases with reaction temperature. However, increasing temperature in milk becomes problematic due to microbial growth. In post-process hydrolysis, UHT milk is sealed aseptically to prevent microbial growth allowing the product to be stored at room temperature. Therefore, aseptic packaging milk containing β- galactosidase can be stored at elevated temperatures without spoilage to increasing the rate of GOS production by transgalactosylation. It should be noted that as the storage temperature increases, so does the potential for milliard reaction in the milk.

For the production of oligosaccharides, there is a clear inverse relationship between sweetness and the degree of polymerization (Ruiz Aceituno L et al 2018). Different enzyme sources produce various glycosidic linkages and different degrees of polymerization (DP) of GOS. Generally, GOS has a relatively low sweetness, 30– 60% that of sucrose (Torres D. P et al 1994). For example, B. bifidum can hydrolyze lactose and produce GOS with a DP-5 to DP-7 (Dumortier V et al 1994). The ability to lower the relative sweetness by increasing the DP, by selective choice of enzyme sources, could allow additional lactose to be added to the milk before hydrolysis. This would increase GOS synthesis due to the direct relationship between lactose concentration and the rate of transgalactosylation. Thus, producing a high yield of GOS without adding excess sweetness or to the milk products. More research should be done on post-process hydrolysis during the storage period. If the temperature rises too high, Maillard reaction can occur and increase furosine concentration, discoloration, and lysine blockage. In the case of GOS, when temperatures rise to the point of Maillard browning, glycosidic linkages start to break, decreasing the degree of polymerization, producing an undesirable product (Schulz P and Rizvi SS 2021; Torres DP et al 1994).

2.8.5. Challenges

Currently, commercially produced milk is produced either with enzymatic batch hydrolysis or enzymatic batch hydrolysis coupled with membrane filtration methods to produce a lactose free milk product higher in protein with the same sweetness as regular milk. The use of enzymatic hydrolysis is more advantageous than filtration as it only increased the price of milk \$0.06-\$0.08 (Rehman SU et al 2009).

The switch from batch process to continuous process would be advantageous economically and environmentally as the enzymes would be recovered, further lowering the price cost of enzymatic hydrolysis. Continuous enzymatic hydrolysis has not been developed commercially due to scaling the batch reactor and recovery and cleaning of the immobilized enzymes. Research on theoretical scale up of enzymatic bioreactors is a critical step forward in commercializing this process. The recent increase in patents for immobilizing enzymes like β-galactosidase, is another promising step forward. Further research should be conducted on the feasibility and development of a large-scale bioreactor employing immobilized β-galactosidase that can be reused at scale.