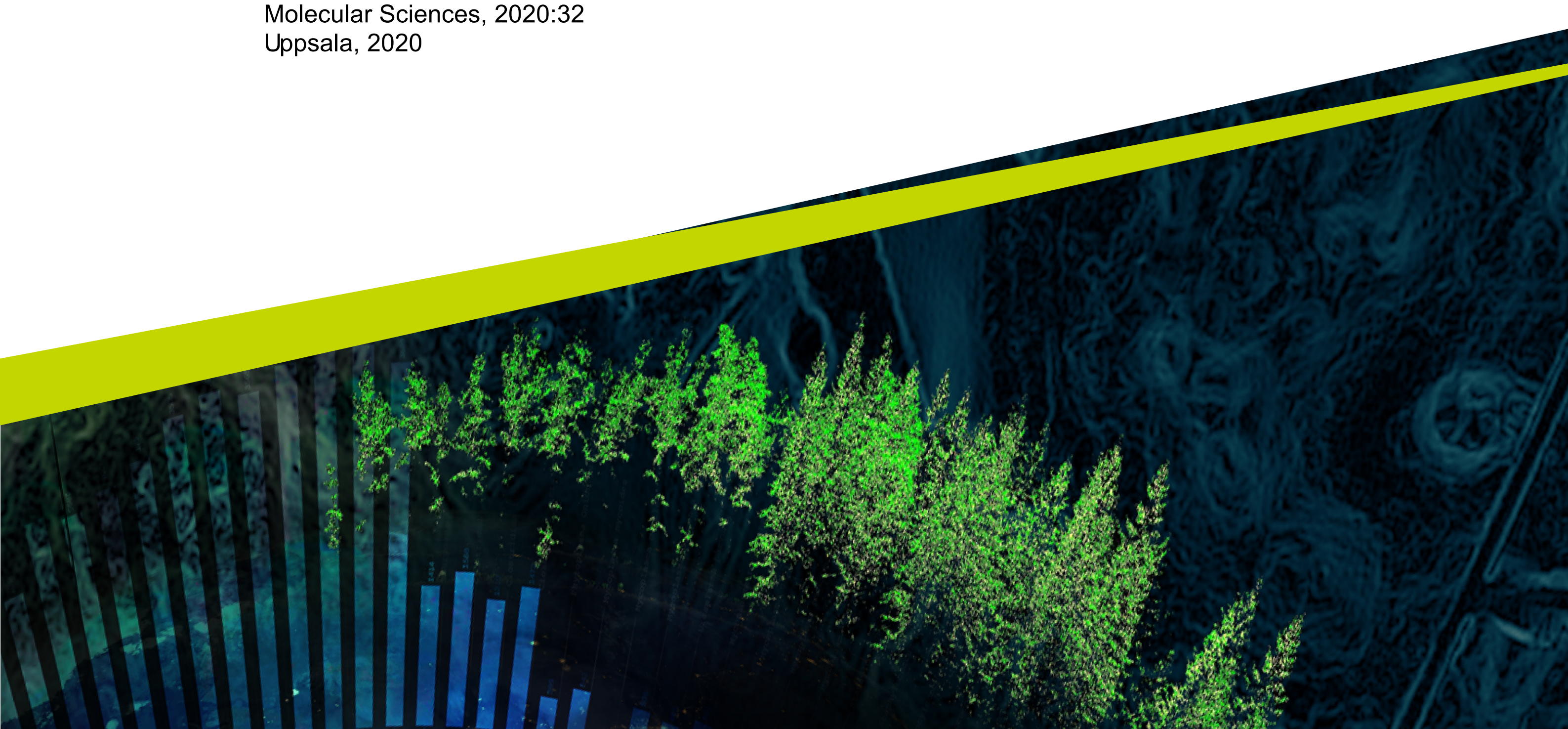
Lactose-free milk

Lactose intolerance, nutrition and process technology

Signe Christerson

Independent Project in Food Science, 15 credits, G2E
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agricultural Programme - Food Science
Molecular Sciences, 2020:32
Uppsala, 2020



Lactose-free milk: Lactose intolerance, nutrition and process technology

Laktosfri mjölk: Laktosintolerans, näring och processteknologi

Signe Christerson   Swedish University of Agricultural Sciences, SLU

Supervisor:	Åse Lundh, Swedish University of Agricultural Sciences, Department of Molecular Sciences
Examiner:	Monika Johansson, Swedish University of Agricultural Sciences, Department of Molecular Sciences
Year of publication:	2020
Place of publication:	Uppsala, Sweden
Title of series:	Molecular Sciences
Part number:	2020:32

Level:	G2E
Course title:	Självständigt arbete i Livsmedelskunskap
Course code:	EX0876
Keywords:	*Lactose free-milk, Maillard reaction, Lactose persistence, lactase, lactose hydrolysed milk, membrane separation*

Swedish University of Agricultural Sciences
NJ Faculty
Department of Molecular Sciences

Arkivering och publicering

Godkända självständiga arbeten (examensarbeten) vid SLU publiceras elektroniskt. Som student äger du upphovsrätten till ditt arbete och behöver godkänna publiceringen. Metadata och fulltext blir då synliga och sökbara på internet. I samband med att dokumentet laddas upp arkiveras det även digitalt.

[X] JA, jag ger härmed min tillåtelse till att föreliggande arbete publiceras

[ ] NEJ, jag ger inte min tillåtelse att publicera fulltexten av föreliggande arbete. Arbetet laddas dock upp för arkivering och metadata och abstract blir synliga och sökbara.

Abstract

The inability to properly digest lactose into its constituent's glucose and galactose, is prevalent worldwide, thus affecting large groups of people, who cannot benefit from the nutritional aspects of consuming dairy products. To enable consumption of dairy products even in adult individuals which suffer from lactase deficiency, the dairy industry has further developed dairy technologies. This has resulted in a growing assortment of the lactose-free dairy products available to the consumer.

The aim of this literature review was to compile current processing methods used in the separation and the hydrolysis of lactose present in bovine milk, as well as to discuss the advantages and disadvantages of these methods. Firstly, lactose intolerance is presented. Then the definition of lactase and different types of lactase deficiencies are given. Thereafter, the worldwide prevalence of lactase persistence and effects on nutritional intake is discussed. Finally, the review is identifying and mapping the principal process technologies available in production of lactose-free and lactose reduced milk, along with a discussion of factors to consider in the process technologies for milk.

In conclusion, there are methods and processes available on the market, which enables production of lactose-free/lactose reduced milk to the consumers. Most new industrial processes for production of lactose-free dairy products rely on the membrane separation techniques, followed by enzymatic hydrolysis of lactose residues. In the future, these processes are likely to continue to develop, which may result in the introduction of new products to the consumer market.

Keywords: Lactose free-milk, Maillard reaction, Lactose persistence, Lactase, Lactose hydrolysed milk, Membrane separation

Sammanfattning

Oförmågan att spjälka laktosmolekylen till dess beståndsdelar glukos och galaktos är spridd över hela världen och påverkar därmed stora människogrupper. Dessa människogrupper kan därmed inte dra nytta av näringsaspekterna som konsumtion av mejeriprodukter innebär. För att möjliggöra konsumtion av mejeriprodukter även i vuxen ålder hos individer som lider av ett underskott i laktasproduktion, har mejerinäringen utvecklat teknologier som resulterat i ett ökande utbud av laktosfria produkter.

Syftet med denna litteraturstudie är att sammanställa processmetoder för separation och hydrolys av laktos i komjölk, samt att diskutera fördelar respektive nackdelar med dessa metoder. Först presenteras laktosintolerans. Därefter ges definitionen av laktas samt olika typer av laktasbrist. Sedan ges en överblick av laktaspersistensens utbredning i världen och effekterna på näringsintag. Slutligen identifierar och kartlägger studien den nuvarande produktionen av laktosfri och laktosreducerad mjölk, följt av en diskussion av faktorer som påverkar processteknologin för mjölk.

Sammanfattningsvis, så finns det idag processer tillgängliga på marknaden, som tillåter produktion av laktosfri konsumtionsmjölk. De flesta nya mejeriprocesser för produktion av laktosfria mejeriprodukter bygger på membranseparationsteknik följt av en enzymatisk hydrolys av återstående laktos. I framtiden kommer sannolikt dessa processer att fortsätta utvecklas och resultera i att nya produkter introduceras på marknaden.

Nyckelord: Laktosfri mjölk, Maillard reaktion, Laktosintolerans, Laktas, Laktos-hydrolys, Membranseparation

Table of contents

List of figures

Abbreviations

ATP	Adenosine Triphosphate
LFM	Lactose-free milk
LF-UHT	Lactose-free Ultra-high-temperature treated
LI	Lactose intolerance
LNP	Lactase non-persistent/persistence
LM	Lactose malabsorption
LP	Lactase persistence
LPH	Lactase-phlorizin hydrolase
NF	Nanofiltration
OCTT	Oro-cecal transit time
RO	Reverse osmosis
UF	Ultrafiltration
UHT	Ultra-high-temperature
UHT-LFM	Ultra-high-temperature treated lactose-free milk

1. Introduction

After weaning (gradually accustoming infants to adult diet, whilst withdrawing the supply of mother's milk), a large part of the world's population experiences a decrease in lactase expression in the small intestines. The decrease in lactase activity results in inadequate hydrolysis of the milk sugar lactose, which is naturally present in milk (Itan *et al.*, 2010; Ingram *et al.*, 2009). Lactase persistence, being the continued production of lactase throughout adulthood, is associated with a specific lactase gene, most commonly found in Europeans (Leonardi *et al.*, 2011).

New dairy technologies, enables the production of lactose-free dairy products, which offers a solution to individuals suffering from lactase deficiency (Le *et al.*, 2014; Walstra *et al.*, 2005a). In recent years, lactose-free dairy has had a growing health appeal to not only those suffering from lactose tolerance, resulting in an increase of demand. However, production of lactose-free milk (LFM) products has much broader consequences for product quality, compared to what one would initially assume. An increase in sweetness, due to hydrolysis of lactose is one of the challenges upon production of LFM. Further, lactose, being a reducing sugar may participate in the Maillard reaction which leads to the formation of flavour compounds and brown pigments (Karlsson *et al.*, 2019). The Maillard reaction may occur in heat-treated dairy products when heated at sterilization conditions (Walstra *et al.*, 2005a). Heat-induced changes within the LFM is further favoured upon storage in high temperatures. Lactose-hydrolysed milk has been proven to be more prone to chemical changes during storage, than conventional Ultra-High-Temperature (UHT) processed milk (Jansson *et al.*, 2014). The acknowledgement of factors affecting the Maillard reaction, has enabled the establishment of preventative measurements (i.e. adapted temperature upon storage and processing) to be taken into consideration when handling lactose-hydrolysed milk (Evangelisti *et al.*, 1999).

The objective in this literature review is to discuss lactose from many aspects, ranging from worldwide prevalence of lactase persistence to advantages and disadvantages of process technologies (e.g. separation of lactose and hydrolysis of lactose).

Firstly, lactose intolerance is presented. Secondly, the definition of lactase and different types of lactase deficiencies are discussed. Thirdly, the worldwide prevalence of lactose persistence is given, as well as the effects on nutritional intake. Fourthly, the principal process technology including separation techniques and hydrolysis is addressed as well as quality changes. Furthermore, advantages and disadvantages of these process technologies will be discussed in this review.

2. Lactose intolerance

Lactose is the principal carbohydrate in milk from most mammals, including humans, and makes up about 40% of the total energy required by infants during the first year of life. Human breastmilk contains about ~7.5% lactose, whereas bovine milk contains about ~4.5% (Vesa *et al.*, 2000). The ability of humans to show high expression of the enzyme lactase at birth for hydrolysation of lactose (Wang *et al.*, 1998), is therefore of major nutritional importance (Troelsen, 2005), as it enables infants to assimilate essential nutrients from the mother's milk.

Lactose intolerance (LI) is a clinical syndrome and defined as the onset of gastrointestinal symptoms following ingestion of lactose by an individual with lactose malabsorption, LM (Suchy *et al.*, 2010; Heyman, 2006). LM is the physiological condition, underlaying LI, caused by insufficient amounts of lactase production. In lactose intolerant (lactase non-persistent, LNP) individuals, lactase activity is insufficient to hydrolyse all the lactose consumed. As a result, some lactose enters the colon where colonic microbiota converts it to fermentable glucose. The glucose is then fermented, consequently producing short chain fatty acids and gases. In combination with the osmotic effects of having undigested lactose in the colon, symptoms characteristic for LI are likely to develop (Ingram *et al.*, 2009). These symptoms may include abdominal pain, diarrhoea, gas, bloating and/or nausea (Deng *et al.*, 2015).

Digestion of lactose by microorganisms may however not be the only factor influencing LI (Misselwitz *et al.*, 2013; He *et al.*, 2008; Vonk *et al.*, 2003b; Szilagyi *et al.*, 2002). He *et al* (2008) analysed the underlaying factors affecting LI and the correlation between lactose digestion capacity and intestinal lactase activity; the results from the study indicated that lactose digestion capacity was not only determined by intestinal lactase activity, but also by its oro-cecal transit time (OCTT) i.e. the period of time needed by the head of the meal to reach to the beginning of the large intestine. The oro-cecal transit time affects the occurrence of the variability of symptoms of LI in individuals diagnosed with low lactase activity. Thus, the major differences in intolerance symptoms is determined by differences in the colonic processing of mal-digested lactose (He *et al.*, 2008; Vonk *et al.*, 2003a). A daily intake of lactose however can lead to colonic adaptation to ferment lactose and inducing a metabolic shift, reducing production of hydrogen by bacteria (Szilagyi *et al.*, 2002). Other authors have stated similar facts; that the onset of symptoms are dependent on the dosage of lactose, the individual's lactase expression, intestinal flora and sensitivity of the gastrointestinal tract (Misselwitz *et al.*, 2013).

2.1. Lactase

The lactase enzyme plays a key role in enabling individuals to properly digest lactose. The enzyme lactase-phlorizin hydrolase (LPH), a β-d-galactosidase, is found in the apical surface of the intestinal microvilli (Szilagyi & Ishayek, 2018; Ugidos-Rodriguez *et al.*, 2018). The enzymatic site, present in the lumen of the gut, enzymatically cleaves the β-1,4-glycosidic bond in the disaccharide lactose, resulting in the two monosaccharides D-glucose and D-galactose. LPH has two enzymatic functions: a lactase and a phlorizin hydrolase activity. The monosaccharides generated are then primarily used for the generation of adenosine triphosphate (ATP) via the citric acid cycle and oxidative phosphorylation (Krebs *et al.*, 1938). The absorption of the monosaccharides by the intestinal epithelium enables the human body to absorb these biosynthetic precursors in order to generate energy (Troelsen, 2005).

The second enzymatic activity, the phlorizin hydrolase activity, is responsible for splitting β-glycosides (glycosidic bonds in complex sugars) with a large hydrophobic alkyl chain. Glycosyl ceramides have been suggested to be the natural substrate of phlorizin hydrolase in milk (Troelsen, 2005; Skovbjerg *et al.*, 1981). Lactase activity has been found in mammals, while phlorizin hydrolase activity has been found in all vertebrates (Leese & Semenza, 1973).

2.2. Lactose deficiencies

There are four different types of lactase deficiency that may lead to LI (Silanikove et al., 2015; Suchy et al., 2010; Savaiano et al., 2001). These lactase deficiencies refer to the decline or absence of intestinal lactase; 1) Primary lactase deficiency (hypolactasia), 2) Secondary lactase deficiency, 3) Developmental lactase deficiency and 4) Congenital lactose deficiency. Treatment of lactase deficiencies includes change of diet, to limit or avoid foods containing lactose. There is considerable variation between individuals in the amount of tolerable lactose. Use of lactase products may help reducing symptoms of LI. Treatment is further dependent on the cause of LI. In cases of secondary lactase deficiency or congenital lactose deficiency, no treatment will aid in regaining production of lactase (Mattar *et al.*, 2012; Szilagyi *et al.*, 2002).

2.2.1. Primary lactase deficiency (hypolactasia)

This type of lactase deficiency, also referred to as lactase non-persistence (LNP), is when the lactase production declines with approximately 90-95% over time (Savaiano *et al.*, 2001). The condition is caused by a common autosomal recessive gene, resulting from a “developmentally regulated” change of the lactase gene expression (Di Costanzo & Berni Canani, 2018). Lactose persistence, however, is inherited as a dominant Mendelian trait (Enattah *et al.*, 2002). The lactase expression declines with age and varies with ethnicity (Leonardi *et al.*, 2011). Measurements to reduce symptoms caused by LI includes adaptation of gut microbiota. This can be achieved through adopting a diet with increasing dose of lactose, resulting in an increase of bacterial β-galactosidase activity (Briet *et al.*, 1997; Johnson *et al.*, 1993). According to Savaiano *et al* (2001), the lactase non-persistence is not a true lactase deficiency disease, but rather a normal expression of human physiology (Savaiano *et al.*, 2001). The permanent loss of lactase, normally takes place at an age of 3-5 years (Montgomery *et al.*, 1991).

Most people with lactase non-persistence retain some lactase activity, allowing them to consume varying amount of lactose in their diets without experiencing symptoms. Individuals with lactose maldigestion may often consume yoghurt and cheeses, since these foods are made through a fermentation process, where the lactose has been reduced to a certain degree (Walstra *et al.*, 2005a). Those affected vary in the amount of lactose tolerance, before symptoms develop (Suchy *et al.*, 2010; Vonk *et al.*, 2003b).

2.2.2. Secondary lactase deficiency (Secondary hypolactasia)

This type of lactase deficiency is temporary, since it may be a result of maldigestion or gastrointestinal infections affecting the villi in the small intestines where lactase is produced (Høst & Halken, 2010). There are many different types of injuries and possible causes affecting the small intestines which may interfere with the lactase production. The most possible causes of secondary lactase deficiency include gastroenteritis, coeliac disease Crohn's disease and chemotherapy. If treatment is implemented on the underlaying cause the secondary lactase deficiency will only be temporary (Di Costanzo & Berni Canani, 2018).

2.2.3. Developmental lactase deficiency

The third type of lactase deficiency is developmental lactase deficiency, which occurs in prematurely borne infants. In the immature gastrointestinal tract, the lactase production is deficient until the fetus is at least 34 weeks of age (Heyman, 2006). Developmental lactase deficiency lasts for a short period of time before the production of the lactase enzymes increases, enabling sufficient levels of lactose hydrolysis. The LI is therefore only regarded as temporary, since the ability to produce lactase improves as the infant ages.

2.2.4. Congenital lactase deficiency

Congenital lactase deficiency or alactasia, is an extremely rare autosomal recessive disorder, in which the small intestines produce very little to zero lactase enzyme from birth (Diekmann *et al.*, 2015). The inability of infants to digest lactose in breast milk or infant formula result in severe diarrhoea, dehydration and weight loss if lactose-free infant formula is not given (Heyman, 2006). This type of lactase deficiency is permanent since it is determined by a specific gene, inherited from the parents (Suchy *et al.*, 2010).

2.3. Worldwide prevalence of lactase persistence

The initial high expression of lactase present at birth, declines with time in most adults. After weaning, large parts of the world population experience a reduced ability to hydrolyse lactose, due to the lost or declined ability to synthesize lactase. Other individuals will maintain the ability to produce lactase as adults and develop lactase persistence (LP) (Venema, 2012). About 35% of the world population are lactase persistent, thus maintaining the capacity to produce lactase throughout adulthood. Thus, they are able to digest the lactose present in the milk without experiencing discomfort (Ingram *et al.*, 2009; Swagerty *et al.*, 2002). LP is governed by a genetic trait, which is highly influenced by cultural food-habits (Leonardi *et al.*, 2011).

The percentage of people with an impaired ability to digest lactose widely varies between countries and continents (Gerbault *et al.*, 2011). The distribution of LP, i.e. continued production of the lactase enzyme in adulthood, is determined by an autosomal dominant trait, which is genetically determined by a single gene (Leonardi *et al.*, 2011; Itan *et al.*, 2009). The geographic distribution of LP phenotype is not uniform, since it is dependent on the historical movement of the continental tiles and keeping of milk producing animals. LP is an example of a human niche construction (Gerbault *et al.*, 2011).

The distribution of LP-phenotype is most prevalent in people of European ancestry. Several mutations associated with African-, Middle Eastern- and Southern Asian groups may also explain the distribution of the phenotype (Gerbault *et al.*, 2011; Leonardi *et al.*, 2011). Elsewhere, LP phenotype is rare and in some cases absent (Gerbault *et al.*, 2011; Itan *et al.*, 2009). The frequency of individuals with the LP trait is highest in the northwest Europe, where individuals in the British islands and Scandinavia have an LP trait prevalence of 89-96%, see *figure 1*. In contrast, in the Eastern Mediterranean the LP trait prevalence can be as low as 15% (Gerbault *et al.*, 2011; Ingram *et al.*, 2009). One can see similar patterns of high LP trait prevalence in India, with higher prevalence of the LP trait in the Northern (63%), than in the Southern parts (10-20%) of the country, see *figure 1* (Itan *et al.*, 2010).

Despite the genetic evidence available, LP genotype data is insufficient to explain the frequency of LP phenotype in some parts of the world (Itan *et al.*, 2010). Analysis of LP-associated genetic variants indicates that the ability to consume appreciable amounts of lactose is a complex process, in which one need to consider the physiological, genetic, social, evolutionary and demographic factors (Leonardi *et al.*, 2011). Further studies validate this claim, by stating that LP, being a genetic trait and linked to animal-husbandry cultural traits, can be seen as a gene-culture co-evolution, where mutual symbiosis between animal and human has prevailed, simultaneously as the event of agriculture evolution (Silanikove *et al.*, 2015). The biological evolution of continued production of lactase throughout adulthood is thus entwined with cultural evolution of dairy production (Leonardi *et al.*, 2011).

The high frequency of lactase non-persistent individuals, is due to natural selection and the replacement of hunter-gather population by sedentary agriculturalists (Malmstrom *et al.*, 2010). The development in the agricultural sector is therefore considered to have greatly influenced modern human dietary habits.

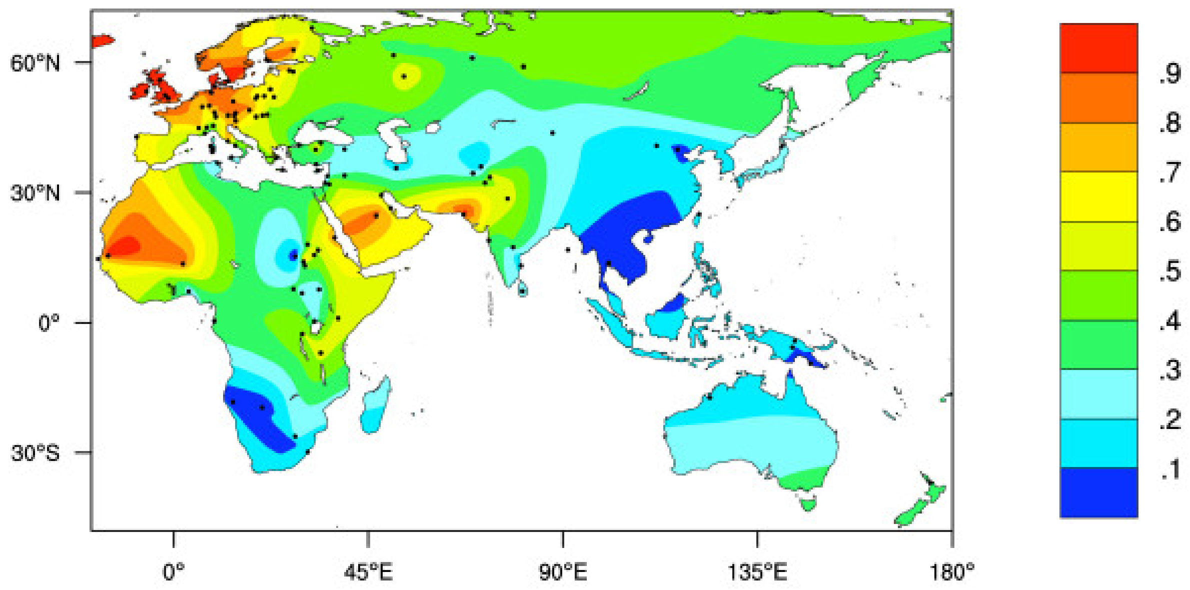


Figure 1. Interpolated map of Old-World Lactase persistence phenotype frequencies (Itan et al, 2010)

2.4. Effects on nutritional intake

The vast majority of individuals with impaired lactase ability may tolerate up to 12g of lactose per serving, before it causes major problems. Many individuals with real or perceived LI avoid dairy and therefore ingest inadequate amounts of calcium and vitamin D, which may result in adverse health outcomes (e.g. increased susceptibility to chronic disease) and nutrient deficiencies (Suchy *et al.*, 2010).

Dairy consumption is associated with maintaining bone dynamics which is constantly remodelled throughout life.

The importance of calcium in milk has been well established as it is responsible for bone modelling and bone health (Heaney, 2009). Calcium strengthens bone-health, as it reduces the risk of osteoporosis (i.e. weakening of the bones, increasing the risk of bone fractions). Additional functions of calcium include participation in blood clotting and muscle contraction as well as the functioning of our nervous system (Thorning *et al.*, 2016). The recognition of calcium's impact on bone health has lead The Swedish National Food Agency (Livsmedelsverket, 2020) to implement food legislation (LIVSFS2018:5) of fortification of vitamin D in milk, as it affect calcium bioavailability (Gallagher *et al.*, 2012; Caroli *et al.*, 2011).

Lactose has been shown to have a synergistic effect on calcium bioavailability. Animal studies have made it evident that lactose has a stimulating effect on calcium absorption (Kwak *et al.*, 2012). Calcium absorption from LFM in lactase deficient adults does not affect the ability to absorb calcium. The dietary calcium intake is yet lower, than that of lactase-persistent in both hydrolysed and unhydrolyzed milk (Tremaine *et al.*, 1986).

The consumer market offers many alternative drinks to those who are LI. The market offers "milk" products which span from animal origin (e.g. lactose-free dairy milk) to plant origin (e.g. oat-, soybean-, and several different types of nut-based drinks). Dairy alternatives of plant origin, are often consumed in similar fashion to that of lactose-free dairy, constituting a nutritious alternative if fortified with vitamin D, riboflavin, B12 and calcium. Calcium-fortified soy drinks have gained popularity over recent years. However, studies comparing the bioavailability of calcium-fortified soy drinks with cow milk suggest choosing lactose-free dairy milk over calcium-fortified soy-drink. In a study by Heaney *et al (*2000), the bioavailability of calcium in soy-drink was only absorbed at 75% the efficiency of calcium from lactose free dairy milk. The result in this study indicates that calcium-fortified soy-drink does not constitute a calcium source comparable to that of lactose free dairy milk. With this discovery in mind, it is not unreasonable that most non-dairy substitutes are fortified with at least 20% more calcium than the daily recommended intake (Dekker *et al.*, 2019). The National Medical Association further supports the claim of lactose-free dairy products being the most ideal substitute for regular dairy products for individuals suffering from LI or LM.

When comparing LFM with regular dairy milk one may notice a slightly sweeter taste in the LFM due to the process requiring hydrolysis of lactose. This perception may however be used to its advantage, as it requires less addition of added sugars in flavoured dairy products by up to 10-15g/kg; thus reducing calorie addition (McCain et al., 2018). Besides the advantages of reduced lactose intake for LI individuals, LFM compared to that of non-lactose hydrolysed milk, is not likely to have different nutritional effects on the human body (Dekker *et al.*, 2019). According to Dekker *et al* (2019a), the pre-digested lactose will enter the small intestines the same way as that of intact lactose is digested. This was further evident in a study investigating possible changes in glycaemic index with calves, suffering from diarrhoea. When fed lactose-hydrolysed cow's milk instead of unprocessed cow milk, the calves exhibited no improved sugar absorption (lactose utilization) (Gutzwiller, 2000). The study suggests that regardless digestion of enzymatically treated milk or regular milk, the glycaemic response will be indifferent in calves, thus not influencing blood glucose levels. A similar result in glycaemic response when consuming enzymatically hydrolysed milk resp. unmodified milk, was observed in a study with individuals suffering from diabetes (Ercan *et al.*, 1993).

3. Principal process technologies

The principal process technologies include separation and hydrolysis of lactose. There are currently three main processes used by the dairy industry for primary separation of lactose in milk, i.e. 1) membrane separation 2) chromatography and 3) crystallization. In the early years of LFM production, separation of lactose by separation technique using chromatography was the primary method used, as it allowed for retaining minerals with the proteins in the milk (Harju *et al.*, 2012). The production method using chromatography was then further developed based on membrane separation techniques (Harju *et al.*, 2012; Jelen & Tossavainen, 2003).The aim with the above-mentioned processes are to separate lactose from the milk. If substantial amounts of lactose are present upon the second step of the lactose-free process; hydrolysis of milk, the net result will be a sweeter milk, as glucose and galactose is substantially sweeter than lactose in its unhydrolyzed form (Walstra *et al.*, 2005b). By separating lactose from milk prior to hydrolysis of lactose in the milk, the final product yielded will have similar attributes and sensory profile compared to its lactose-containing counterpart. Thus, following the removal of lactose from the milk is the hydrolysis of lactose using soluble enzyme. Addition of lactase to the milk enzymatically hydrolyses the remaining lactose, resulting in a LFM (Harju *et al.*, 2012).

There are currently two processes using hydrolysis of lactose to glucose and galactose with soluble enzymes, applied in the production of LFM, i.e. 1) pre-hydrolysis (batch processing) and 2) post-hydrolysis (aseptic processing). The process of lactose hydrolysis by lactase is a low-tech solution, not requiring specialized equipment (Dekker *et al.*, 2019). By combining separation of lactose and hydrolysis of lactose, manufacturers wish to reduce the lactose content sufficiently to meet the demands from the Food agency authorities (Troise *et al.*, 2016).

3.1. Separation techniques

3.1.1. Membrane separation

Within the dairy industry today, ultrafiltration (UF) and nanofiltration (NF) processing is mainly applied to separate lactose from the milk. The membrane filtration technology is a non-thermal technology applied in the processing of LFM (Tetrapak, 2015). The filtration process efficiently removes unwanted compounds (e.g. lactose, microorganisms, drug residues in milk), that may interfere with the milk’s product quality, texture and shelf-life. The membrane separation process results in a 40% reduction of lactose by separation (Jansson *et al*., 2014)

The UF membrane separates the skim milk into two streams, allowing water, dissolved salts, lactose and acids to pass through it in either direction, while retaining proteins and fat. Whereas the NF membrane separates a range of minerals from the milk, primarily allowing the fluid and certain monovalent ions to pass through the membrane (Tetrapak, 2015).

To produce a LFM, the milk is first ultrafiltrated, the permeate of the UF is then further nanofiltrated and the permeate from the NF is then further concentrated using reverse osmosis (RO) (concentrating all total solids whilst allowing water to pass through the membrane). The retentate of the reverse osmosis is then returned back to the UF-retentate (returning all the minerals), after which residual lactose is hydrolysed using either pre-hydrolysis or post-hydrolysis (Harju *et al.*, 2012).

The gained popularity with filtration process using UF/NF in the dairy industry, is suggested to be due to the method requiring very low maintenance. The filtration process is also said to be easily operated, thus does not require specialized knowledge in order to be operated (Kumar *et al.*, 2013). Among the disadvantages with membrane separation is that organic membranes, depending on material, is limited to work in a certain range of temperature, pH, and transmembrane pressure (the pressure balance between the retentate side and permeate side). Inorganic membranes have the disadvantage of being more expensive and having lower packing capacity. However, inorganic materials have the advantage of withstanding operating in more extreme conditions, thus having a longer service life (Le *et al.*, 2014).

3.1.2. Chromatography

Chromatography may be used to separate components (e.g. lactose) based on differences in the flow velocities of different components of a liquid. By using a resin with different affinities to different components or a resin which will separate compounds based on size, different fractions can be collected through their differences in elution time (Harju *et al.*, 2012). This method has been developed to specifically separate lactose from milk and whey by strong cation exchange resins (Jelen & Tossavainen, 2003). The disadvantages with using chromatography compared to current membrane separation techniques when producing LFM, is that the chromatography technology is more difficult for dairy plants to adopt and harder to licence to other dairies.

3.1.3. Crystallization

Lactose may be separated from milk using a crystallization process. The lactose in the milk is concentrated to a high total solid content so that the lactose becomes saturated and crystallizes, the lactose crystals is subsequently separated using centrifugation (Holsinger, 1997). Production of LFM using the crystallization method is not feasible due to the high viscosity of concentrated milk, resulting in a low yield of lactose crystals. The method is therefore mainly applied in commercial production of lactose (Harju *et al.*, 2012; Holsinger, 1997).

3.2. Hydrolysis

The lactases used for commercial production of LFM is available from a number of sources, e.g. microbial- or fungal origin. The lactase of fungal origin, is traditionally derived from the dairy yeast *Kluyveromyces lactis* and its close relatives *Saccharomyces lactis, K. marxianus* and *K. fragilis (Saqib et al., 2017; Pivarnik et al., 1995).* Other commercial lactases from bacterial- (*Bacillus circulans*) and fungal origin (*Aspergillus oryzae)* are less suitable for hydrolysis of lactose, thus these lactases are primarily used as nutritional enzymes (Dekker *et al.*, 2019). Depending on the source, the lactase activity differs. Optimal activity is dependent on the temperature of the milk, the pH, time and dosage of enzyme used in the production process (Dekker, 2019).

3.2.1. Pre-hydrolysis

In the batch process (pre-hydrolysis), soluble lactase enzyme is added to a tank with thermized milk, i.e. milk which has been heat-treated at 65°C for 15 min. The batch is subsequently incubated for 24 h during refrigerated conditions (4-8°C), allowing for hydrolysis of lactose to take place and at the same time preventing microbial growth to occur in the non-sterile product. After incubation with the lactase enzyme, the milk is pasteurized (to destroy residual lactase), homogenized, standardized and packaged (Dekker *et al.*, 2019).

There are a number of aspect that are important to consider when producing LFM using batch processing, e.g. 1) the ratio between substrate and enzyme, 2) the pH of the milk 3) maximum temperature as well as 4) contact time permissible, 5) enzyme activity and 6) cost of the enzyme (Harju *et al.*, 2012; Walstra *et al.*, 2005b).

The ratio between the substrate and enzyme needs to be considered as adequate amount of lactase are required to sufficiently reduce lactose content within a given timeframe and temperature. Yet, there is no global consensus on the regulatory requirements for lactose-free claims in drinking milk (van Scheppingen *et al.*, 2017). As an indicator of adequate lactose reduction, The National food agency in the Scandinavian countries have set the limit for residual lactose content to be less than 10 mg per 100 gram of product (<0.01%), which is now generally labelled as "lactose free". Treatment with lactases (e.g. β-D-galactosidases) reduces the lactose content in milk to <0.01% (van Scheppingen *et al.*, 2017). The ratio between the substrate and enzyme to obtain the final milk product with such low concentrations of lactose present subsequently requires attention to the processing of the milk used and the activity and dosage of the enzyme. In the batch process, the enzyme dosage is relatively high, with an enzyme being selected for high activity at neutral pH and low temperature.

Another factor that needs to be considered is the pH of the milk, where an increase/decrease in pH leads to non-optimal enzymatic activity (Walstra *et al.*, 2005a). The temperature will greatly influence the rate of which the enzyme interacts with the substrate. However, one needs to take into consideration that optimal temperature for enzyme and substrate interactions, will also yield undesirable microbial growth. In the dairy industry, when producing LFM according to the batch process, milk is kept overnight at refrigeration temperature, to reduce the risk of microbial spoilage (Zadow, 2012). It is important to take the extensive contact-time needed between enzyme and the substrate into consideration, allowing adequate time for the lactase to hydrolyse the lactose.

Enzymatic activity is a factor greatly affecting the rate at which batch processing can be carried out. A lactase product with high specific activity is favoured, which may help to shorten the production time, hence saving costs. The enzyme can only be utilized once in batch processing, as it is destroyed upon pasteurization of the milk, both activity and cost of the enzyme needs to be considered in the process. The batch process, being a discontinuous process, requires the hold-up of a tank during 24h. This may pose a problem if the number of available tanks in the factory is limited, since it will affect the cost effectiveness of the production technology.

3.2.2. Post-hydrolysis

In the aseptic process (post-hydrolysis), the milk is thermally sterilized according to UHT procedure and subsequently packaged into previously sterilized containers under sterile conditions. The packages are subsequently put in quarantine and stored at ambient temperatures for 3 days prior to 3-6 months of storage at ambient temperature (Dekker *et al.*, 2019). The minimal amounts of enzyme added to the sterile milk, stored at ambient temperature, are adequate for complete hydrolysis of lactose present in the milk, resulting in a LFM with long-lasting shelf-life (Dekker *et al.*, 2019; Dahlqvist *et al.*, 1977).

Factors that advocate for aseptic processing of LFM is the lower production cost. The aseptic processing of UHT-LFM requires minimal amounts of sterile filtered lactase into the milk packages, which hydrolyses the lactose during storage

(Dahlqvist *et al.*, 1977). In LF-UHT milk, lactose hydrolysis takes place over the first week or two of storage of the product. Other authors have recommend hydrolysis of lactose after heat-treatment as a repercussive method to avoid the occurrence of Maillard reaction, which results in undesirable changes in the taste and the smell of the milk. (Mendoza *et al.*, 2005).

As with pre-hydrolysis (batch-processing), there are many aspects that are important to consider when producing LFM using post-hydrolysis (aseptic-processing). One of the factors to consider in aseptic processing is the dosage of enzyme, which is substantially lower compared to the batch process. The reason for this is because the incubation time and storage temperature is higher in milk produced using aseptic processing (Walstra *et al.*, 2005b). Other challenges and factors to consider regarding lactose-free UHT milk is the presence of active enzyme at the time of consumption, the challenge is in the uncontrolled condition of the milk, i.e. the inability to control the degree of hydrolysis, thus possible side effects affecting texture and sensory attributes. The aseptic process is also less cost effective than the batch processing method, as it requires special equipment which is not economically justifiable for smaller dairy processing plants. To prevent microbial contamination, the process additionally requires highly skilled operators. When operated under optimal conditions, the process is a fully-continuous ~~process~~, which is a major advantage when producing LFM on a larger scale (Dekker *et al.*, 2019)

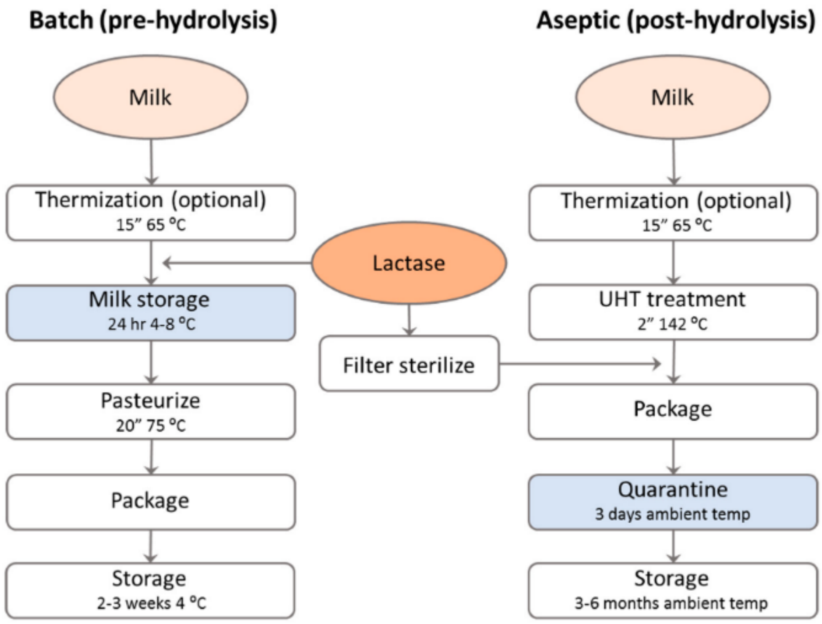


Figure 2. A schematic illustration of batch and aseptic processes that are used to produce lactose-free milk. The process step where lactose hydrolysis takes place is indicated in blue. Process conditions may vary from one factory to another, and additional process steps (e.g. homogenization and standardization) are commonly included before the heat-treatment (Dekker et al., 2019).

3.3. Quality changes

One major chemical reaction, impacting consumer perception of LFM is the Maillard reaction, which is responsible for the formation of brown pigments and caramelised flavours in hydrolysed milk (Schiano *et al.*, 2017). The changed carbohydrate composition in lactose-hydrolysed milk (yielding reducing sugars i.e. glucose and galactose) enables the reaction between a carbonyl group of a reducing sugar (mainly lactose in open chain structure) and the amino group (mainly lysine in milk proteins), known as the Maillard reaction. The reaction may lead to sensory changes in the milk, e.g. colour, flavour, texture and reduction of nutritional values (loss of lysine which is an essential amino acid) of the hydrolysed milk (Paques & Lindner, 2019; Walstra *et al.*, 2005b). Due to these unwanted changes, heat treatment of LFM should be as gentle as possible as the Maillard reaction is known to increase with changes in pH and temperature. The temperature of which the processed LFM is stored in may also influence the Maillard reaction as well as the loss of stability in the product. Karlsson *et al (*2019) amongst others, have established that LF-UHT milk is especially susceptible to Maillard reactions (Karlsson *et al.*, 2019; Paques & Lindner, 2019). Tossavainen *et al* (2008) states in a study that the quality and stability of LFM is highly dependent on storage temperature, as the Maillard reaction can proceed significantly during the time on the shelf when stored at ambient temperatures. Thus, the temperature of which the final product is stored in is of higher significance than the actual UHT technology used (Tossavainen & Kallioinen, 2008).

Other factors favouring the occurrence of Maillard reactions are proteases present in milk or proteases stemming from lactase preparation (mainly a problem in LFM using post-hydrolysis). The protease presence may cause proteolysis, which enhance the Maillard reaction, due to the higher level of free amino acids (Jansson *et al.*, 2014).

To avoid Maillard reactions in LF-UHT milk, Mendoza *et al (2005)* recommend hydrolysis of lactose after heat-treatment. They further suggest; limiting the degree of lactose-hydrolysis to 80-90%, based on avoiding excessive sweetness (Mendoza *et al.*, 2005). In 1996, Vasala *et al.* also patented a method (wo/1996/022695) to reduce the perceived sweetness in lactose-hydrolysed UHT milk using addition of sweetness-suppressing additives (e.g potassium salts of an organic acid such as citric acid, malic acid, gluconic acid and lactic acid).

4. Discussion

Inventing a process technology which does not confer a sweet taste to the milk upon hydrolysis of lactose to monosaccharides, has been one of the biggest challenges upon production of LFM. For individuals sensitive or intolerant to the normal lactose content in milk (being about 4.6-4.9%), LFM constitute a nutritious and good alternative. Especially important is LFM to infants with Developmental or Congenital lactase deficiency, as the invention allows for substituting regular mothers milk with milk where the lactose has been converted. LFM is a nutrient-rich beverage which may benefit health as it is packed with important nutrients (e.g. calcium, vitamin D, phosphorous, B vitamins and potassium.

Dairy process technologies aiming to reduce the lactose content in milk is well-established in modern dairy industry, many different approaches exist to modify the composition of milk. The enzymatic process approach (addition of lactase to milk) have difficulties in production, as this type of process result in conversion of lactose to monosaccharides, which increases the taste of the milk to undesirable sweet levels. The addition of a prior process step (membrane separation) has therefore been suggested to reduce the lactose content in milk to about 3% (*Lange et al*, 2000). When using ultrafiltration/nanofiltration and reverse osmosis (filtration processes), one can obtain a skimmed milk with the possibility to increase the protein content to about 3.8-4.0% or greater. The addition of proteins to the skimmed milk improves the organoleptic properties of the milk. Organoleptic meaning the aspects of food which create the individual experience via senses, including taste, sight, smell, and touch. A process plan including ultrafiltration of milk, followed by an enzymatic process approach is therefore the most used approach for production of LFM without conferring an excessive sweetness to the milk.

The quality of a LFM is affected by many variable factors e.g. the ratio between substrate and enzyme, the pH of the milk, maximum temperature, contact time permissible and the activity and efficiency of the enzyme. The quality is further affected by storage temperature and time as well as the amount of dissolved oxygen available in milk, the milk composition and the activity of heat-resistant enzymes (Karlsson *et al.*, 2019).

For the thermal treatment to be beneficial for LFM quality, maximum temperature and time needs to be carefully monitored. Inadequate thermal treatment may result in active pathogenic agents in the milk responsible for shortened shelf life and changes in organoleptic and nutritional properties (Lange *et al,* 2000; Tikanmäki *et al* 2005). The thermal treatment is further implemented to increase the permeation speed during the following ultrafiltration step, thus reducing the clogging rate at the filtering walls. If the temperature of the milk is not stabilized prior to filtration, the permeation speed may be affected. The filtration speed is affected even by an increase of 1 ° C, which increase the permeation-speed by about 2.5%. The equipment used to conduct the thermal treatment and the filtration process, may vary depending on systems used, producers of LFM should therefore carefully follow equipment-manufacturers recommendations. When producing LFM one therefore need to take all these aspects into consideration to ensure product quality.

In the overall production of LFM, there will always be a risk of chemical reactions (Maillard reaction) involving lactose, affecting the stability and shelf-life of the milk. As one might assume, the milk stability will gradually change over time, which will impact the products shelf-stability and sensory attributes (Karlsson *et al.*, 2019).

New technological improvements are frequently introduced to the LFM processing industry. Recent studies have been concentrated on developing membrane filtration to the use of such filtrated, low-carbohydrate milk in the production of other dairy products. Current problems with the membrane techniques in general is that in UF, not only lactose is filtered out from the milk, but also some of the minerals that have an impact on taste. Thus, controlling the mineral content in LFM when using UF is a particularly problematic in the field. The membrane filtration process Is further known to have extensive loss of minerals, thus in which the minerals must be added separately. UF also produce sugar- and mineral containing secondary flows, which increases the wastewater load and further requires processing, and thus addition of costs. New technological improvements focusing on these areas would therefore be beneficial for development of current processing methods applied in the production of LFM.

As a conclusion many new processes are bound to develop in time to improve current dairy processing methods. In the future it is likely that we will see an increase in demand from countries where LP is low (high incidence of lactose intolerant individuals) tentatively in e.g. Asian and African countries because of disposable income in developing countries. For developed countries where the LFM-production is already well-established, there seems to be an opening for innovative new products that may compete with plant-based alternatives if nutritional importance is highlighted.

5. Conclusion

In conclusion, although there are challenges in the manufacture of lactose-free dairy products, there are today methods and processes available which enable production of lactose-free and/or lactose-reduced drinking milk. With regards to countries where the prevalence of lactase persistence is low, the processes utilized to produce lactose-free milk is of high importance since it facilitates the consumption of milk. Milk is rich in important vitamins and calcium and benefits public health. The dairy process method mainly applied in modern manufacturing plants rely on membrane techniques followed by enzymatic hydrolysis of lactose residues. As the dairy technology advances, new processes to produce lactose-free milk are to develop, and consequently, new products will be introduced to the consumer in the future.

6. References

Briet, F., Pochart, P., Marteau, P., Flourie, B., Arrigoni, E. & Rambaud, J.C. (1997). Improved clinical tolerance to chronic lactose ingestion in subjects with lactose intolerance: a placebo effect? *Gut,* 41(5), pp. 632-5.

Caroli, A., Poli, A., Ricotta, D., Banfi, G. & Cocchi, D. (2011). Invited review: Dairy intake and bone health: A viewpoint from the state of the art¹. *Journal of Dairy Science,* 94(11), pp. 5249-5262.

Dahlqvist, A., Asp, N.G., Burvall, A. & Rausing, H. (1977). Hydrolysis of lactose in milk and whey with minute amounts of lactase. *Journal of Dairy Research,* 44(3), pp. 541-548.

Dekker, P.J.T. (2019). Enzymes Exogenous to Milk in Dairy Technology: β-d-Galactosidase☆. In: *Reference Module in Food Science* Elsevier. Available from: http://www.sciencedirect.com/science/article/pii/B9780081005965007435.

Dekker, P.J.T., Koenders, D. & Bruins, M.J. (2019). Lactose-Free Dairy Products: Market Developments, Production, Nutrition and Health Benefits. *Nutrients,* 11(3), p. 551.

Deng, Y., Misselwitz, B., Dai, N. & Fox, M. (2015). Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. *Nutrients,* 7(9), pp. 8020-8035.

Di Costanzo, M. & Berni Canani, R. (2018). Lactose Intolerance: Common Misunderstandings. *Annals of Nutrition and Metabolism,* 73(suppl 4)(4), pp. 30-37.

Diekmann, L., Pfeiffer, K. & Naim, H.Y. (2015). Congenital lactose intolerance is triggered by severe mutations on both alleles of the lactase gene. *BMC Gastroenterol,* 15, p. 36.

Enattah, N.S., Sahi, T., Savilahti, E., Terwilliger, J.D., Peltonen, L. & Jarvela, I. (2002). Identification of a variant associated with adult-type hypolactasia. *Nat Genet,* 30(2), pp. 233-7.

Ercan, N., Nuttall, F.Q., Gannon, M.C., Redmon, J.B. & Sheridan, K.J. (1993). Effects of glucose, galactose, and lactose ingestion on the plasma glucose and insulin response in persons with non-insulin-dependent diabetes mellitus. *Metabolism,* 42(12), pp. 1560-1567.

Evangelisti, F., Calcagno, C., Nardi, S. & Zunin, P. (1999). Deterioration of protein fraction by Maillard reaction in dietetic milks. *J Dairy Res,* 66(2), pp. 237-43.

Gallagher, J.C., Yalamanchili, V. & Smith, L.M. (2012). The effect of vitamin D on calcium absorption in older women. *The Journal of clinical endocrinology and metabolism,* 97(10), pp. 3550-3556.

Gerbault, P., Liebert, A., Itan, Y., Powell, A., Currat, M., Burger, J., Swallow, D.M. & Thomas, M.G. (2011). Evolution of lactase persistence: an example of human niche construction. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* 366(1566), pp. 863-877.

Gutzwiller, A. (2000). Glucose and Galactose Absorption after Ingestion of Milk Containing Hydrolysed Lactose in Calves with Diarrhoea. *Journal of Veterinary Medicine Series A,* 47(8), pp. 495-500.
Harju, M., Kallioinen, H. & Tossavainen, O. (2012). Lactose hydrolysis and other conversions in dairy products: Technological aspects. *International Dairy Journal - INT DAIRY J,* 22.
He, T., Venema, K., Priebe, M.G., Welling, G.W., Brummer, R.J. & Vonk, R.J. (2008). The role of colonic metabolism in lactose intolerance. *Eur J Clin Invest,* 38(8), pp. 541-7.
Heaney, R.P. (2009). Dairy and bone health. *J Am Coll Nutr,* 28 Suppl 1, pp. 82s-90s.
Heyman, M.B. (2006). Lactose intolerance in infants, children, and adolescents. *Pediatrics,* 118(3), pp. 1279-86.
Holsinger, V.H. (1997). Physical and Chemical Properties of Lactose. In: Fox, P.F. (ed. *Advanced Dairy Chemistry Volume 3: Lactose, water, salts and vitamins*. Boston, MA: Springer US, pp. 1-38. Available from: https://doi.org/10.1007/978-1-4757-4409-5_1.
Høst, A. & Halken, S. (2010). CHAPTER 46 - Approach to Feeding Problems in the Infant and Young Child. In: Leung, D.Y.M., Sampson, H.A., Geha, R. & Szefler, S.J. (eds) *Pediatric Allergy: Principles and Practice (Second Edition)*. Edinburgh: W.B. Saunders, pp. 487-493. Available from: http://www.sciencedirect.com/science/article/pii/B9781437702712000468.
Ingram, C.J., Mulcare, C.A., Itan, Y., Thomas, M.G. & Swallow, D.M. (2009). Lactose digestion and the evolutionary genetics of lactase persistence. *Hum Genet,* 124(6), pp. 579-91.
Itan, Y., Jones, B.L., Ingram, C.J., Swallow, D.M. & Thomas, M.G. (2010). A worldwide correlation of lactase persistence phenotype and genotypes. *BMC Evol Biol,* 10, p. 36.
Itan, Y., Powell, A., Beaumont, M.A., Burger, J. & Thomas, M.G. (2009). The origins of lactase persistence in Europe. *PLoS computational biology,* 5(8), pp. e1000491-e1000491.
Jansson, T., Clausen, M.R., Sundekilde, U.K., Eggers, N., Nyegaard, S., Larsen, L.B., Ray, C., Sundgren, A., Andersen, H.J. & Bertram, H.C. (2014). Lactose-hydrolyzed milk is more prone to chemical changes during storage than conventional ultra-high-temperature (UHT) milk. *J Agric Food Chem,* 62(31), pp. 7886-96.
Jelen, P. & Tossavainen, O. (2003). Low lactose and lactose-free milk and dairy products - Prospects, technologies and applications. *Australian Journal of Dairy Technology,* 58, pp. 161-165.
Johnson, A.O., Semenya, J.G., Buchowski, M.S., Enwonwu, C.O. & Scrimshaw, N.S. (1993). Adaptation of lactose maldigesters to continued milk intakes. *Am J Clin Nutr,* 58(6), pp. 879-81.
Karlsson, M.A., Langton, M., Innings, F., Malmgren, B., Höjer, A., Wikström, M. & Lundh, Å. (2019). Changes in stability and shelf-life of ultra-high temperature treated milk during long term storage at different temperatures. *Heliyon,* 5(9), p. e02431.
Krebs, H.A., Salvin, E. & Johnson, W.A. (1938). The formation of citric and alpha-ketoglutaric acids in the mammalian body. *Biochem J,* 32(1), pp. 113-7.
Kumar, P., Sharma, N., Ranjan, R., Kumar, S., Bhat, Z.F. & Jeong, D.K. (2013). Perspective of membrane technology in dairy industry: a review. *Asian-Australasian journal of animal sciences,* 26(9), pp. 1347-1358.
Kwak, H.-S., Lee, W.-J. & Lee, M.-R. (2012). Revisiting lactose as an enhancer of calcium absorption. *International Dairy Journal,* 22(2), pp. 147-151.
Le, T., Cabaltica, A.D. & Bui, V.M. (2014). Membrane separations in dairy processing. *J. Food Res. Technol.,* 2.

Leese, H.J. & Semenza, G. (1973). On the identity between the small intestinal enzymes phlorizin hydrolase and glycosylceramidase. *J Biol Chem,* 248(23), pp. 8170-3.
Leonardi, M., Gerbault, P., Thomas, M. & Burger, J. (2011). The evolution of lactase persistence in Europe. A synthesis of archaeological and genetic evidence. *International Dairy Journal,* (in press).
Malmstrom, H., Linderholm, A., Liden, K., Stora, J., Molnar, P., Holmlund, G., Jakobsson, M. & Gotherstrom, A. (2010). High frequency of lactose intolerance in a prehistoric hunter-gatherer population in northern Europe. *BMC Evol Biol,* 10, p. 89.
Mattar, R., de Campos Mazo, D.F. & Carrilho, F.J. (2012). Lactose intolerance: diagnosis, genetic, and clinical factors. *Clinical and experimental gastroenterology,* 5, pp. 113-121.
McCain, H.R., Kaliappan, S. & Drake, M.A. (2018). Invited review: Sugar reduction in dairy products. *J Dairy Sci,* 101(10), pp. 8619-8640.
Mendoza, M.R., Olano, A. & Villamiel, M. (2005). Chemical Indicators of Heat Treatment in Fortified and Special Milks. *Journal of Agricultural and Food Chemistry,* 53(8), pp. 2995-2999.
Misselwitz, B., Pohl, D., Frühauf, H., Fried, M., Vavricka, S.R. & Fox, M. (2013). Lactose malabsorption and intolerance: pathogenesis, diagnosis and treatment. *United European gastroenterology journal,* 1(3), pp. 151-159.
Montgomery, R.K., Büller, H.A., Rings, E.H.H.M. & Grand, R.J. (1991). Lactose intolerance and the genetic regulation of intestinal lactase-phlorizin hydrolase. *The FASEB Journal,* 5(13), pp. 2824-2832.
Paques, M. & Lindner, C. (2019). *Lactose: Evolutionary Role, Health Effects, and Applications*: Elsevier Science. Available from: https://books.google.se/books?id=W76NDwAAQBAJ.
Pivarnik, L.F., Senecal, A.G. & Rand, A.G. (1995). Hydrolytic and transgalactosylic activities of commercial beta-galactosidase (lactase) in food processing. *Adv Food Nutr Res,* 38, pp. 1-102.
Saqib, S., Akram, A., Halim, S.A. & Tassaduq, R. (2017). Sources of β-galactosidase and its applications in food industry. *3 Biotech,* 7(1), pp. 79-79.
Savaiano, D., Hertzler, S., Jackson, K.A. & Suarez, F.L. (2001). CHAPTER 37 - Nutrient Considerations in Lactose Intolerance. In: Coulston, A.M., Rock, C.L. & Monsen, E.R. (eds) *Nutrition in the Prevention and Treatment of Disease*. San Diego: Academic Press, pp. 563-575. Available from: http://www.sciencedirect.com/science/article/pii/B9780121931551500398.
Schiano, A.N., Harwood, W.S. & Drake, M.A. (2017). A 100-Year Review: Sensory analysis of milk. *Journal of Dairy Science,* 100(12), pp. 9966-9986.
Silanikove, N., Leitner, G. & Merin, U. (2015). The Interrelationships between Lactose Intolerance and the Modern Dairy Industry: Global Perspectives in Evolutional and Historical Backgrounds. *Nutrients,* 7(9), pp. 7312-31.
Skovbjerg, H., Sjostrom, H. & Noren, O. (1981). Purification and characterisation of amphiphilic lactase/phlorizin hydrolase from human small intestine. *Eur J Biochem,* 114(3), pp. 653-61.
Suchy, F.J., Brannon, P.M., Carpenter, T.O., Fernandez, J.R., Gilsanz, V., Gould, J.B., Hall, K., Hui, S.L., Lupton, J., Mennella, J., Miller, N.J., Osganian, S.K., Sellmeyer, D.E. & Wolf, M.A. (2010). NIH consensus development conference statement: Lactose intolerance and health. *NIH Consens State Sci Statements,* 27(2), pp. 1-27.
Swagerty, D.L., Jr., Walling, A.D. & Klein, R.M. (2002). Lactose intolerance. *Am Fam Physician,* 65(9), pp. 1845-50.
Szilagyi, A. & Ishayek, N. (2018). Lactose Intolerance, Dairy Avoidance, and Treatment Options. *Nutrients,* 10(12), p. 1994.

Szilagyi, A., Rivard, J. & Shrier, I. (2002). Diminished efficacy of colonic adaptation to lactulose occurs in patients with inflammatory bowel disease in remission. *Dig Dis Sci,* 47(12), pp. 2811-22.

Thorning, T.K., Raben, A., Tholstrup, T., Soedamah-Muthu, S.S., Givens, I. & Astrup, A. (2016). Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. *Food & nutrition research,* 60, pp. 32527-32527.

Tossavainen, O. & Kallioinen, H. (2008). Effect of lactose hydrolysis on furosine and available lysine in UHT skim milk. *Milchwissenschaft,* 63, pp. 22-26.

Tremaine, W.J., Newcomer, A.D., Riggs, B.L. & McGill, D.B. (1986). Calcium absorption from milk in lactase-deficient and lactase-sufficient adults. *Dig Dis Sci,* 31(4), pp. 376-8.

Troelsen, J.T. (2005). Adult-type hypolactasia and regulation of lactase expression. *Biochim Biophys Acta,* 1723(1-3), pp. 19-32.

Troise, A.D., Bandini, E., De Donno, R., Meijer, G., Trezzi, M. & Fogliano, V. (2016). The quality of low lactose milk is affected by the side proteolytic activity of the lactase used in the production process. *Food Res Int,* 89(Pt 1), pp. 514-525.

Ugidos-Rodriguez, S., Matallana-Gonzalez, M.C. & Sanchez-Mata, M.C. (2018). Lactose malabsorption and intolerance: a review. *Food Funct,* 9(8), pp. 4056-4068.

van Scheppingen, W.B., van Hilten, P.H., Vijverberg, M.P. & Duchateau, A.L.L. (2017). Selective and sensitive determination of lactose in low-lactose dairy products with HPAEC-PAD. *Journal of Chromatography B,* 1060, pp. 395-399.

Vasala et al (1996). WO1996022695- A method of surpressing sweetness of a lactose-hydrolyzed milk product. Finland. Publication nr: WO/1996/022695

Venema, K. (2012). Intestinal fermentation of lactose and prebiotic lactose derivatives, including human milk oligosaccharides. *International Dairy Journal,* 22(2), pp. 123-140.

Vesa, T.H., Marteau, P. & Korpela, R. (2000). Lactose intolerance. *J Am Coll Nutr,* 19(2 Suppl), pp. 165s-175s.

Vonk, R., Priebe, M., Koetse, H., Stellaard, F., Lenoir-Wijnkoop, I., Antoine, J.M., Zhong, Y. & Huang, C.Y. (2003a). Lactose intolerance: Analysis of underlying factors. *European Journal of Clinical Investigation,* 33, pp. 70-5.

Vonk, R.J., Priebe, M.G., Koetse, H.A., Stellaard, F., Lenoir-Wijnkoop, I., Antoine, J.M., Zhong, Y. & Huang, C.Y. (2003b). Lactose intolerance: analysis of underlying factors. *European Journal of Clinical Investigation,* 33(1), pp. 70-75.

Walstra, P., Geurts, T.J., Noomen, A., Jellema, A. & Boekel, M. (2005a). Dairy Technology-Principles of Milk Properties and Processes. *New York : Marcel Dekker inc., 1999.*

Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2005b). *Dairy science and technology, second edition*.

Wang, Y., Harvey, C.B., Hollox, E.J., Phillips, A.D., Poulter, M., Clay, P., Walker-Smith, J.A. & Swallow, D.M. (1998). The genetically programmed down-regulation of lactase in children. *Gastroenterology,* 114(6), pp. 1230-6.

Zadow, J.G. (2012). *Whey and Lactose Processing*: Springer Netherlands. Available from: https://books.google.se/books?id=SYT1CAAAQBAJ.