Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Galactose in dairy products

Agnes Abrahamson

Agronomy Program – Food Science
Independent Project in Food Science• Master Thesis • 30 hec • Advanced A2E
Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap, no 401
Uppsala, 2015

Galactose in dairy products

Agnes Abrahamson

Supervisor:	Åse Lundh, Professor at Department of Food science
Assistant Supervisor:	Henrik Hansson, Researcher at Department of Chemistry and Biotechnology
Examiner:	Monika Johansson, Researcher at Department of Food science

Credits: 30 hec
Level: Advanced A2E
Course title: Independent Project in Food Science
Course code: EX0425
Programme/education: Agronomy – Food Science

Place of publication: Uppsala
Year of publication: 2015
Cover picture: Sara Larsson
Title of series: Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap
Serie no: 401
Online publication: http://stud.epsilon.slu.se

Keywords: Milk, galactose, glucose, lactose, HPAE-PAD, Swedish dairy products

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Abstract

Milk and milk consumption has been subject of discussion for a long time, and is still a hot topic. Recently, as study was published that observed a correlation between milk intake and increased risk of fractures and mortality. The authors proposed the milk's content of D-galactose as the possible mechanism, since D-galactose is used indicated ageing in animal models. Therefore, this study aims to develop a method to determine the amount of galactose, glucose and lactose in milk and fermented milk and apply it on to dairy products on the Swedish market. Low-pasteurized milk, lactose free milk, UHT milk, yogurt, lactose free yogurt, Filmjölk, Onaka and A-fil were subjected to analysis, stored in different temperatures until their expiration date and sampled during this time period to analyse changes in carbohydrate content. A method for HPAE-PAD analysis, a method for quantification of carbohydrates, was developed using CarboPac SA10 column. The amount of galactose in milk is 7.12 mg/100 g. No changes in carbohydrate content could be observed, except for UHT milk stored at 30° C (12.22 mg/100 g) compared to 20° C (9.90 mg/100 g) and 4° C (9.39 mg/100 g) storage temperature. The amount of galactose in yogurt products are generally higher, compared to other types of fermented milks (1583.33 mg/100 g to 62.95 mg/100 g). The analysis method need further development regarding sample dilution.

Keywords: Milk, galactose, glucose, lactose, HPAE-PAD, Swedish dairy products

Sammanfattning

Mjölk och konsumtion av mjölk och mejeriprodukter har länge diskuterats, och är ännu ett aktuellt ämne. Nyligen publicerades en studie där ett samband mellan mjölkkonsumtion och risk för frakturer och dödlighet observerats. Författarna föreslog mjölkens innehåll av D-galaktos att vara orsaken, då D-galaktos används för att inducera åldrande i djurstudier. Syftet med denna studie är att utveckla en metod att analysera mängden galaktos, glukos och laktos i mjölk och fermenterade mjölkprodukter som finns på den svenska marknaden. Lagpastöriserad mjölk, laktosfri mjölk, mjölk med lång hållbarhet, yoghurt, laktosfri yoghurt, Filmjölk, Onaka och A-fil lagrades i olika temperaturer till sitt Bäst-föredatum och prover togs under tiden, för att se om mängden av de olika kolhydraterna ändrades under lagring. En metod för HPAE-PAD-analys utvecklades för att passa CarboPac SA10-kolonnen. Mängden galaktos i mjölk uppmättes till 7,12 mg/100 g. Ingen skillnad i kolhydratinnehållet kunde mätas under lagringen, förutom i mjölk med lång hållbarhet lagrad i 30° C (12,22 mg/100 g), jämfört med lagring i 4° C (9,39 mg/100 g) och 20° C (9,90 mg/100 g). Mängden galaktos i yoghurt är generellt högre, jämför med filprodukter (1 583,33 mg/100 g jämfört med 62,95 mg/100 g). Analysmetoden behöver fortsatt utveckling med avseende på spädning av prover.

Nyckelord: Mjölk, galaktos, glukos, laktos, HPAE-PAD, svenska mejeriprodukter

Table of contents

List of Tables

List of Figures

Abbreviations

AGE	Advanced Glycation End Products
CaP	Calcium Phosphate
E_{DET}	Detection Potential
E_{OX}	Oxidation Potential
EPS	Exopolysaccharides
E_{RED}	Reduction Potential
GALE	UDP-Galactose 4-Epimerase
GALK	Galactokinase
GALM	Galactose Mutarotase
GalNAc	N-Acetyl-D-Galactoseamine
GALT	Galactose-1-Phosphate Uridyltransferas
GlcNAc	N-Acetyl-D-Glucosamine
HCT	Heat Coagulation Time
HPAE	High Performance Anion-Exchange Chromatography
IMCU	International Milk Clotting Units
MFG	Milk Fat Globule
MFGM	Milk Fat Globule Membrane
NANA	N-Acetyl Neuraminic Acid
NF	Nanofiltration
PAD	Pulsed Amperometric Detection
PPI	Proton Pump Inhibitor
t_{DEL}	Delay Time
t_{INT}	Integration Time
UDP	Uridine-Diphosphate
UF	Ultrafiltration

1 Milk and dairy products

The interest in milk and its effect on human health is an ongoing concern. The controversial topic of milk and milk consumption has raised questions ever since the first dairies opened. From the start, milk was an unsafe food which spread tuberculosis and other diseases. After introduction of pasteurization, milk was praised as a healthy drink that saved people from malnourishment. Among with new processes, such as homogenisation, new health concerns appeared. For instance, the content of saturated fat in milk is still being debated.

Recently, a study claiming that high consumption of milk contributes to fractures and increased risk of mortality was published. In the study by Michaëlsson, et al. (2014) a correlation between milk intake and higher risk of fractures and mortality among middle aged and elder Swedes was observed. The authors suggested one of the milk sugars, galactose, as a possible mechanism. Galactose is used to study ageing in mice and rats. One glass of milk contains equal amounts of galactose per body weight as those used in animal studies, calculated as a split lactose molecule. This raises the question of how much galactose different dairy products contain and provided that galactose is bad for us, which products we should avoid.

The total milk delivered to Swedish dairies in 2013 was 2 870 000 tonnes. About 43% of this milk was processed into drinking milk or fermented products, e.g. yogurt and sour milk (*filmjölk* in Swedish). The milk production shows a declining trend since the 1980's (Jordbruksverket, 2013).

Also, the consumption of milk is decreasing, from top note 182.0 kg per capita and year in 1980, to 121.9 kg 2011, fermented milk included. The consumption of cheese, on the other hand, has increased from 14 kg per capita and year in 1980, to 18.2 kg in 2011 (Jordbruksverket, 2013). The average intake of milk including fermented milk was 245 g per person and day in 2011 according to the food query "Riksmaten" (2012). The survey was performed on 1797 Swedish women and men age 18-80. In the study it was reported that the intake of cream and crème fraiche was 8 g, and 25 g cheese per person and day. The consumption of butter and spreads was 11 g per person and day (ibid, 2012). The sale of lactose free dairy products increased with 12% compared with the previous year (Svensk Dagligvaruhandel, 2013)

1.1 Major milk components

Milk is an emulsion of milk fat globules dispersed in an aqueous phase. It is produced as feed for the young, and provides all required nutrients for growth (Table 1). Unless otherwise stated, only bovine milk is discussed in this report.

Table 1. *Major components of milk*

Component	Average in milk (g/100 g)	
	Walstra, Wouters, & Geurts (2006)	Lindmark Månsson (2012)
Water	87.10	86.86
Lactose	4.60	4.73
Lipids	4.00	4.18
Protein	3.30	3.47
Minerals	0.70	0.76
Organic acids	0.17	0.15*

*only citric acid

1.1.1 Lactose

Lactose is the main carbohydrate in milk and accounts for 54% of the total non-fat milk solids (Saxelin, Korpea, & Mäyrä-Mäkinen, 2003). Lactose consists of one glucose unit and one galactose unit, connected by a glycosidic linkage in β-configuration, described as β-D-galactopyranosyl-(1→4)-D-glucopyranose (Figure 1). Lactose is a reducing sugar (Coultate, 2009) unique to milk, and it has been found in milk from nearly all mammals.

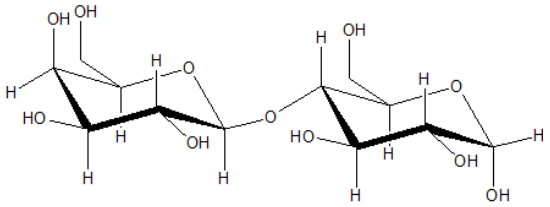


Figure 1. The structure of lactose. Picture: Agnes Abrahamson.

Lactose is synthesized in the Golgi apparatus of the lactating cell. Lactose is synthesised by the proteins α-lactalbumin and galactosyltransferase, which catalyses the reaction whereby lactose is formed from glucose and uridine-diphosphate-galactose (UDP-galactose). Glucose derived from blood glucose is used as a precursor for lactose. Glucose is phosphorylated into UDP-glucose, and then converted to UDP-galactose in the cytosol of the lactating cell. This molecule is transported into the Golgi lumen by active transport. Glucose is transported into Golgi using glucose transporter GLUT1.

The lactose biosynthesis:

Glucose + UDP-Galactose → Lactose + UDP

The Golgi membrane is impermeable to lactose, and so water is drawn in to the Golgi vesicle via osmosis (Walstra, Wouters, & Geurts, 2006).

The monomers of lactose occurs predominantly in the pyranose ring form. The O-C linkages in glucose can break, since it is a reducing end, and form an aldehyde.

The open ring form can convert lactose from α- to β-anomer. This process is known as mutarotation. Under normal conditions in milk, less than 0.1% of the lactose is in the open chain form, but high pH and temperature increase the levels. The galactose unit can only be in β-pyranose form. The reaction α ↔ β equilibrium takes hours at room temperature, but only a few minutes at 70° C. The equilibrium at room temperature is 37% α- lactose and 63% β-lactose (Walstra, Wouters, & Geurts, 2006).

Lactose is readily metabolised by microorganisms, which makes milk an easily fermentable substrate. A large number of bacterial species are able to ferment lactose into lactic acid, and are therefore named lactic acid bacteria (LAB). Controlled fermentation by LAB is used in production of dairy products such as yogurt and cheese (Kelly & Bach Larsen, 2010).

1.1.2 Milk fat

Nearly all lipids in milk are found within the milk fat globules (MFG). Milk fat globules are spherical colloidal assemblies of milk lipids with a core rich in triacylglycerol. Triacylglycerol make up 98% of the milk fat, but also small amounts of di- and monoacylglycerol, cholesterol and cholesterol esters, free fatty acids, and phospholipids are present. The fat composition varies with species, breed, feed, stage of lactation, number of lactations, etc. The milk fat is enclosed by a membrane (MFGM) built up by phospholipids, proteins and glycoproteins which prevent aggregation and coalescence. The size of the MFG varies from 0.1 - 20 µm (Lopez, 2011). Milk fat contains approximately 400 different fatty acids, of which approximately 70% are saturated fats (Lindmark Månsson, 2008), and was thought to have a negative impact in cardiovascular health, although later reviews has failed to find an increased risk of milk intake and cardiovascular disease and stroke (Huth & Park, 2012).

1.1.3 Milk proteins

The proteins of milk are divided into caseins and whey proteins. Caseins include α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein and make up 80% of the total protein content. The other 20% consists of whey proteins, including β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulins. The distinction between the two groups is based on solubility at pH 4.6 (Kelly & Bach Larsen, 2010).

The casein fraction is organized in micelles, a large network with a hydrophobic core and hydrophilic outer layer allowing the caseins to remain suspended in the aqueous phase. Caseins are small proteins, form little tertiary structure and form hydrophobic bonds with each other which makes them relatively heat stable. Clusters of calcium (Ca) and phosphate (P) form as milk is synthesized, and due to their low solubility the caseins rapidly bind CaP to prevent nucleation. K-casein differs

from the other caseins with its ability to become glycosylated with oligosaccharides consisting of galactose, and one or two N-acetyl neuraminic acid (NANA) residues. These groups have negative charges, and are thus hydrophilic. The κ-casein works as a chain terminator in the 3D structure of the casein micelle. The peptide bond in the glucomacropeptide is readily hydrolysed in presence of enzymes such as rennet, and this property is used in cheese making process (Walstra, Wouters, & Geurts, 2006).

Whey proteins build a heterogeneous fraction with the common feature that they are soluble in the serum phase of milk. They are typically globular proteins and the two major ones are β-lactoglobulin and α-lactalbumin, which are both synthesized by the secretory cells. β-lactoglobulin makes up the major part of the whey proteins, α-lactalbumin, as previously mentioned, is a coenzyme in the synthesis of lactose. Other major whey proteins include the immunoglobulins, and IgG, IgA and IgM occur at high levels in colostrum with the biological function to provide protection to the newly born calf (Walstra, Wouters, & Geurts, 2006).

1.1.4 Vitamins and minerals

Milk is a good source of essential vitamins and minerals (Table 2). Skimmed milk, but also some fermented milks, are often fortified with vitamin A. This is done to counteract the loss of vitamin A during the separation of the cream (Livsmedelsverket, 2013). Vitamin C is degraded during pasteurization of the milk.

Table 2. *The average content of vitamins, minerals and trace elements in Swedish milk, adapted from Lindmark Månsson (2012)*

Fat soluble vitamins (µg/100g)		Water soluble vitamins (µg/100g)		Minerals and trace elements (mg/100g)	
α-tocopherol	107.00	Vitamin C (mg)	0.80	Potassium	159.00
Retinol eq.	38.60	Niacin eq.	740.00	Calcium	119.00
Retinol	36.00	Pantothenic acid	477.00	Phosphorus	101.00
Vitamin K	1.22	Riboflavin	143.00	Chloride	93.00
Vitamin D	0.01	Thiamine	49.00	Sodium	39.00
		Vitamin B6	43.30	Magnesium	11.00
		Folate	14.90	Zink	0.04
		Biotin	1.29	Iron	0.02
		Vitamin B12	0.57	Cupper	0.01
				Manganese	0.00
				Iodine (µg)	11.70
				Molybdenum (µg)	4.68
				Selenium (µg)	1.60
				Chromium (µg)	<0.01

Milk contains inorganic and organic salts. The salts in milk serum are distributed as dissolved salts. In the colloidal phase, on the contrary, they are undissolved, i.e. CaP in casein micelles. The most important cations are sodium, potassium, calcium, magnesium, whereas the anionic ones are chloride, sulphate, carbonate, phosphate and citrate (Walstra, Wouters, & Geurts, 2006). Changes in lactose levels will be counteracted by changes in milk salt levels, to preserve constant osmotic pressure (Kelly & Bach Larsen, 2010).

1.2 Milk carbohydrates in human nutrition

Lactose provides 30% of the energy from milk (Saxelin, Korpea, & Mäyrä-Mäkinen, 2003), and gives milk its sweet taste, although lactose has low relative sweetness (Table 3). Digested sugars provide approximately 4 kJ/g. Since not all lactose is degraded and absorbed, but fermented in the colon the real energy value will be lower, approximately 2-4 kJ/g. The energy from colon fermentation comes from volatile fatty acids produced by the intestinal microbiota (Schaafsma, 2008).

Table 3. *Relative sweetness of milk sugar, compared to sucrose. Adapted from Schaafsma (2008)*

Relative sweetness of milk sugars	
Sucrose	1.0
Lactose	0.2-0.4
Glucose	0.6-0.7
Galactose	0.5-0.7

1.2.1 Lactose metabolism by the Leloir pathway

In humans, lactose metabolism begins with hydrolysis of lactose by lactase, an enzyme developed in the brush border of the small intestine. Lactase, or β-galacto-sidase, catalyses the hydrolysis of the β-1→4 linkage in lactose, leaving glucose and galactose to be absorbed by the small intestine. The sugar monomers are transported through the portal vein to the liver, where galactose is converted to glucose via the Leloir pathway. The Leloir pathway starts with the β-D-galactose being epimerized to α-D-galactose with the enzyme galactose mutarotase (GALM). β-D-galactose is then phosphorylated into galactose 1-phosphate by ATP dependent galactokinase (GALK). The enzyme galactose 1-phosphate uridylyltransferase (GALT) converts galactose 1-phosphate into UDP-galactose, which is epimerized into UDP-glucose, by UDP-galactose 4-epimerase (GALE) (Holden, et al., 2003). UDP-glucose can enter glycogenesis with glycogen being the end product, i.e. storage energy.

1.2.2 Lactose intolerance

While countries in the northern hemisphere such as Sweden in general have lactose tolerant population approximately 65% of the world population has developed lactose intolerance (Asp, 2006). A person with lactose intolerance experiences intestinal discomfort after lactose is ingested, due to lactase deficiency. The lactose is not digested in the small intestine, but fermented in the colon, causing bloating, flatulence and diarrhoea. There are two different types of lactose intolerance; primary and secondary acquired intolerance. The common lactase deficiency, which is genetically determined, is acquired after the first years of life, with a reduction in lactase production to 5-10% (Asp, 2006). Secondary acquired lactose intolerance can occur as a result from disease in the small intestine, where the digestion enzyme is located (Vesa, et al., 2000). Lactose intolerance is not to be confused with milk allergy, in which the milk proteins are the cause of illness (Turinen & Korpela, 2004).

1.2.3 Galactosemia

After absorption in the small intestine, galactose is transported to the liver and metabolized via the Leloir pathway (chapter 1.2.1). This process requires the enzyme GALT, otherwise galactose 1-phosphate will accumulate in the tissues and eventually lead to blindness and brain damage (Asp, 2006). The consequences of GALT deficiency are known as galactosemia. Galactosemia is a rare genetically determined disease, with only 40 known cases in Sweden in total (Socialstyrelsen, 2013).

High concentration of galactose 1-phosphate in the tissues leads to formation of galactitol, a sugar alcohol, in the lens of the eye, causing cataracts. Galactitol is a dead-end pathway (Leslie, 2003). Accumulation in the tissues may also lead to reduced effect of various enzymes, which can explain the impact on kidneys, liver, and central nervous system. When GALT is not functioning, the production of UDP-galactose is inhibited. UDP-galactose is necessary for appropriate glycosylation of proteins, and hence the correct function of hormones, receptors and structures in the nervous system (Ridel, et al., 2005).

Galactosemia in infants is found by screening of new-borns'. Patients are treated with a galactose restricted diet, e.g. galactose free formula. Galactose occurs in most foodstuffs, therefore total exclusion is complicated. There is also an endogenous production of galactose to consider, and studies suggest that the endogenous production is more important than galactose from the diet. Even a galactose restricted diet may eventually lead to mental retardation and ovarian failure. Exclusion of galactose-rich dairy foods from the diet can lead to less bone density due to low calcium intake (Ridel, et al., 2005).

However, fruits and vegetables are also sources of dietary galactose, according to Gross & Acosta (1991), with amounts ranging from 0.1 mg/100 g fresh weight in artichokes, to 35.4 mg/100 g fresh weight in persimmon.

1.2.4 Milk carbohydrates as prebiotics

Prebiotics are compounds that stimulate growth and/or the function of beneficial microbiota in the gastrointestinal tract, and are shown to have positive health effects on the host (Vyas & Ranganathan, 2012).

Galacto-oligo-saccharides (GOS) consists of several galactose units joined together with a terminal glucose (gal-$(gal)_n$-glu). GOS are produced from lactose by the enzyme galactosyltransferase and act as a prebiotic, since they are resistant to digestive enzymes (Schaafsma, 2008). They occur naturally in human milk, and stimulate the growth of bifidobacteria and lactobacilli in breast-fed infants (Saxelin, Korpea, & Mäyrä-Mäkinen, 2003). GOS of bovine origin are commercially used and have various applications in the food industry, such as infant formula and ice cream. The interest in GOS is increasing, since it can be used in functional foods (Schaafsma, 2008). The amount of GOS in bovine milk increases during fermentation (Toba, et al., 1983).

Exopolysaccharides (EPS) are a diverse group of long-chain saccharides that bacteria synthesize and secrete outside their cell. EPS consists of branched, repeated units of sugars and sugar derivates, e.g. glucose, galactose, mannose, N-acetylglucoseamine (GlcNAc), N-acetylgalactoseamine (GalNAc) and rhamnose. During growth the bacteria secrete the EPS as a loose slime into the surroundings protecting the microbe from adverse conditions. In fermented milk products, EPS improve the rheological properties, stability and mouth feel. In human metabolism, EPS increase the gastrointestinal transit time, and thus facilitate the colonization by Bifidobacteria and Lactobacilli and is considered a prebiotic (Patel, et al., 2012: Badel, et al., 2011).

1.2.5 Galactose and ageing

A recently published epidemiological study by Michaëlsson, et al. (2014) concluded that high milk consumption was associated with higher mortality and incidence of fractures, especially in women. The study used two large Swedish cohorts where the participants had answered a food frequency questionnaires and collected blood samples had been analysed for biomarkers of oxidative stress and inflammation. The food frequency questionnaires were performed once in the male cohort, and twice with 20 years in between in the female cohort. The fracture incidence was higher in women who consumed more than 3 glasses a day. Both men and women with high milk consumption were associated with higher risk of mortality. The proposed mechanism for the observations was related to the lactose and D-galactose content in non-fermented dairy products, e.g. liquid milk (ibid, 2014).

The reason for suggestion this mechanism is that D-Galactose has been used to induce ageing in rats and mice models (Song, et al., 1999). Galactose is, as earlier stated, a reducing sugar and can participate in the Maillard reaction. When the reaction takes place *in vivo*, the products are called advanced glycation end products, AGE. The formation of AGE is proportional to the glucose concentration in the blood and is regarded as a long-term effect of high blood glucose levels. Glycated haemoglobin, HbA_{1C}, is used to measure the average glucose concentration in the blood. Ageing and degenerative diseases, like atherosclerosis, diabetes mellitus and Alzheimer's disease are thought to have a connection to AGE (Asp, 2006).

In a study by Song et al (1999), mice injected with D-galactose showed an ageing process similar to "naturally" aged rats, which suggest that AGE is involved in the ageing process. The rats were injected with 50 mg/kg of D- galactose. The effect of the D-galactose model is dependent on gender, exposure time and age of the animal, where females are thought to be more resistant to the oxidative stress induced by the galactose treatment (Haoa, et al., 2014).

1.3 Effects on milk carbohydrates in dairy processes

After the milk is delivered to the dairy, it undergoes several treatments in the processing to various dairy products in order to protect the consumer from potentially harmful bacteria, inactivate enzymes and preserve the quality of the milk. Microbiological changes occur during storage of milk, but also enzymatical, chemical and physical changes (Walstra, Wouters, & Geurts, 2006).

1.3.1 Heat treatment

Various combinations of temperature and time are applied to protect the consumer from potentially pathogenic and/or product spoiling bacteria, inactivate enzymes and preserve the quality of the milk. Microbiological changes occur during storage of milk, but also enzymatical, chemical and physical changes. Pasteurization is a process by which the milk is heated to temperatures above 70° C in order to kill pathogens and spoilage bacteria, but also enzymes such as lipases and proteases are inactivated. The time/temperature conditions have been established to ensure the inactivation of *Mycobacterium tuberculosis* (Lewis, 2010). Low pasteurization, 15 sec at 72° C, kills almost all pathogens and inactivates the enzyme alkaline phosphatase. High pasteurization, from 85° C up to 100° C for 20 sec, inactivates most of the enzymes in milk, and all vegetative microorganisms. Sterilization, 30 min at 110° C, kills all microorganisms, including spores. One type of sterilization is ultra-high-temperature, short time (UHT) where temperatures above 130° C are applied for a short period of time, e.g. 10 sec (Walstra, Wouters, & Geurts, 2006). In production of UHT milk, either direct or indirect heat treatment is applied. Direct heat

treatment is the most efficient, and less chemical changes occur (Jansson, et al., 2014). Production of UHT milk is associated with problems such as aggregation and sedimentation, age gelation, fat separation, and poor flavour (i.e. cooked flavour).

Changes in milk caused by heat treatment can be reversible or irreversible. A number of reactions take place during heat treatment (Table 4). The changes are caused by a combination of temperature and time.

Table 4. *Examples of the changes in milk during heat treatment, depending on temperature and time. After Walstra, Wouters & Geurts (2006)*

Changes in milk during heat treatment
Gases are partly removed
The amount of colloidal calcium increases (calcium moves from serum into micelle phase)
The amount of inorganic phosphate increases
pH decreases
Serum proteins are denatured, the insoluble part of them binds to κ-casein
Formation of sulfhydryl groups
Enzymes are inactivated
Maillard reactions occur
Lactose reactions, e.g. isomerization into lactulose and formation of formic acid
The redox potential decreases
Some vitamins are degraded

Reactions involving lactose include the Maillard reactions and the isomerization of lactose. These have implications for flavour, colour, nutritive value and pH (Walstra, Wouters, & Geurts, 2006), and also for the stability of the milk products (Jansson, et al., 2014).

The Maillard reaction involves a carbonyl group of lactose and the nucleophilic amino group of an amino acid, most commonly lysine residues participate. Maillard reactions include a wide range of reactions that involve sugar and amino acids, and form a variety of end products. In milk, the advanced reaction forms melanoidins, causing browning of the milk, change in taste and decrease of nutritive value. The reaction occurs during intense heating of milk, e.g. 120° C in UHT-treatment. Once the Maillard reaction has started, it can continue at room temperature during storage (ibid, 2006).

During heat treatment, lactose undergoes isomerization, e.g. the Lobry de Bruyn-van Ekenstein reaction, to lactulose. The reaction involves isomerization of the glucose unit into fructose, thus lactulose consists of fructose and galactose. In sterilized milk, 0.3-1 g/l is formed. Further on, the lactulose can split, leaving a free galactose and formic acid, formed from fructose (Walstra, Wouters, & Geurts, 2006). The

amount of lactulose in milk is positively correlated to the intensity of the heat treatment, and is therefore used as a reliable heat treatment index (Deeth, 2010). Lactulose is not found in nature, and is not absorbed by the human digestive system. It is regarded as a functional ingredient and could stimulate the growth of bifido bacteria (Özer, et al., 2004).

The heat stability of milk can be determined by investigation of the heat coagulation time (HCT). This is done by incubation of milk in a sealed tube at a high temperature, usually approximately 130° C, and visual determination of the time it takes for the milk to coagulate. Major factors of importance for the heat stability of the milk include pH, protein content, and minerals such as calcium and phosphate (Williams, 2002).

1.3.2 Fermentation

Lactic acid bacteria (LAB) are able to ferment the lactose in milk into lactic acid and flavour compounds such as acetaldehyde and diacetyl, and carbon dioxide. The lactic acid lowers the pH of the milk from 6.7 to about 4.3-4.5 (Fondén, Saarela, Mättö, & Mattila-Sandholm, 2003). Fermented milks are better tolerated by lactose intolerant individuals (Alm, 1982), and are believed to have a probiotic effect (Fondén, Saarela, Mättö, & Mattila-Sandholm, 2003). Traditional strains used in yogurt production are *Lactobacillus delbrueckeii* subsp. *bulgaricus* and *Streptococcus thermophilus*. In the Scandinavian countries, mesophilic starter cultures such as *Lactococcus* spp. and *Leuconostoc* spp are often used. LABs are attributed to alleviate lactose intolerance symptoms, viral diarrhoea, infant gastroenteritis, treat antibiotic associated diarrhoea, alleviate dermatitis in children, have positive effects on bladder and cervical cancer, modulation of intestinal microbiota, modulation of the immune system and lowering biomarkers of inflammation, according to the review by Kailasapathy (2013). Not all positive effects can be attributed to all LABs.

The bacteria start the fermentation by transporting lactose into the cell either as it is, or by phosphorylation and use of transport proteins. Hydrolysis of lactose into glucose and galactose is catalysed by β-galactosidase. Further on, the mode of fermentation pathways differs, depending on LAB strain; if it is a homofermentative or heterofermentative bacteria. Homofermentative bacteria ferment glucose via the Embden-Meyerhof pathway and galactose via the tagatose pathway, producing lactic acid. Some bacteria do not ferment the galactose moiety, which instead will be secreted and accumulate in the media. Heterofermentative LAB lack one key enzyme and ferment lactose via the phosphoketolase pathway, which produces lactate, ethanol and carbon dioxide (Adams & Moss, 2006).

In the production of traditional Kefir, neither glucose nor galactose can be detected during the fermentation. This suggests that the monosaccharides are metabolized immediately after lactose degradation. Lactose decreases significantly during

the first 24h of fermentation, and then more slowly until the end of fermentation (~8 days). In the study, a commercial starter culture, containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Kluyveromyces marxianus* subsp. *fragilis* was used (García Fontán, et al., 2006).

Lactose, galactose and glucose content of some common dairy products are presented in Table 5.

Table 5. *Lactose, galactose and glucose content of commercially available dairy products. The methods for determining the carbohydrate content differs, and also sampling in relation to fermentation time*

Dairy product	Lactose g/100 g	Galactose g/100 g	Glucose g/100 g	Reference
Milk	5.00	0.00	-	Alm (1982)
	5.00	0.00	0.00	Goodenough & Kleyn (1976)
	4.10	0.01	0.02	Cataldi, et al. (2003)
Lactose free milk	0.42	1.96	2.06	Pirisino (1983)
Yogurt	2.40	0.98	-	Alm (1982)
	4.06	1.85	0.05	Goodenough & Kleyn (1976)
	4.05	1.30	0.00	Li, et al. (1983)
	4.51	1.29	0.34	Toba, et al. (1983)
Sour milk	3.70	0.05	-	Alm (1982)
	3.97	0.81	0.01	Goodenough & Kleyn (1976)
A-fil	2.60	0.70	-	Alm (1982)

Not all lactic acid bacteria are able to ferment galactose (Table 6). This has implications for the galactose content in the final dairy product. There are exceptions, e.g. Anbukkarasi, et al. (2014) reported that it is possible to use galactose fermenting strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* in youghurt production. Incomplete galactose fermentation gives excess amounts of galactose in the product, which has been assosciated with poor quality. For instance, galactose contributes to the browning of cheese when cooked, which is not always desired (Neves, et al., 2010).

Table 6. *Galactose fermentation properties of the most common strains of lactic acid bacteria used in fermented dairy products found on the Swedish market*

Strain	Galactose fermentation	Reference
Streptococcus thermophilus	-	Anbukkarasi, et al. (2014); Nishimura, (2014)
Lactobacillus delbrueckii subsp. *bulgaricus*	-	Anbukkarasi, et al. (2014); Nishimura, (2014)
Lactococcus lactis ssp. *lactis*	+	Åkerberg, et al. (1998);Cocaign-Bousquet, et al. (1996)
Lactococcus lactis ssp. *cremoris*	+	Garrigues, et al. (1997);Cocaign-Bousquet, et al. (1996)
Lactococcus lactis ssp. *lactis* var.*diacetylactis*	+	Cocaign-Bousquet, et al. (1996)
Leuconostoc cremoris	+	Cogan & Jordan (1994)
Bifidobacterium longum	+	Abbad Andaloussi, et al. (1995)
Lactobacillus acidophilus	+	Holzapfel & Wood (2014)

1.3.3 Lactose reduction

Individuals with lactose intolerance have less acceptance for dairy products. Yogurt is generally well tolerated in these individuals, as well as cheese matured for at least 6 months (Suarez, et al., 1995). During the last decade, however, the dairy industries have increased the production of lactose reduced dairy products to meet the demand from these consumers. To claim a product is "lactose free" in Scandinavia, the upper limit of lactose content is 100 mg/100 g product, but no internationally recognized limit exists (Harju, et al., 2012). The upper threshold for lactose free infant formula is 10 mg/100 kcal (European Food Safety Authority, 2010). Lactose reduction can be performed by adding enzyme, e.g. β-galactosidase, to degrade lactose. Filtration or chromatographic methods are alternative techniques, whereby sugars are removed. It is common to use a combination of techniques in order not to alter the taste too much (Harju, et al., 2012), since the different milk sugars has different sweetness (Table 3).

Hydrolysis of lactose into galactose and glucose was the first applied method to reduce the lactose content. Hydrolysis of 70% of the lactose will increase the sweetness of the milk to the same extent as adding 2% of sucrose (ibid, 2012). Lactose hydrolysed milk is associated with several difficulties in the processing, i.e. the pH of milk promotes growth of microorganisms, milk proteins tend to adhere on the surfaces and cause fouling, and the β-galactosidase enzyme is not very stable in the classical methods (Harju, Kallioinen, & Tossavainen, 2012).

Lactose can be removed from milk using chromatographic separation (Turinen & Korpela, 2004), combined with lactose hydrolysis. The hydrolysis takes places in an ion exchange chromatogram column, where β-galactosidase from bacterial origin

is attached to the resin. A minimum of 80% of the lactose in a product is degraded this way. Fermentation of lactose hydrolysed milk is shown to give rise to galacto-oligo-saccharide formation (Toba, et al., 1983).

Lactose reduction based on membrane techniques is a combination of ultrafiltration (UF) and nanofiltration (NF). Other smaller compounds are also separated along with lactose, which reduces the mineral content of the milk. The minerals can be returned with reverse osmosis of the retenate (Harju, et al., 2012). Around 40 % of the lactose is removed this way, the residual lactose is hydrolysed with β-galactosidase (Jansson, et al., 2014). Studies suggests that lactose hydrolysed milk is more prone to chemical changes during processing and storage, than conventional milk. The milk is more prone to Maillard reactions, and the added lactase enzyme may have proteolytic activity (ibid, 2014).

1.4 Determination of carbohydrates by HPAE-PAD

HPAE-PAD (High Performance Anion-Exchange Chromatography, Pulsed Amperometric Detection) can be used to determine anionic substances with high sensitivity and selectivity. It is most commonly used for determination of carbohydrates, including mono-, di-, oligo- and polysaccharides, sugar alcohols and amino sugars for food science purposes. The major advantage of the method is that it is quantitative and that there is no need for derivatization (Peris-Tortajada, 2013).

HPAE separates anionic substances, which are either anions in their common stage, or can become anions at higher pH, such as carbohydrates. Anion-exchange columns have a polymer-based stationary phase and an alkaline eluent is used to create the high pH required. Most carbohydrates are slightly acidic in normal conditions. At high pH they are partially ionized, and can be separated in anion-exchange chromatography. As an example, the pKa for galactose is 12.39. Sodium hydroxide is often used as an eluent (Nardinello, Palermo, Quinto, & Centonze, 2012).

The separation of carbohydrates with similar structures such as galactose and glucose is problematic due to the co-elution, since their retention time does not differ significantly (Cataldi, et al., 1999). Closely related carbohydrates such as glucose and galactose can be separated more efficiently if the pH is close to their pKa values, i.e. pH 12. Post column addition of a strong alkaline eluent is needed to provide the requirements of an alkaline environment for the detection electrode (Corradini, et al., 2012). Isocratic conditions are required to separate similar carbohydrates (Nardinello, Palermo, Quinto, & Centonze, 2012). Moreover, milk is a complex matrix which further complicates the separation. The concentration of sodium hydroxide can alter the retention time of the carbohydrates. It is important to prevent the contamination of carbonate, which is detrimental to the chromatography

(Cataldi, et al., 1999). At the high pH applied in Ion Exchange columns, carbonate forms a divalent anion and binds strongly to the column and interferes with the retention of the carbohydrates (Corradini, et al., 2012). Fresh eluents must be prepared at least once a week, and always kept in an inert gas atmosphere. Post-column addition of sodium hydroxide after each run is common, to regenerate the column (Nardinello, Palermo, Quinto, & Centonze, 2012).

PAD detects the eluted carbohydrates, by applying different potentials during a given time period. This method is favourable under alkaline conditions. The detection is performed at a detection cell, which consists of a gold electrode and an Ag|AgCl reference electrode (ibid, 2012). The potential variations, known as waveforms, create oxidizing and reducing conditions on the surface of the gold electrode (Thermo Fisher, 2014). First, a positive potential is applied (detection potential, E_{DET}) and the hydroxyl groups on a carbohydrate are oxidized, and the current is measured at a certain potential. The generated current is proportional to the amount of analyte. The detection potential ranges from 0-200 mV in this step. When the carbohydrates are oxidized, by-products will attach to the surface of the electrode. After oxidation, a reduction step is performed by raising the potential enough to oxidize the gold at the surface of the electrode whereby the carbohydrate by-products will desorb. The potential (oxidation potential, $E_{OX} > E_{DET}$) is 650-800 mV. Finally, the restoration of the electrode by reduction of the gold-oxide is performed by a low potential at -300 mV (reduction potential, E_{RED}). The current E_{DET} is only measured during a short sampling interval (integration time, t_{INT}), after a delay period (delay time, t_{DEL}) that allows the current to decay (Nardinello, Palermo, Quinto, & Centonze, 2012). The PAD method for detection is highly specific and sensitive with a detection limit of 10 pmol (Peris-Tortajada, 2013).

1.5 Objectives

The recent interest in milk consumption and health, and especially galactose as a possible substance involved in age-related conditions, raises the question of how much galactose, glucose and lactose different dairy products contain. The aim of this study is to describe the bovine milk with focus on galactose, the composition of milk carbohydrates and the impact by various dairy processes. A sensitive method for determination of the amount of galactose, glucose and lactose has been developed and applied to common Swedish dairy products.

The specific aims of this study were:

1. To optimize a method for quantification of galactose, glucose and lactose in milk and fermented milk using HPAE-PAD
2. To apply the method for determination of the amount of galactose, glucose and lactose in different dairy products found on the Swedish market and to study if there are changes in content during storage until their expiration date. Products included in the analysis: regular milk (low pasteurized, 1.5% fat), UHT milk, lactose free milk, yogurt, lactose free yogurt, Filmjölk, A-fil, and Onaka.

Hypotheses:

3. Galactose, glucose and lactose in low pasteurized milk will not change during storage, due to its short shelf life and lack of microorganisms.
4. Lactose free milk will contain higher level of monosaccharides, and the levels may increase if the lactase enzyme is still active.
5. Lactose is expected to decrease due the heat-induced reactions in UHT treated milk. The amount of monosaccharides is not expected to change in milk stored at 4° C, but increase in milk stored at 30° C.
6. Fermented milk is expected to contain lower amounts of lactose to start with, due to the activity of the lactic acid bacteria. The amount of lactose is also expected to decrease during storage.
7. The amount of galactose in the yogurt products is expected to be higher, compared to the other types of fermented milks.

2 Experimental

2.1 Chemicals

Sodium hydroxide, 50% solution in water (1.515 g/ml) (Merck, Darmstadt, Germany), sodium acetate 99,9%, reagent grade water (Sigma-Aldrich), 18.2 MΩ-cm resistance, filtered through a 0.2 µm membrane prior to use, and helium, 4.8 grade 99.998%. Potassium hexacyanoferrate(II) trihydrate (Carrez solution I), zinc sulphate heptahydrate (Carrez solution II), lactose, lactulose, glucose, galactose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine were obtained from Sigma-Aldrich (St- Louis, MO, USA). Calf rennet, i.e. chymosin was obtained from Chr. Hansen A/S (Chy-max Ultra, Hørsholm, Danmark).

2.2 Apparatus

All experiments were performed using a Thermo Scientific Dionex system ICS-3000 (Sunnyvale, CA, USA) equipped with a DP Dual Pump. The samples were injected using a 10µl injection loop. The carbohydrates were separated using a Dionex CarboPac SA10, PA10 and PA10 separation column (all of them 2×250mm), coupled with a guard column (2×50 mm) at 25° C. The detection cell included a disposable gold electrode with PTFE gasket and a pH-Ag|AgCl reference electrode. Data acquisition and processing was done using Chromeleon Chromatography Data version 6.80 (Thermo Fischer Dionex) and a personal computer.

2.3 Standard preparation

External standards of lactose, galactose, and glucose were prepared in concentration of 20, 10, 5, 2.5 and 1 mg/l and dissolved in unionized water. 200 µl Carrez solution I and II was added. In addition, solutions of 10 mg/l of lactulose, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine were prepared.

2.4 Milk samples

The dairy products (Table 7) were purchased at local supermarkets in Uppsala. All milks were used as available, e.g. not all of them were freshly produced. Fresh milk was stored for 8 days, and sampling was performed at day 1, 4 and 8. The fermented milks and lactose free milk was stored for 20 days, and sampling was done on day 1, 4, 8, 12, 16 and 20. The UHT milk was stored for 10 weeks, and sampling were done at day 1, 8, 15, 22, 29, 36, 43, 50, 57, and 64. Additionally, the

UHT milk was stored at different temperatures (4° C, 20° C and 30° C) in order to simulate different storage scenarios. All other products was stored at 4° C. On each sampling occasion an un-opened package was used and the samples aliquots were frozen (-20° C) until the day of analysis. The milks were sampled within four days from the production date, which means day 1, 4, etc. does not indicate the age of the product, only how long it has been stored for the analysis. Sampling was performed in duplicates.

Table 7. *The dairy products, all manufactures by Arla Foods (Sweden), are listed along with ingredients, according to the package label. Starter culture after Lo (2014)*[1]

Product	Ingredients	Starter culture
Milk 1.5% fat	Low-pasteurized milk, vitamin A and D, of which carbohydrates 4.9 g/100 g	-
Lactose free milk 1.5% fat	High-pasteurized milk, lactase, vitamin A and D, of which carbohydrates 2.7 g/100 g	-
UHT milk 1.5% fat	Ultra-high pasteurized milk, of which carbohydrates 5.0 g/100 g	-
Yogurt 3% fat	High-pasteurized milk, starter culture, of which carbohydrates 3.6 g/100 g	*Streptococcus thermophilus, Lactobacillus bulgaricus*
Lactose free yogurt 1.5% fat	High-pasteurized milk, milk protein, lactase, starter culture, of which carbohydrates 2.9 g/100 g	*Streptococcus thermophilus, Lactobacillus bulgaricus*
Filmjölk 3% fat	High-pasteurized milk, starter culture, of which carbohydrates 3.8 g/100 g	*Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris, Lactococcus lactis* ssp. *lactis* var. *Diacetylactis, Leuconostoc cremoris*
Onaka 1.5% fat	High-pasteurized milk, milk protein, starter culture, vitamin A and D, of which carbohydrates 3.8 g/100 g	*Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris, Lactococcus lactis* ssp. *lactis* var. *Diacetylactis, Leuconostoc cremoris, Bifidobacterium longum, Bifidobacterium lactis*
A-fil 3% fat	High-pasteurized milk, starter culture, of which carbohydrates 3.8 g/100 g	*Lactococcus lactis* ssp. *lactis, Lactococcus lactis* ssp. *cremoris, Lactococcus lactis* ssp. *lactis* var. *Diacetylactis, Leuconostoc cremoris, Lactobacillus acidophilus*

2.5 Preparation of samples

The regular milk and lactose free milk samples were prepared by adding 200 µl calf rennet (200 IMCU) to 10 ml milk followed by incubation for 20 minutes in 30°

1. Lo, Winnie, Arla Foods (2014) E-mail October 24.

C. The clotted milk was cut with a knife, and allowed to rest for approximately 2 minutes. The whey was separated using a filter (Whatman no.3) and 1 ml whey was diluted 10 times in deionized water. 1.75 ml each of Carrez solution I (2.7 g $K_4Fe(CN)_6$•3H2O in 100 ml water) and II (5.5 g $ZnSO_4$•$7H_2O$ in 100 ml water) was added, followed by centrifugation (Sorvall Super T21 Centrifuge, Sunnyvale, CA, USA) at 4000 rpm for 30 minutes at 4° C. The supernatant was filtered through a 0.2 µm membrane. 1 g de-proteinized milk was collected and diluted with 1 ml deionized water.

The fermented milk and UHT milk were prepared by adding 1.75 ml each of Carrez solution I and II to 10 ml milk, followed by centrifugation at 4000 rpm for 30 minutes at 4° C. The supernatant was diluted 100 times and filtered through a 0.2 µm membrane. 1 g de-proteinized milk was collected, diluted with 1 ml deionized water after the procedure developed by Cataldi, et al. (2003).

2.6 Analytical procedure

The method was optimized with regards to the parameters presented in Table 8.

Table 8. *Method optimization parameters*

Parameters	After Cataldi, et al. (2003)	Modified Cataldi, et al. (2003)	Sugar analysis in lignocellulose	Modified lignocellulose method	Last run on PA1	Final HPAE-PAD method
Milk sample preparation	Addition of Carrez solution I and II, centrifugation, filtration	Addition of Carrez solution I and II, centrifugation, filtration	Precipitation of casein proteins, addition of Carrez solution I and II, centrifugation, filtration	Precipitation of casein proteins, addition of Carrez solution I and II, centrifugation, filtration	Precipitation of casein proteins, addition of Carrez solution I and II, centrifugation, filtration	Precipitation of casein proteins, addition of Carrez solution I and II, centrifugation, filtration
Sample dilution	100 times	10 times	10 times	10 times	10 times	100 times
Columns	Dionex CarboPac PA10	Dionex CarboPac PA10	Dionex CarboPac PA1	Dionex CarboPac PA1	Dionex CarboPac PA1	Dionex CarboPac SA10
Flow rate	0.35 ml/min	0.35 ml/min	0.35 ml/min	0.35 ml/min	0.38 ml/min	0.38 ml/min
Run time	20 min	20 min	50 min	41 min	20 min	17 min
Injection volume	10 µl	20 µl	20 µl	20 µl	10 µl	10 µl
Amino trap	No	No	Yes	Yes	Yes	Yes
Ion trap	No	No	No	No	No	Yes

Detection	PAD	PAD	PAD, post-column addition of 0.1 M NaOH	PAD, post-column addition of 0.1 M NaOH	PAD, post-column addition of 0.1 M NaOH	PAD, post-column addition of 0.3 M NaOH
Waveform	Modified*	Modified*	Carbohydrate (standard quad)	Carbohydrate (standard quad)	Carbohydrate (standard quad)	Carbohydrate (standard quad)
Eluents	Deionized water, 10 mM NaOH, 10 mM NaOH and 50 mM NaAc	Deionized water, 10 mM NaOH, 10 mM NaOH and 50 mM NaAc	Deionized water, 100 mM NaOH, 100 mM NaOH and 0.25 M NaAc	Deionized water, 100 mM NaOH, 100 mM NaOH and 0.04 M NaAc	Deionized water, 10 mM NaOH, 10 mM NaOH and 50 mM NaAc	Deionized water, 10 mM NaOH and 0.1 M NaOH
Eluent gradients	Gradients with NaOH concentrations ranging from 4-15 % were used	Gradients with NaOH concentrations ranging from 4-15 % were used	Gradients with NaOH concentrations ranging from 4-15 % were used	Gradients with NaOH concentrations ranging from 4-8 % were used	Gradients with NaOH concentrations ranging from 4-10 % were used	Regeneration 2 minutes before sample injection, and a NaOH gradient from 12 minutes

*The modified potential waveform is described in table 11.

During the optimization of the procedure, samples were run on PA1, PA10 and SA10 columns. However, the SA10 column gave the best separation of the monosaccharides, and shortest run time. The flow rate through the column was 0.38 ml/min and sugars were eluted isocratically with water, followed by a gradient of 10 mM NaOH after 12 minutes. A 2 minutes regeneration step consisting of flushing with water was performed before each run. Post column addition of 0.3 M NaOH before the detection electrode was used. For details, see Table 8. The waveform used was Carbohydrate (standard quad), for details see Table 9.

The data was processed using Microsoft Excel and statistical software R.

Table 9. *High Performance Anionic Exchange-Pulsed Amperometric Detection conditions*

Parameters	Conditions
Columns	Dionex CarboPac SA10
Flow rate	0.38 ml/min
Injection volume	10 µl
Tray temperature	4° C
Detection	Integrated pulsed amperometry Au and PTFE disposable electrode
Waveform	Carbohydrate (standard quad)
Background	<20 nC
Temperature	25° C
Eluents	A) Deionized water B) 10 mM NaOH C) 0.1 M NaOH

3 Results

The optimization of the HPAE-PAD method is presented as the parameters that were improved (Table 8). The result from the analysis is presented in Table 10.

3.1 Optimization of HPAE-PAD method for quantification of galactose, glucose and lactose in milk and fermented milk

The analytical procedure was optimized with respect to separation of monosaccharides, run time of analysis and elution of lactose in UHT milk. All peaks in the chromatograms were identified. The run time was 19 minutes, including a 2 minute regeneration step in before the sample injection. Close to base-line separation of monosaccharides could be achieved (Figure 2).

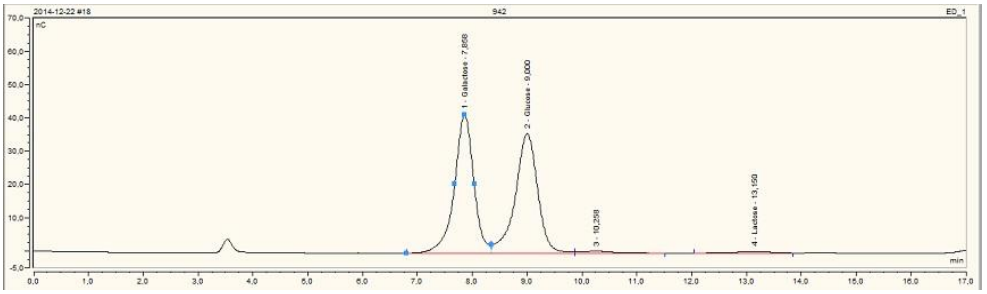


Figure 2. Lactose free milk run on CarboPac SA10 column with almost base-line separation of monosaccharides (peak 1 galactose, peak 2 glucose), one unidentified peak with retention time 10.258 and traces of lactose (peak 4).

In order to identify unknown peaks in lactose free milk and fermented milks, samples with GalNAc, GlcNAc was run (Figure 3). The unknown peaks had retention time approximately 10.258 (Figure 2), and could not be identified as GalNAc or GlcNAc. However, the unidentified peaks are rather small and will not interfere with the results of the analysis.

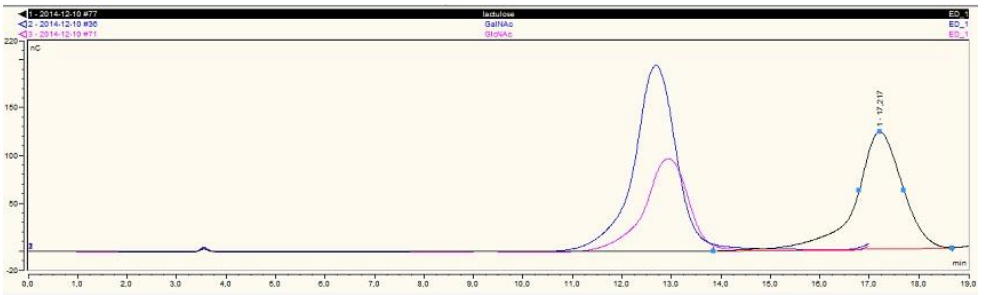


Figure 3. The over-layered chromatograms of (from the left) GalNAc, GlcNAc and lactulose. Retention times were 12.692, 12.934 and 17.217, respectively. The lactulose sample was run on a program with 2 additional minutes due to its long elution time.

Lactulose did not co-elute with lactose in the UHT milk samples (Figure 4). The unknown peak at the end of the chromatogram (retention time 16.834), and was probably lactulose (retention time 17.217). Although, it is possible, when analysing lactose free products, that some sugars (lactose, lactulose and iso-maltose) co-elute. This will overestimate the lactose in lactose free dairy products.

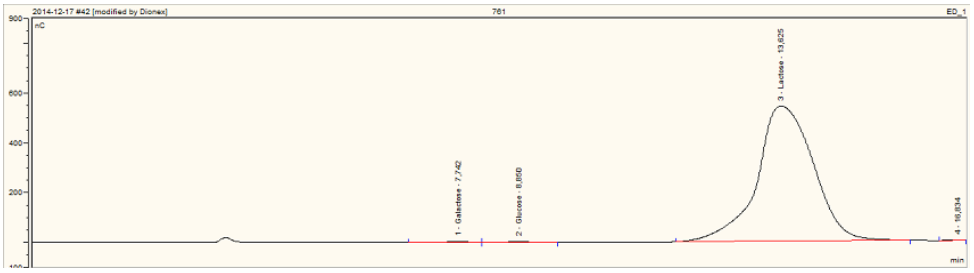


Figure 4. Analysis of UHT milk shows galactose, glucose, lactose and a small unidentified peak at the end of the chromatogram (retention time 16.834).

3.2 Quantification of galactose, glucose and lactose in milk and fermented milk using HPAE-PAD

The results from the HPAE-PAD analysis is shown in Table 10. The amount of galactose, glucose and lactose in milk is presented as initial values from the first measurement, and mean values over the whole storage period. The median values are also presented, to express the variation in data. Calculations are presented in Appendix 4.

Table 10. *Galactose, glucose and lactose concentration in different dairy products from the initial measuring point during storage until their expiration date*

	Galactose (mg/100g)			Glucose (mg/100g)			Lactose (g/100g)		
	Initial	Mean	Median	Initial	Mean	Median	Initial	Mean	Median
A-fil (n=12)	51.22	51.86	50.22	0.00	1.19	0.00	3619.48	4.14*	3.99
Onaka (n=12)	67.19	84.91	83.87	0.00	8.52	9.76	4160.16	4.00*	4.00
Filmjölk (n=12)	56.39	52.10	53.73	0.00	0.00	0.00	4594.69	3.96*	4.07
Lactose free yogurt (n=12)	1885.78	1840.22*	1824.08	1136.54	1068.69*	1052.46	2.94	0.00	0.00
Yogurt (n=12)	1029.35	1583.33*	1630.13	0.00	13.34	15.09	2190.78	3.12*	3.22
UHT milk 30° C (n=20)	8.33	12.22	11.31	8.30	11.35	9.60	3751.01	3.95*	3.80
UHT milk 20° C (n=20)	8.33	9.90	9.03	8.30	10.02	8.96	3751.01	3.73*	3.73
UHT milk 4° C (n=20)	8.33	9.39	8.26	8.30	9.73	8.45	3751.01	3.68*	3.66
Lactose free milk (n=12)	1066.33	1581.18*	1632.12	1082.38	1723.96*	1804.15	0.00	0.09	0.13
Milk (n=2)	7.12	7.12	7.12	9.34	8.34	8.34	4.26	4.26*	4.26

* The values are outside the standard curve and have not been quantified correctly.

The galactose content varies from 7.12 to 12.22 mg/100 g in different milk products. In fermented milk the amount ranges from 51.86 to 84.91 mg/100 g. The glucose content ranges within the same intervals. The amount of galactose in the yogurt-type of fermented milks are generally higher than the others (Filmjölk, Onaka and A-fil). The amount of lactose in most products is not reliable, due to the measured values being outside the standard curve. The same goes for monosaccharides in lactose free milk and yogurt.

The sugar composition of lactose free milk is presented in Figure 5. No major changes occur during storage, the monosaccharide content remains approximately 1.5 g/100 g.

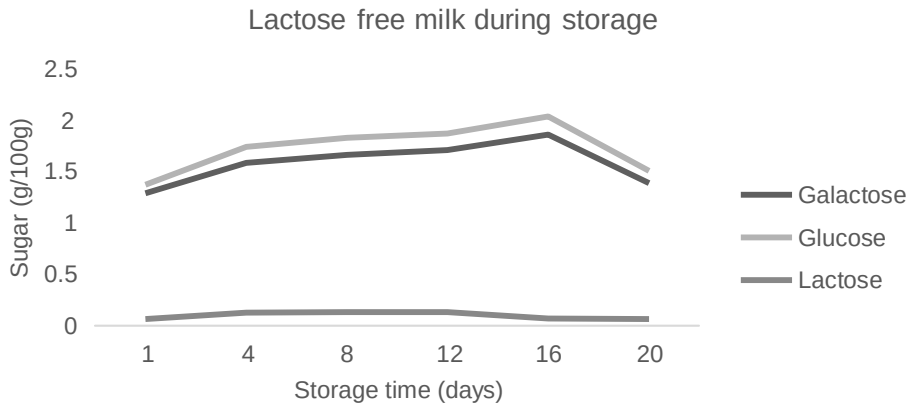


Figure 5. Galactose, glucose and lactose in lactose free milk during storage.

The galactose and glucose content in UHT milk co-varies dependent of each other when stored at 4° C (Figure 6). From 36 days and forward, the monosaccharide content does not change. A similar pattern for UHT milk stored at 20° C and 30° C

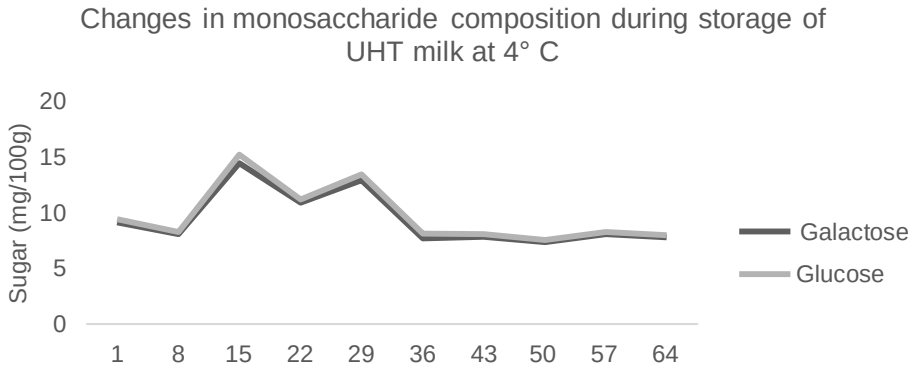


Figure 6. Galactose and glucose content in UHT milk stored at 4 ° C.

is present (Appendix 2). The difference from the first sampling to the last is approximately -2 mg/100 g.

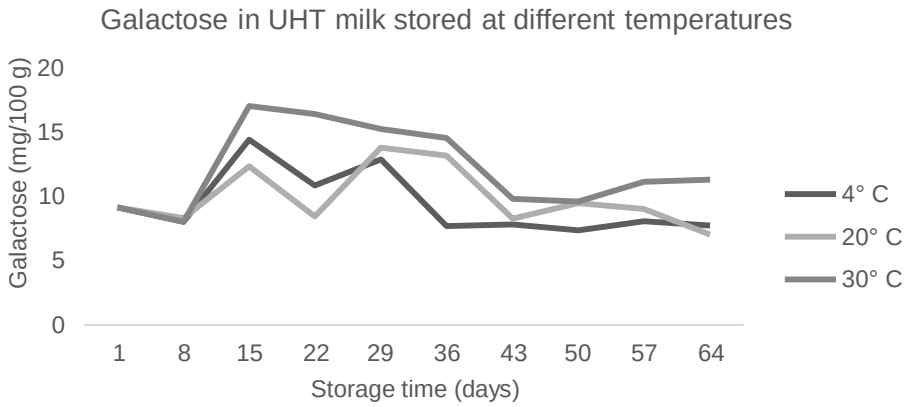


Figure 7. Changes in galactose content in UHT milk stored in different temperatures.

The effect of storage temperature on galactose content in UHT milk is illustrated in Figure 7. Irrespective of the temperature, the content is fairly stable from day 43 and forward. The galactose content is lowest in milk stored at 20° C, 7.02 mg/100 g, and highest in milk stored at 30° C, 11.30 mg/100 g. In the 4° C milk, the final galactose content was 7.74 mg/100 g. The initial value was 9.12 mg/100 g galactose, which indicates that milk stored at 4° C and 20° C has lower galactose content, compared to milk stored at 30° C. The amount of galactose increases from one week of storage, and then shows a declining trend. No statistical analysis has been performed to confirm whether the differences are significant or not.

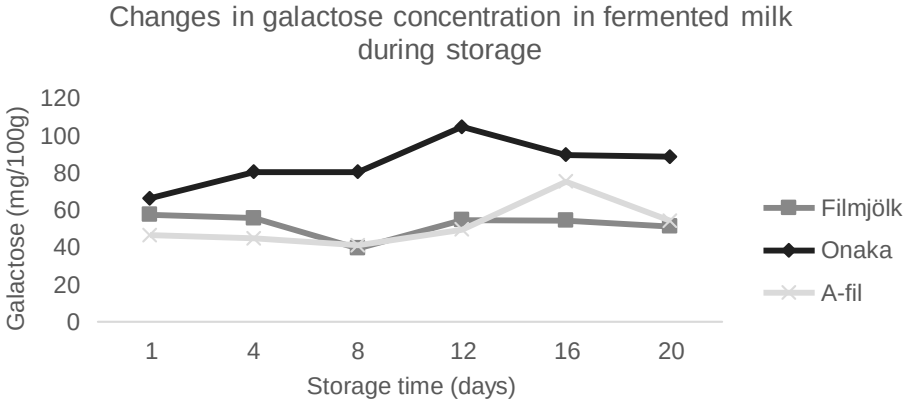


Figure 8. Changes in galactose concentration in fermented milks during storage.

In fermented milk, the changes in galactose concentration are presented in Figure 8. In Onaka, the amount of galactose increases from 66.20 mg/100 g to 88.49

mg/100 g. A-fil and Filmjölk is fairly constant at approximately 60 mg/100 g (Appendix 1).

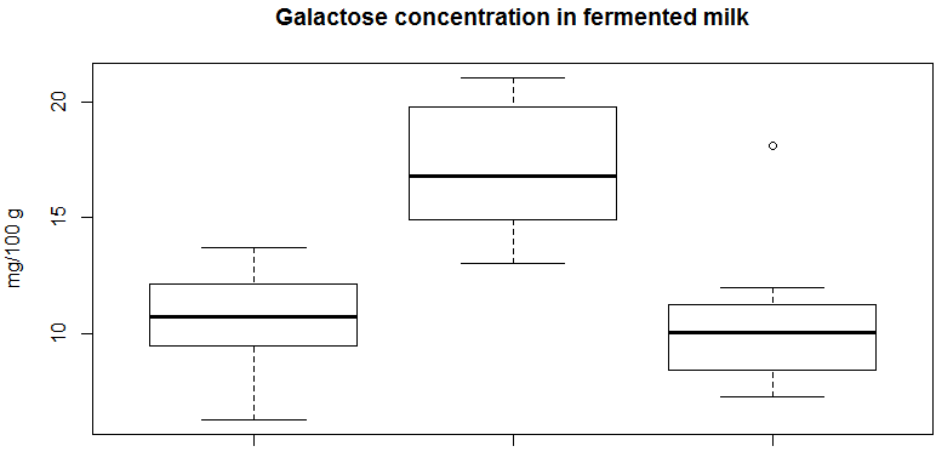


Figure 9. Variations in galactose concentration in three of the fermented milks: Filmjölk, Onaka and A-fil.

A presentation of the variations in the fermented milk is presented in figure 9. A large variation indicates a larger change during storage, illustrated as the bars. The size of the box indicates the variation in the second and third quantile, and the line the median. A-fil has less variation, and also less distribution of data points.

4 Discussion

The objectives of this study was to optimize a method for quantification of lactose, glucose and galactose in milk and fermented milk, and apply the method of analysis on some of the dairy products found on the Swedish market.

4.1 Optimization of HPAE-PAD method for quantification of galactose, glucose and lactose in milk and fermented milk

Regarding the selection of milk products, the study design could have been more elaborated. For example, UHT milk is usually not consumed in large amount in the Nordic countries, due to its taste, but a large amount of the sampling were done on this milk. In addition, storage at 30° C temperature is too hot to apply to Swedish climate and storage conditions, but in a world perspective UHT milk is generally not stored at refrigerated temperatures. Taking Swedish consumption patterns into account, more low pasteurized milk 1.5% fat from different manufacturers would have been plausible. Different pasteurization techniques can cause different changes in the carbohydrate content.

Also, analysis of lactose free and lactose reduced milk from different manufacturers is interesting, since there are produced by different processing methods which can affect the sugar composition of the final product. The selection of fermented milks is slightly better, but still only one manufacturer is represented. Other manufacturers may use different strains of LAB with different galactose fermenting properties. Other dairy products such as cheese, cream, cream cheese, butter, crème fraiche, spreads etc. are outside the scope of this study, but are of course interesting to look at regarding carbohydrate content.

In order to get better validation of these products, several tests based on different packages is required. Since the tests only were performed in duplicates from the same milk package, only an indication of the carbohydrate composition could be obtained.

The analytical method had serious flaws that needed to be developed, i.e. the filtration step was inefficient due to protein contamination, the resolution of the lactose peak was poor and base-line separation of the monosaccharides could not be made properly (see Table 8 for details on the method parameters that were optimized). In order to improve the efficiency of the filtration step, rennet precipitation of the caseins was introduced. The lactose peak was still extended, and no base-line separation of the monosaccharides in the fermented milk samples could be achieved. Only UHT-treated milk was analysed using this method. The sample dilution was too extensive, and the amount of injected samples could be increased from 10 to 20 µl.

Fermented milk and lactose free milk was analysed using a modified version of the Cataldi method (2003), where the sample dilution was decreased and the injection volume increased. The lactose peak in the fermented milk was extended, and the monosaccharides in the lactose free milk did not separate appropriately. Also, unidentified peaks could be detected.

A method used to separate monosaccharides in lignocellulose was introduced. The column used in this analysis was CarboPac PA1. Four eluents, instead of three were used, and also the potential waveform used was Carbohydrate (standard quad). To further improve the results, an amino trap column was included in this analysis. To make the detection more efficient, post-column addition of NaOH was performed. The run time for the analysis was extended to 41 minutes. The elution of the carbohydrates was completed during the first 10 minutes in the fermented milk samples, but without separation. The same problems were observed in the modified version of the lignocellulose method, where the retention time was not consistent, with poor separation the assumed lactose peak was split in two. Lactose was thought to co-elute with lactulose. Additional changes in the sample preparation was introduced, i.e. rennet precipitation of casein. The removal of proteins made the filtration step more efficient, but rennet precipitation did not work for the UHT milk due to the extensive heat treatment applied on this milk. A modified version of this method was tried with lower concentration of sodium acetate and different gradients. This method showed to be time consuming and not very efficient with regards to separation of mono-saccharides.

The last run on the PA1 column included the same eluents as in the Cataldi method (2003), but instead of the modified waveform, the Carbohydrate standard quad was used. The flow rate was slightly increased to 0.38 ml/min to agree with the manufacturer's recommendation. The retention time for the carbohydrates was not consistent and the monosaccharides did not separate properly.

The final method optimization was performed on the CarboPac SA10 column. The sample dilution was increased due to the sensitivity of the method. An ion trap to reduce the background noise from the eluents was introduced successfully.

Using the right potential waveform is crucial, otherwise the disposable electrode will deteriorate. The Carbohydrate (standard quad) was by far the most sensitive, and gave more stable results. A comparison of the potential waveform is presented in Table 11.

Table 11. *The potential waveform used in the first experiments, after Cataldi et al. (2003), and Carbohydrate (standard quad) as used in the following experiments*

Modified waveform				Carbohydrate (standard quad)			
Time (s)	Potential (V) vs. Ag|AgCl		Current integration	Time (s)	Potential (V) vs. Ag|AgCl		Current integration
0.00	E_{DET}	+0.05		0.00	E_{DET}	+0.10	
0.24		+0.05	Start	0.20		+0.10	Start
0.44		+0.05	End	0.40		+0.10	End
0.45	E_{OX}	+0.80		0.41	E_{OX}	-2.00	
0.63		+0.80		0.42		-2.00	
0.64	E_{RED}	-0.22		0.43		+0.60	
1.00		-0.22		0.44	E_{RED}	-0.10	
				0.50		-0.10	

4.2 Quantification of galactose, glucose and lactose in milk and fermented milk using HPAE-PAD

It is possible to quantify galactose, glucose and lactose in milk and fermented milk products, but not at the same time. A further development of the method is required. Due to large differences in concentration (approximately 4.5 g/100 g lactose, but only 7.12 mg/100 g galactose) both mono- and disaccharides cannot be quantified in the same run. In order to quantify the sugar composition of milk, a sample needs to be analysed in two dilutions; approximately 100x and 1000x. A broader span of the standard solutions can also be applied, where amounts of sugar outside the 1-20 mg range can be quantified.

The regular drinking milk was not subjected to a storage analysis, due problems in sample preparation and lack of time. Milk has a short shelf life, approximately 8 days, and nothing was expected to happen during such short time. The possible explanation to the lack of changes in the carbohydrate content is that no extensive heat treatment is applied in the production, and no addition of LAB. The literature review (Table 5) identified galactose amounts ranging from 0.00 to 4.00 mg/100g, approximately twice as much was identified in this study (7.12 mg/100g). A larger number of samples is needed in order to confirm this result, but one important explanation is the use of different analytical methods.

No changes in the sugar composition were seen in lactose free milk during storage. Approximately 1.5 g/100 g of each monosaccharide was quantified, which gives in total approximately 3 g/100 g. According to the package label, lactose free milk contains 2.7 g carbohydrates per 100 g. The constant content of galactose and glucose indicates that the lactase enzyme is not active in the package. The HPAE-

PAD analysis of lactose free milk had an unexpected problem; the electrode signal increased at the end of the sequence. Usually, the signal deteriorates due to the high pH. This has probably affected the result, and made it less reliable. The R-value from the standard curve was 0.98, whereas the others were 0.99 (Appendix 3). Analysis of lactose free milk presented the same problems with low and high concentrations of different compounds. Analysis of a dilution series would solve the problem. However, due to the erratic behaviour of the electrode and the need of another dilution in order to quantify the monosaccharides properly, the result from the lactose free milk analysis is not reliable. A precious study by Pirisino (1983) identified around 2000 mg/100g of galactose and glucose respecitvely, which is close to 1800 mg/100g identified in this study. The electrode tend to underestimate the amount of sugar when the concentration exceeds the standard curve, which is also seen in the lactose quantification. The amount of lactose is quantified to 4.2 g/100 g in milk, but should be 4.5-5 g/100 g. If these improvements are in place, the suggested method of analysis should be reliable.

The analysis of the UHT milk indicated that milk stored at 4° C and 20° C has lower galactose content, compared to milk stored at 30° C. First, the amount of galactose increases during storage, and then shows a declining trend. The first analysis was only performed on one milk package, since no storage had taken place yet. At the other sampling points, a new package stored at each temperature was opened, therefore the differences. Statistical analysis need to be performed in order to confirm the results. But it agrees with the hypothesis that the monosaccharide content would increase in UHT milk stored at warmer temperatures, since heat induced reactions Maillard reactions occurs. Lactose isomerization into lactulose, i.e. the Lobry de Bruyn-van Ekenstein reaction, often causes the lactulose molecule to split, leaving two monosaccharides. Therefore, the monosaccharides content is believed to increase during storage. The Maillard reactions would only decrease the lactose content. These reactions continue in an extensive period of time, whereas a 10 week trial may be too short. It is not possible to conclude if the amount of lactose has changed in the analysis, due to the unreliable quantification. Lactose and lactulose was thought to co-elute, based on the shape of the peaks in the first chromatograms. This was later rejected as a case of bad sample preparation.

The amount of lactose in fermented milk was expected to decrease during storage. Due to lack of reliable quantification, no such statements could be made. But, taking into account that the low pH inhibits growth of microorganisms, the amount of lactose is probably not going to decrease. Unidentified peaks in the fermented milk samples were thought to be GalNAc and GlcNAC – building blocks of GOS and EPS (chapter 1.2.4). No such identification could be made.

Yogurt has higher levels of galactose and glucose (1500 mg/100g galactose 13 mg/100 g glucose), compared to the other fermented milks. This is because the use

of LAB strains which do not metabolize galactose. The amount of glucose in yogurt is approximately 100 times lower, which indicates that the LAB strains used in yogurt production, *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* use glucose as a carbon source but not galactose (Table 6). In lactose free yogurt, the amount of glucose is higher than in regular yogurt, but still not as high as the amount of galactose. This agrees with the hypothesis, and is consistent with the literature review (980-1800 mg/100g galactose, 0.0-340 mg/100 g glucose). Although, LAB metabolize some galactose but to a lower extent. Though, the numbers are varying and indicates the problem with quantifying a complex substrate such as milk. There is no glucose detected in Filmjölk, which indicates that LAB prefer glucose to galactose in general and not only in yogurt. The same pattern is seen in Onaka (8.5 mg/100 g galactose) and A-fil (1.2 mg/100 g galactose) and in the literature review.

4.3 Galactose as a possible mechanism

When lactose is digested in the body, it will be hydrolysed into one moiety galactose, and one moiety glucose via the Leloir pathway. Regardless off consumed in the form as mono- or disaccharide, galactose will enter the body as monosaccharide. The total galactose content in milk product should therefore be calculated as galactose + the galactose from lactose to give the whole galactose content. One glass of milk (2 dl) would then contain 14.2 mg galactose, 16.7 mg glucose and 8.5 g lactose, and the total galactose content (galactose + the lactose molecule split in two) would then be 4.3 g. This is the equal amount to a 50 mg/body weight dose of an 86 kg human (chapter 1.2.5 for details). To be correct, not all lactose is digested in the small intestine but some of it is fermented by the colonic microbiota which also needs to be taken into account. Also, lactose intolerant people metabolise lactose to a much lower extent, which gives an even lower galactose load. It is also important to remember that the correlation of galactose and ageing is seen in only animal studies, where the dose was given subcutaneously. The same effect may not be achieved when ingested orally.

Milk and dairy products is not the only source to galactose in the diet. Fruits and vegetables is also a common source, and there is also an endogenic production to take into account. To people with galactosemia, the galactose content of food is a crucial knowledge, whereas the possibility to modulate LAB to produce galactose free yogurt out of lactose free milk is tempting requiring reliable analytical methods in order to ensure the galactose elimination.

There was no correlation between yogurt intake and negative health effects observed in the study by Michaëlsson, et al. (2014), which could possibly be explained by the pro- and prebiotics effect on fermented milk. Galactose is an important part

in the LAB production of GOS and EPS, which have observed health effects (Saxelin, Korpea, & Mäyrä-Mäkinen, 2003;Patel, Majumder, & Goyal, 2012;Badel, Bernardi, & Michaud, 2011). However, these are not digestible and will not enter the body as monosaccharides, suggesting that the galactose content of foods and its effect on health should be carefully evaluated.

In the rapid response section in the online publication by Michaëlsson (2014), several interesting comments from scientists, doctors, nutritionists etc. all around the world have been published. The comments question the galactose mechanism as an explanation, but also the study per se. Some claim that in order to have a reliable and valid food intake study, the questionnaire must be supplemented with quantifiable measures. A lot of critique is given to the fact that the study design, and ask for experimental results. Another respondent comments upon the fact that the reported energy intake in the food frequency questionnaire is too low, and would lead to nutrient deficiency, and claims that the reliability of the study is affected by this weakness. Vitamin D status is suggested as a cofounding factor. Also, someone thinks that the high incidence of reflux disease in Scandinavia and the intake of proton pump inhibitors (PPI) can explain the high risk of fractures. PPI reduces the calcium uptake, and PPI medication should be taken into account. On the other hand, there are also thoughts that the galactose mechanism could explain the French paradox; lower incidence of cardio-vascular disease in spite of diet with lots of saturated fat (The British Medical Journal, 2014).

This study will be debated for years to come, and new studies are needed in order to conclude whether galactose is important or not.

4.4 Conclusion

It was possible to quantify galactose, glucose and lactose using HPAE-PAD and the procedure that was optimized in this study. Attention needs to be put in the dilution of the samples. In milk, the amount of galactose is the equal amount as the dose used in animal studies to study ageing. I UHT milk and lactose free milk, the change in galactose during storage is minimal. Fermented milks contain various amounts of galactose, more in yogurt than in other types. The content of galactose in fermented milk is generally higher than glucose. The galactose content of food and its implications on human health should be carefully evaluated.

Acknowledgement

I would like to thank my supervisors, Henrik Hansson and Åse Lundh, SLU for their time and effort.

References

Abbad Andaloussi, S., H., T., Marczak, R., & Bonaly, R. (1995). Isolation and characterization of exocellular polysaccharides produced by Bifidobacterium longum. *Applied Microbiology and Biotechnology, 43*, 995-1000.

Adams, M., & Moss, M. (2006). *Food Microbiology. Second Edition*. Cambridge: The Royal Society of Chemistry.

Åkerberg, C., Hofvendahl, K., Zacchi, G., & Hahn-Hägerdal, B. (1998). Modelling the influence of pH, temperature, glucose and lactic acid concentrations on the kinetics of lactic acid production by Lactococcus lactis ssp. lactis ATCC 19435 in whole-wheat flour. *Applied Microbiology and Biotechnology, 49*(6), 682-690.

Alm, L. (1982). Effect of Fermentation on Lactose, Glucose and Galactose Content in Milk and Suitability of Fermented Milk Products for Lactose Intolerant Individuals. *Journal of Dairy Science, 65*(3), 346-352.

Anbukkarasi, K., UmaMaheswari, T., Hemalatha, T., Kumar Nanda, D., & Singh, P. (2014). Preparation of low galactose yoghurt using cultures of Gal+ Streptococcus thermophilus in combination with Lactobacillus delbrueckii ssp. Bulgaricus. *Journal of Food Science Technology, 51*(9), 2183-2189.

Asp, N.-G. (2006). Kolhydrater. In Abrahamsson, Lillemor, A. Andersson, W. Becker, & G. Nilsson (Eds.), *Näringslära för högskolan* (pp. 94-96). Liber AB.

Badel, S., Bernardi, T., & Michaud, P. (2011). New perspectives for Lactobacilli exopolysaccharides. *Biotechnology advances, 29*(1), 54-66.

Cataldi, T., Angelotti, M., & Bianco, G. (2003). Determiantion of mono- and disaccharides in milk and milk products by high-performance anion-exchange chromatography with pulsed amperometric detection. *Analytica Chimica Acta*(485), 43-49.

Cataldi, T., Angelotti, M., & Bufo, S. (1999). Method Development for the Quantitative Determination of Lactulose in Heat-Treated Milks by HPAEC with Pulsed Amperometric Detection. *Analytical Chemistry, 71*(21), 4919-4925.

Cocaign-Bousquet, M., Garrigues, C., Loubiere, P., & Lindley, N. (1996). Physiology of pyruvate metabolism in Lactococcus lactis. *Antonie van Leeuwenhoek, 70*, 253-267.

Cogan, T., & Jordan, K. (1994). Metabolism of Leuconostoc bacteria. *Journal of Dairy Science, 77*, 2704-2717.

Corradini, C., Cavazza, A., & Bignardi, C. (2012). High-Performance Anion-Echange Chromatography Coupled with Puled Electrochemical Detection as a Powerful Tool to Evaluate Carbohydrates of Food Interest: Principles andApplications. *International Journal of Carbohydrate Chemistry*, 1-13.

Coultate, T. (2009). *Food - The Chemistry of its Components* (5:th ed.). Cambridge: The Royal Society of Chemistry.

Deeth, H. (2010). Improving UHT processing and UHT milk products. In M. W. Griffiths (Ed.), *Improving the safety and quality of milk. Volume 1: Milk production and processing* (pp. 303-325). Cambridge: Woodhead Publishing Limited.

European Food Safety Authority. (2010). Scientific Opinion on lactose thresholds in lactose intolerance and galactosaemia. *EFSA Journal, 8*(9), 1-28.

Fondén, R., Saarela, M., Mättö, J., & Mattila-Sandholm, T. (2003). Lactic acid bacteria (LAB) in functional dairy products. In T. Mattila-Sandholm, & M. Saarela (Eds.), *Function dairy products* (pp. 244-257). Cambridge: Woodhead Publishing Limited.

García Fontán, M., Martínez, S., Franco, I., & Carballo, J. (2006). Microbiological and chemical changes during the manufacture of Kefir made from cows' milk, using a commerical starter culture. *International Dairy Journal, 16*, 762-767.

Garrigues, C., Loubiere, P., Lindley, N., & Cocaign-Bousquet, M. (1997). Control of the shift from homolactic acid to mixed-acid fermentation in Lactococcus lactis: predominant role of the NADH/NAD+ ratio. *Journal of Bacteriology, 179*(17), 5282-5287.

Gésan-Guiziou, G. (2010). Removal of bacteria, spores and somatic cells from milk by centrifugation and microfiltration techniques. In M. Griffithsl (Ed.), *Improving the safety and quality of milk. Volume 1: Milk production and processing* (pp. 349-372). Cambridge: Woodhead Publishing Limited.

Goodenough, E., & Kleyn, D. (1976). Qualitative and Quantitative Changes in Carbohydrates During the Manufacture of Yogurt. *Journal of Dairy Science, 59*(1), 45-47.

Gross, K., & Acosta, P. (1991). Fruits and Vegetables are a Source of Galactose: Implications in Planning the Diets of Patients with Galactosaemia. *Journal of Inherited Metabolic Disease, 14*, 253-258.

Haoa, L., Huanga, H., Gao, J., Marshall, C., Chen, J., & Xiao, M. (2014). The influence of gender, age and treatment time on brain oxidative stress and memory impairment induced by d-galactose in mice. *Neuroscience Letters*(571), 45–49.

Harju, M., Kallioinen, H., & Tossavainen, O. (2012). Lactose hydrolysis and other conversions of dairy products: Technological aspects. *International Dairy Journal, 22*(2), 104-109.

Holden, H., Rayment, I., & Thoden, J. (2003). Structure and Function of Enzymes of the Leloir Pathway for Galactose Metabolism. *The Journal of Biological Chemistry*(278), 43885-43888.

Holzapfel, W., & Wood, B. (2014). *Lactic Acid Bacteria : Biodiversity and Taxonomy.* Somerset, NJ, USA: Wiley.

Huth, P., & Park, K. (2012). Influence of Dairy Product and Milk Fat Consumption on Cardiovascular Disease Risk: A Review of the Evidence. *Advances in Nutrition*(3), 266-285.

Jansson, T., Clausen, M., Sundekilde, U., Eggers, N., Nyegaard, S., Larsen, L., . . . Bertram, H. (2014). Lactose-Hydrolyzed milk is more prone to chemical changes during storage than conventional ultra-high-temperature (UHT) milk. *Journal of Agricultural and Food Chemistry*, 7886-7896.

Jordbruksverket. (2013, April). *Jordbruksverket*. Retrieved 10 3, 2014, from http://www.jordbruksverket.se/webdav/files/SJV/Amnesomraden/Statistik%2C%20fakta/Livsmedel/Statistikrapport2013_4/201304._tabeller8.htm

Kailasapathy, K. (2013). Commercial sources of probiotic strains and their validated and potential health benefits - a review. *International Journal of Fermented Foods*, 1-17.

Kelly, A., & Bach Larsen, L. (2010). Milk Biochemistry. In M. W. Griffiths (Ed.), *Improving the safety and quality of milk. Volume 1: Milk production and processing* (pp. 3-27). Woodhead Publishing Limited.

Leslie, D. N. (2003). *Insights into the pathogenesis of Galactosemia*. Retrieved 10 09, 2014, from http://www.annualreviews.org/doi/pdf/10.1146/annurev.nutr.23.011702.073135

Lewis, M. (2010). Improving pasteurised and extended shelf-life milk. In M. W. Griffiths (Ed.), *Improving the safety and quality of milk. Volume 1: Milk production and processing* (pp. 277-301). Cambridge: Woodhead Publishing Limited.

Lindmark Månsson, H. (2008). Fatty acids in bovine milk fat. *Food and nutrition research*(52).

Lindmark Månsson, H. (2012). *Den svenska mejerimjölkens sammansättning 2009.* Retrieved 10 06, 2014, from http://www.lrf.se/Global/LRF%20Mj%C3%B6lk/Forskningsrapporter/FoR_7094_Den_svenska_mejerimjolkens_sammansattning_2009.pdf

Livsmedelsverket. (2013). *Berikning av mjölk med vitamin A (retinol)*. Retrieved 12 03, 2014, from http://www.slv.se/sv/grupp1/Mat-och-naring/Vad-innehaller-maten/Vitaminer/Vitamin-A/Berikning-av-mjolk-med-vitamin-A-retinol1/

Lopez, C. (2011). Milk fat globules enveloped by their biological membrane: Unique colloidal assemblies with a specific composition and structure. *Current Opinion in Colloid & Interface Science*(16), 391–404.

LRF Mjölk. (2013). *LRF*. Retrieved 10 03, 2014, from http://www.lrf.se/PageFiles/138059/milk_key_figures_sweden.pdf

Michaëlsson, K., Wolk, A., Langenskiöld, S., Basu, S., Warensjö Lemming, E., Melhus, H., & Byberg, L. (2014). Milk intake and risk of mortality and fractures in women and men: cohort studies. *The British Medical Journal*(349).

Nardinello, D., Palermno, C., Quinto, M., & Centonze, D. (2012). Determination of dietary sugars by ion chromatography and electrochemical detection: a focus on galactose, glucose, fructose and sucrose. In V. Preedy (Ed.), *Food and Nutritional Components in Focus* (pp. 269-285). The Royal Society of Chemistry.

Neves, A., Pool, W., Solopova, A., Kok, J., Santos, H., & Kuipers, O. (2010). Towards Enhanced Galactose Utilization by Lactococcus lactis. *Applied and Environmental Microbiology*, 7048-7060.

Nishimura, J. (2014). Exopolysaccharides produced from Lactobacillus delbrueckii supsp.bulgaricus. *Advances in Microbiology*(4), 1017-1023.

Özer, D., Akin, S., & Özer, B. (2004). Effect of Inulin and Lactulose on Survival of Lactobacillus AcidophilusLA-5 and Bifidobacterium Bifidum BB-02 in Acidophilus-Bifidus Yoghurt. *Food Science and Technology International, 11*(19), 19-24.

Patel, S., Majumder, A., & Goyal, A. (2012). Potentials of Exopolysaccharides from Lactic Acid Bacteria. *Indian Journal of Microbiology, 52*(1), 3-12.

Peris-Tortajada, M. (2013). HPLC Determination of Carbohydrates in Food. In L. Nollet, & F. Toldrá (Eds.), *Food Analysis by HPLC* (pp. 233-249). Boca Raton: Tayol and Francis Group.

Pirisino, J. (1983). High Performance Liquid Chromatographic Determination of Lactose, Glucose, and Galactose in Lactose-Reduced Milk. *Journal of Food Science, 48*(3).

Ridel, K. R., Leslie, N. D., & Gilbert, D. L. (2005). An Updated Review of the Long-Term Neurological Effects of Galactosemia. *Pediatric Neurology, 33*(3), 153–161.

Riksmaten. (2012). *Livsmedelsverket*. Retrieved 10 3, 2014, from http://www.slv.se/upload/dokument/rapporter/mat_naring/2012/riksmaten_2010_2011.pdf

Saxelin, M., Korpea, R., & Mäyrä-Mäkinen, A. (2003). Introduction: classifying functional dairy products. In T. Mattila-Sandholm, & M. Saarela (Eds.), *Functional dairy products* (pp. 1-17). Cambridge: Woodhead Publishing Limited.

Schaafsma, G. (2008). Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal , 18*(5), 458–465.

Socialstyrelsen. (2013). *Ovanliga diagnoser*. Retrieved 10 09, 2014, from http://www.socialstyrelsen.se/ovanligadiagnoser/galaktosemi

Song, X., Bao, M., Diandong, L., & Yong Ming, L. (1999). Advanced glycation in D-galactose induced mouse. *Mechanisms of ageing and development*(108), 239–251.

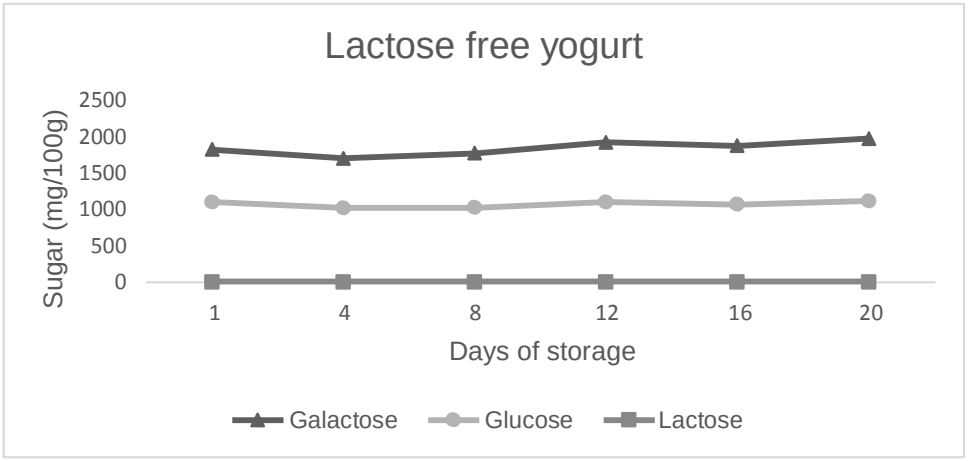
Suarez, F. L., Savaiano, D. A., & Levitt, M. D. (1995, July 6). A Comparison of Symptoms after the Consumption of Milk or Lactose-Hydrolyzed Milk by People with Self-Reported Severe Lactose Intolerance. *New England Journal of Medicine*, pp. 1-4.

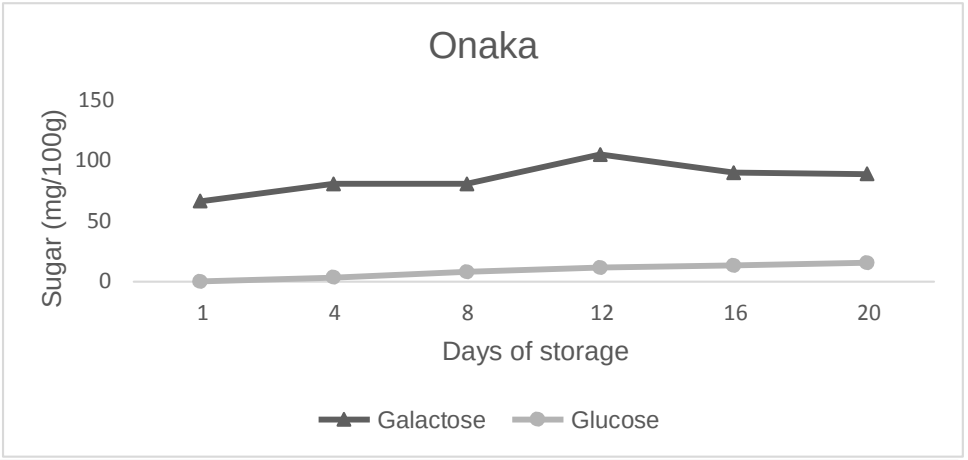
Svensk Dagligvaruhandel. (2013). *Svensk Dagligvaruhandel*. Retrieved 10 03, 2014, from http://www.svenskdagligvaruhandel.se/MediaBinaryLoader.axd?MediaArchive_FileID=dce1feb0

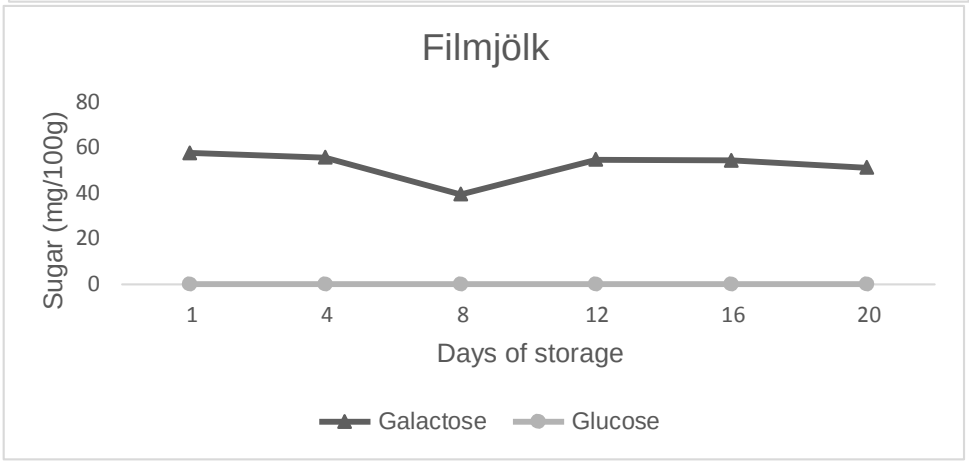
-9a08-435a-8030-37cf54fe9a0b&FileName=H%C3%A4lsan+och+maten+2013-f%C3%B6r+utskrift.pdf
The British Medical Journal. (2014). *All rapid responses*. Retrieved 11 11, 2014, from http://www.bmj.com/content/349/bmj.g6015
Thermo Fisher. (2014). *High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection*. Retrieved 10 1, 2014, from http://www.dionex.com/en-us/products/ion-chromatography/ic-rfic-solutions/hpae-pad/lp-111613.html
Toba, T., Watanabe, A., & Adachi, S. (1983). Quantitative changes in sugars, especially oligosaccharides, during fermentation and storage of youghurt. *Journal of Dairy Science*(66), 17-20.
Turinen, T., & Korpela, R. (2004). Lactose intolerance and low lactose dairy products. In C. Shortt, & J. O'Brien (Eds.), *Handbook of Functional Dairy Products* (pp. 71-85). CRC Press. Vesa, T. H., Marteau, P., & Korpela, R. (2000). Lactose intolerance. *Journal of the American College of Nutrition*, *19*(2), 165-175.
Vyas, U., & Ranganathan, N. (2012). Probiotics, Prebiotics and Synbiotics: Gut and Beyond. *Gatroenterology Research and Practice*, 1-16.
Walstra, P., Wouters, J., & Geurts, T. (2006). *Dairy Science and Technology* (2:a ed.). Taylor and Francis.
Williams, R. (2002). The relationship between the composition of milk and the properties of bulk milk products. *Austrialian Jorunal of Dairy Technology*, *57*(1), 30-44.

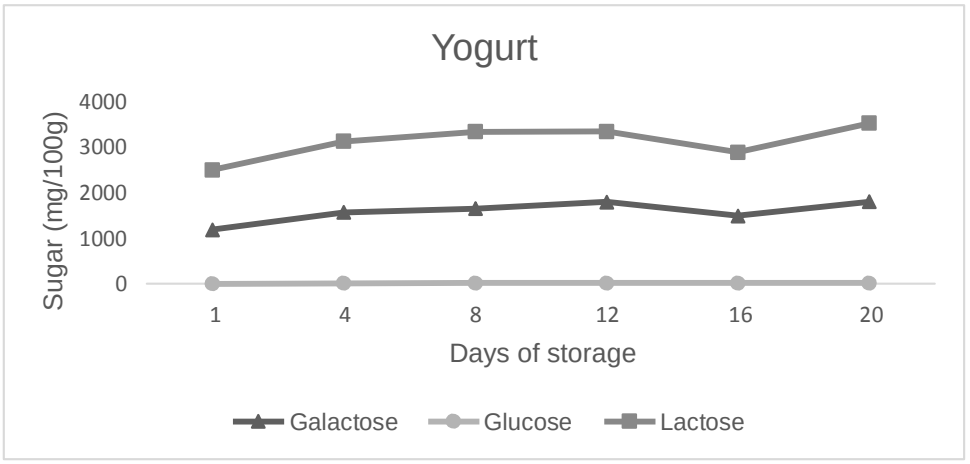
Appendix 1

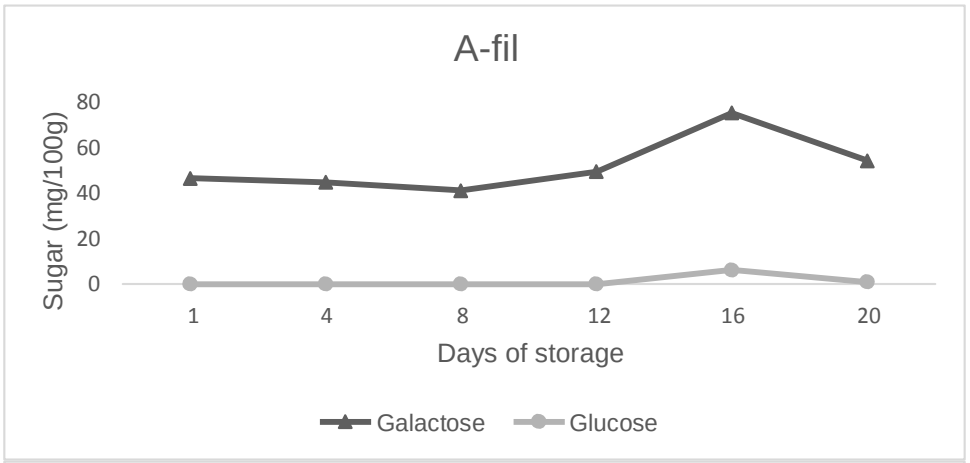
Changes in galactose, glucose and lactose in fermented milks during storage

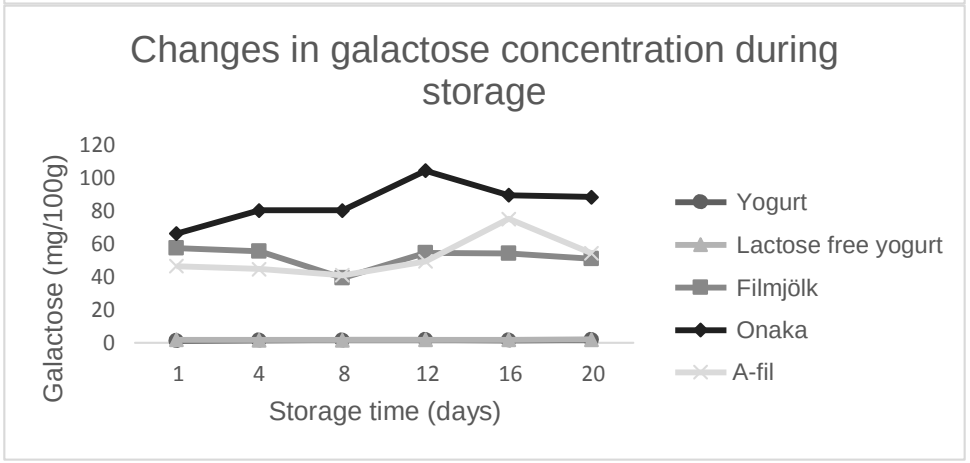


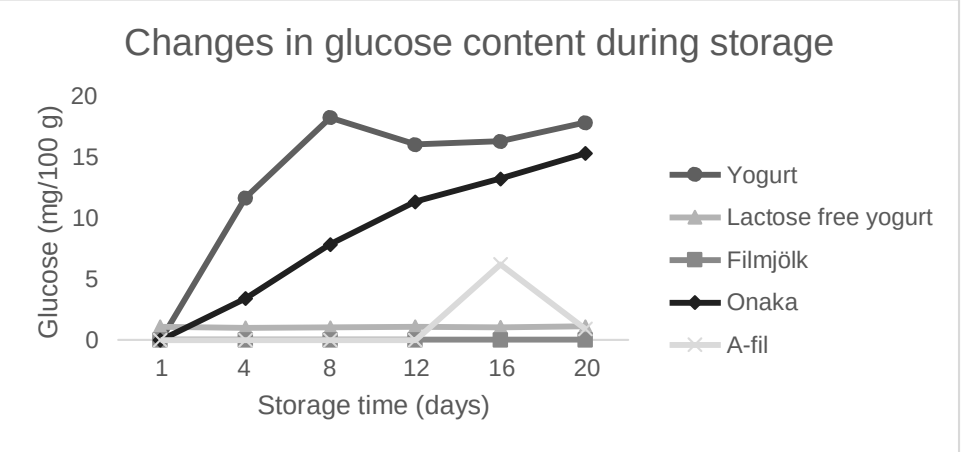





Yogurt
Sugar (mg/100g)
4000
3000
2000
1000
0
1
4
8
12
16
20
Days of storage
Galactose
Glucose
Lactose

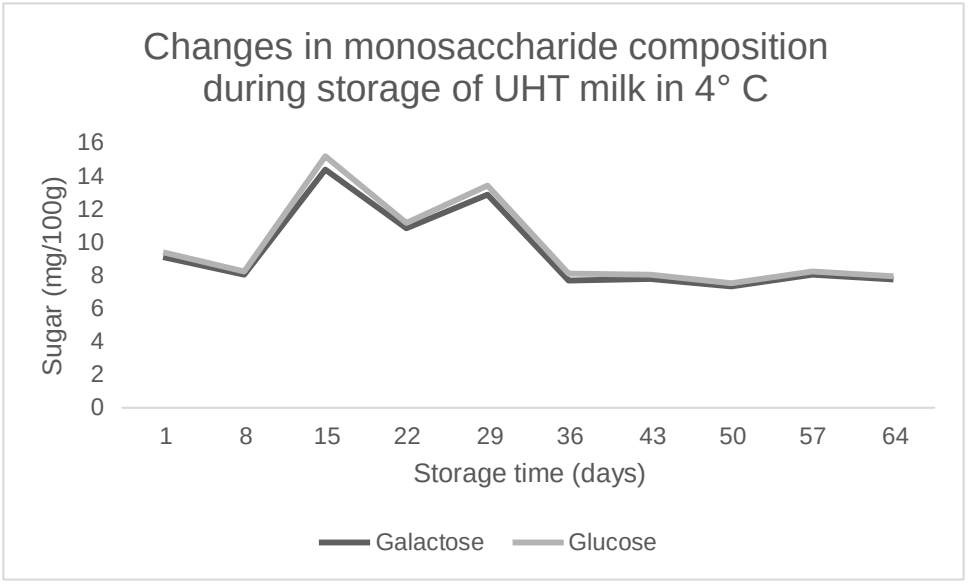

A-fil
Sugar (mg/100g)
80
60
40
20
0
1
4
8
12
16
20
Days of storage
Galactose
Glucose

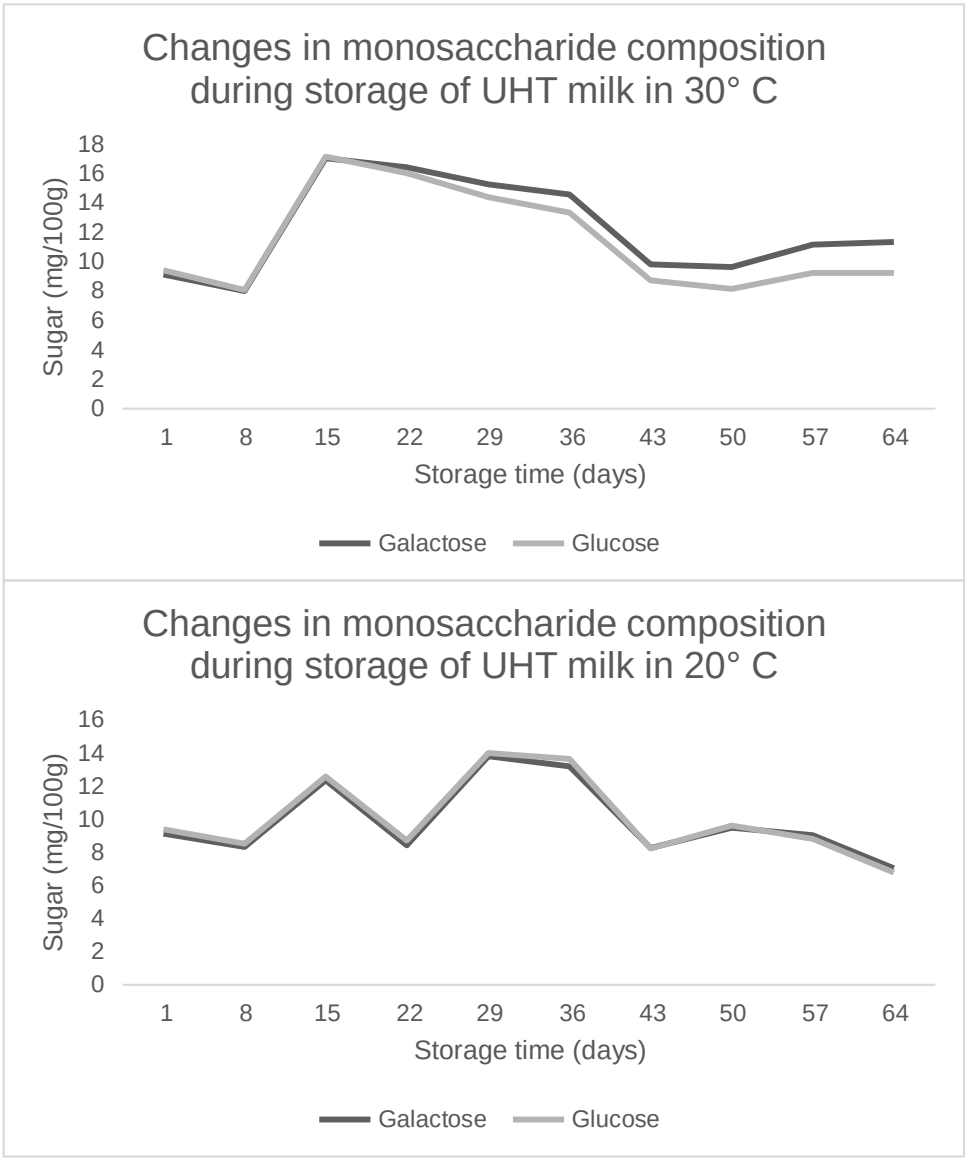

Changes in galactose concentration during storage
Galactose (mg/100g)
120
100
80
60
40
20
0
1
4
8
12
16
20
Storage time (days)
Yogurt
Lactose free yogurt
Filmjölk
Onaka
A-fil


Changes in glucose content during storage
Glucose (mg/100 g)
20
15
10
5
0
1
4
8
12
16
20
Storage time (days)
Yogurt
Lactose free yogurt
Filmjölk
Onaka
A-fil

Appendix 2

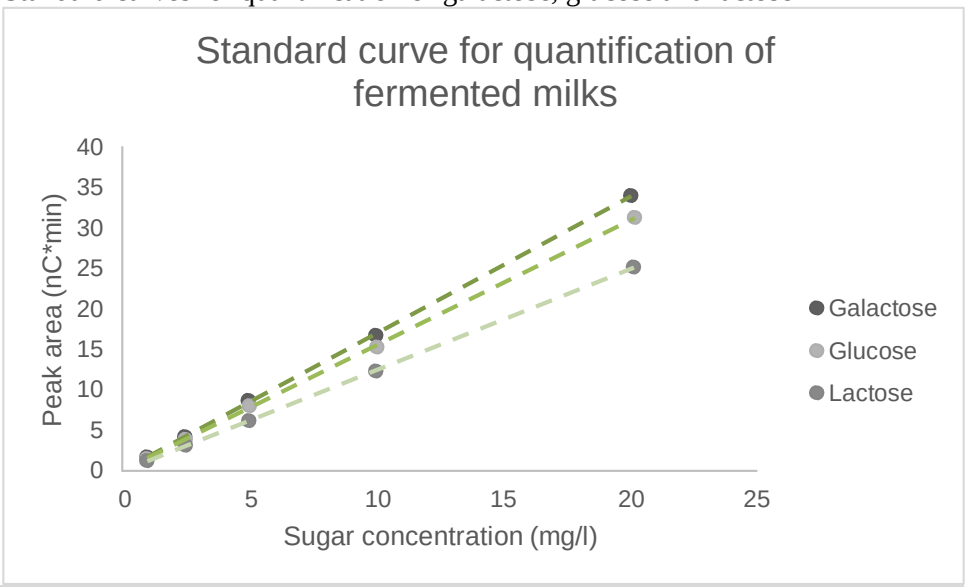
Changes in monosaccharide content in UHT milk stored in different temperatures



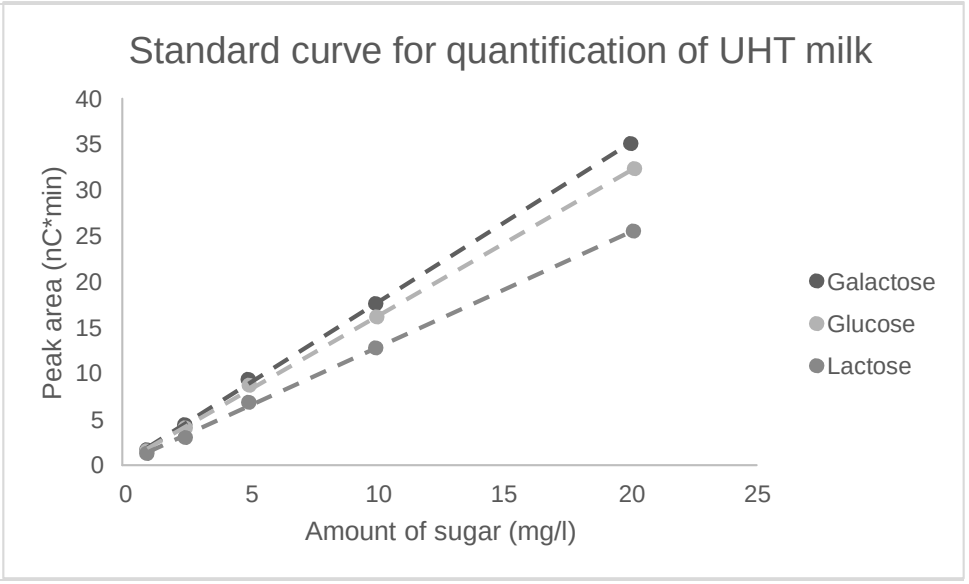

Changes in monosaccharide composition during storage of UHT milk in 30° C
Sugar (mg/100g)
18
16
14
12
10
8
6
4
2
0
1
8
15
22
29
36
43
50
57
64
Storage time (days)
Galactose
Glucose
Changes in monosaccharide composition during storage of UHT milk in 20° C
Sugar (mg/100g)
16
14
12
10
8
6
4
2
0
1
8
15
22
29
36
43
50
57
64
Storage time (days)
Galactose
Glucose

Appendix 3

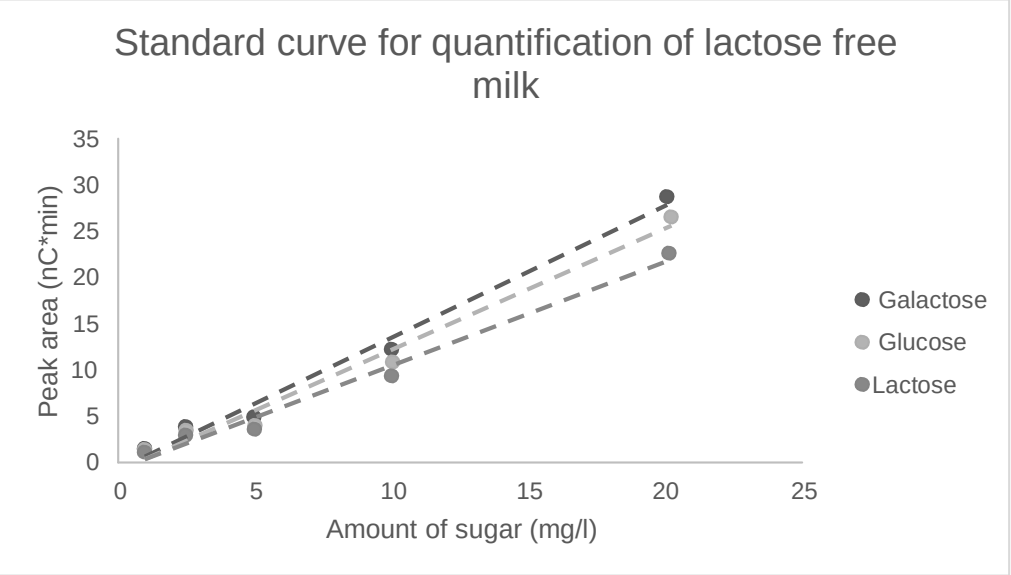
Standard curves for quantification of galactose, glucose and lactose



Galactose	y = 1,6886x + 0,066	R^2 = 0,9999
Glucose	y = 1,5429x + 0,0333	R^2 = 0,9998
Lactose	y = 1,2483x - 0,0461	R^2 = 0,9999



Galactose	y = 1,7421x + 0,2377	R^2 = 0,9996
Glucose	y = 1,5928x + 0,2319	R^2 = 0,9994
Lactose	y = 1,2642x + 0,1417	R^2 = 0,9993



Galactose	y = 1,4272x - 0,779	R^2 = 0,9867
Glucose	y = 1,3148x - 0,9714	R^2 = 0,9811
Lactose	y = 1,1224x - 0,7821	R^2 = 0,984

Appendix 4

Result from the HPAE PAD analysis and Excel calculations

Yogurt																
Galctose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
539.42	232.499	254.723	128.031	0.324	0.222	137.6483	82.95917	0.214772	13764.83	8295.917	21.47721	10.2		1885.782	1136.541	2.942378
503.514	211.405	237.524	116.656			125.1563	75.58669		12515.63	7558.669		10.5		1752.189	1058.214	0
458.363	197.677	215.209	108.046			117.0265	70.00629		11702.65	7000.629		10.6		1650.074	987.0886	0
488.259	208.952	229.278	114.569			123.7037	74.23404		12370.37	7423.404		10.6		1744.222	1046.7	0
602.825	256.937	276.589	137.97			152.1207	89.40093		15212.07	8940.093		10.3		2099.266	1233.733	0
432.936	182.857	193.838	93.956			108.25	60.87413		10825	6087.413		9.8		1439.725	809.626	0
523.454	225.524	237.372	117.993			133.5177	76.45324		13351.77	7645.324		10		1802.489	1032.119	0
602.631	255.967	269.799	133.923			151.5463	86.77795		15154.63	8677.795		9.9		2030.72	1162.825	0
550.227	232.424	245.668	121.095			137.6039	78.46374		13760.39	7846.374		9.7		1816.372	1035.721	0
538.319	232.189	239.934	120.483			137.4648	78.06708		13746.48	7806.708		10.5		1924.507	1092.939	0
613.946	257.701	272.69	133.741			152.5731	86.65999		15257.31	8665.999		10.3		2105.509	1195.908	0
531.75	229.19	235.133	118.083			135.6887	76.51157		13568.87	7651.157		10		1831.798	1032.906	0
													MEAN	1840.221	1068.693	0.245198
Lactosefree yogurt													MEDIAN	1824.085	1052.457	0
Galctose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
299.686	123.34			197.644	193.908	73.00367		155.3746	7300.367		15537.46	10.6		1029.352	0	2190.782
376.491	159.943			236.919	246.629	94.68021		197.6088	9468.021		19760.88	10.7		1344.459	0	2806.045
509.359	213.114	2.513	1.227	302.13	311.627	126.1684	0.773673	249.678	12616.84	77.3673	24967.8	10.3		1741.124	10.67669	3445.557
412.391	176.321	2.709	1.489	252.205	261.622	104.3794	0.943483	209.6196	10437.94	94.34831	20961.96	9.9		1398.684	12.64267	2808.902
446.29	190.263	3.512	1.932	272.496	286.641	112.6359	1.230605	229.662	11263.59	123.0605	22966.2	10.6		1588.166	17.35153	3238.234
473.262	204.264	3.812	2.11	281.178	302.203	120.9274	1.345972	242.1286	12092.74	134.5972	24212.86	10.7		1717.169	19.1128	3438.226
572.813	247.438	3.727	2.032	309.76	332.818	146.4953	1.295418	266.6539	14649.53	129.5418	26665.39	10.5		2050.935	18.13585	3733.155
452.622	189.948	2.972	1.6	265.277	269.131	112.4494	1.015425	215.6349	11244.94	101.5425	21563.49	10.2		1540.556	13.91133	2954.199
379.715	163.059	2.857	1.547	225.372	236.478	96.52552	0.981075	189.477	9652.552	98.10746	18947.7	10.1		1312.747	13.34261	2576.887
482.281	207.676	3.984	2.215	275.575	293.441	122.948	1.414026	235.1094	12294.8	141.4026	23510.94	10.1		1672.093	19.23075	3197.488
513.344	215.967	3.994	2.183	305.128	319.012	127.858	1.393285	255.5941	12785.8	139.3285	25559.41	10.4		1777.226	19.36667	3552.758
504.24	215.852	3.386	1.789	287.212	304.88	127.7899	1.137922	244.2731	12778.99	113.7922	24427.31	10.8		1827.395	16.27229	3493.105
													MEAN	1583.326	13.33693	3119.612
Filmjölk													MEDIAN	1630.13	15.09181	3217.861
Galctose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
17.357	6.966			380.695	415.574	4.086225		332.9489	408.6225		33294.89	10.3		56.38991	0	4594.695
18.14	7.405			379.227	421.375	4.346204		337.596	434.6204		33759.6	10		58.67375	0	4557.546
13.279	7.116			283.641	290.274	4.175056		232.5724	417.5056		23257.24	10.9		60.12081	0	3349.042
15.331	6.269			346.263	372.333	3.673457		298.309	367.3457		29830.9	10.4		51.06106	0	4146.495
14.061	5.802			327.091	339.221	3.396897		271.7833	339.6897		27178.33	10.5		47.55656	0	3804.966
9.348	3.843			236.498	237.731	2.236764		190.4807	223.6764		19048.07	10.5		31.3147	0	2666.73
14.104	5.764			324.784	348.811	3.374393		279.4658	337.4393		27946.58	10.8		48.25382	0	3996.36
19.123	7.588			391.031	437.527	4.454578		350.5352	445.4578		35053.52	10.2		61.02772	0	4802.332
13.501	6.094	n.a.	n.a.	309.297	301.925	3.569821		241.9059	356.9821		24190.59	9.7		47.12164	0	3193.158
18.598	7.689			375.109	414.225	4.514391		331.8682	451.4391		33186.82	10.1		61.39571	0	4513.408
10.462	4.348			248.377	252.404	2.535828		202.2351	253.5828		20223.51	9.8		33.72652	0	2689.727
20.133	8.101			404.18	447.807	4.75838		358.7704	475.838		35877.04	10.9		68.52067	0	5166.294
													MEAN	52.0969	0	3956.729
Onaka													MEDIAN	53.72548	0	4071.428
Galctose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
19.511	7.945			340.167	360.588	4.665995		288.9002	466.5995		28890.02	10.9		67.19034	0	4160.163
19.754	8.346			347.28	365.901	4.90347		293.1564	490.347		29315.64	9.8		65.21616	0	3898.98
22.825	9.544	0.807	0.405	360.226	389.043	5.612934	0.24091	311.6952	561.2934	24.091	31169.52	9.9		75.21331	3.228194	4176.715
24.771	10.385	0.839	0.428	368.216	405.357	6.11098	0.255817	324.7642	611.098	25.5817	32476.42	10.5		85.55371	3.581438	4546.698
24.658	10.303	1.928	0.95	346.278	361.19	6.062419	0.594141	289.3824	606.2419	59.41409	28938.24	10.3		83.66138	8.199144	3993.478
22.843	9.565	1.78	0.88	333.501	341.527	5.62537	0.548772	273.6306	562.537	54.87718	27363.06	10.2		77.06757	7.518174	3748.739
30.308	12.858	3.093	1.291	380.711	416.047	7.575506	0.815153	333.3278	757.5506	81.51533	33332.78	10.4		105.2995	11.33063	4633.257
29.521	12.478	3.039	1.276	373.488	409.858	7.350468	0.805431	328.3699	735.0468	80.54313	32836.99	10.6		103.6416	11.35658	4630.015
26.312	11.77	3.785	1.6	325.913	344.527	6.931186	1.015425	276.0339	693.1186	101.5425	27603.39	10.2		94.95724	13.91133	3781.664
23.666	10.429	3.424	1.448	297.84	311.819	6.137037	0.91691	249.8319	613.7037	91.69097	24983.19	10.2		84.0774	12.56166	3422.696
28.538	12.572	4.759	2.023	336.332	360.647	7.406135	1.289585	288.9474	740.6135	128.9585	28894.74	10.4		102.9453	17.92523	4016.37
20.351	9.061	3.377	1.44	263.365	270.017	5.326898	0.911725	216.3447	532.6898	91.17247	21634.47	10.4		74.04388	12.67297	3007.191
													MEAN	84.90562	8.523779	4001.331
A-fil													MEDIAN	83.86939	9.764888	4004.924
Galctose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
12.253	6.425			314.933	332.174	3.765842		266.138	376.5842		26613.8	10.1		51.21544	0	3619.477
12.267	5.003			346.055	369.283	2.923724		295.8657	292.3724		29586.57	10.8		41.80925	0	4230.879
15.407	6.422			383.765	419.185	3.764065		335.8416	376.4065		33584.16	10.6		53.07332	0	4735.367
10.9	4.527			305.889	305.889	2.641833		245.0814	264.1833		24508.14	10.3		36.4573	0	3382.123
12.588	5.159			331.514	344.179	3.016108		275.7551	301.6108		27575.51	10.5		42.22551	0	3860.571
12.187	4.987	n.a.	n.a.	332.794	338.239	2.914248		270.9966	291.4248		27099.66	10.2		39.9252	0	3712.654
13.92	5.803			341.034	360.389	3.397489		288.7408	339.7489		28874.08	10.1		46.20585	0	3926.874
15.165	6.28			367.998	385.639	3.679972		308.9683	367.9972		30896.83	10.8		52.62359	0	4418.246
19.68	11.299			377.926	415.6	6.652256		332.9697	665.2256		33296.97	10.1		90.47069	0	4528.388
16.2537	7.1834	3.2545	1.3858	414.68	469.375	4.214971	0.876596	376.0483	421.4971	87.6596	37604.83	10.7		59.85259	12.44766	5339.886
15.616	7.372	0.404	0.243	338.356	361.55	4.326661	0.135913	289.6708	432.6661	13.59129	28967.08	10.2		59.27526	1.862007	3968.49
14.791	5.96			341.23	354.434	3.490465		283.9703	349.0465		28397.03	10.6		49.21556	0	4003.981
													MEAN	51.86246	1.192473	4143.911
													MEDIAN	50.2155	0	3986.236

UHT milk 4 degrees C																
Galactose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
5.814	2.371	4.676	2.176	560.222	697.498	1.224557	1.220555	551.6187	61.22783	61.02775	27580.93	10.1		8.326985	8.299774	3751.007
6.299	2.672	5.325	2.583	654.648	850.562	1.397337	1.47608	672.6944	69.86683	73.80399	33634.72	10.7		9.921089	10.48017	4776.13
5.497	2.28	4.455	2.141	543.99	680.005	1.172321	1.198581	537.7814	58.61604	59.92906	26889.07	10.4		8.147629	8.330139	3737.581
5.411	2.194	4.371	2.071	544.764	659.245	1.122955	1.154633	521.36	56.14775	57.73167	26068	10.6		7.916833	8.140165	3675.588
13.067	5.308	10.589	4.997	690.119	1132.42	2.910453	2.99165	895.6481	145.5226	149.5825	44782.4	5.1		12.51495	12.86409	3851.287
17.152	7.075	14.582	6.947	875.282	1526.198	3.924746	4.215909	1207.132	196.2373	210.7955	60356.6	4.8		16.2877	17.49602	5009.598
12.222	4.955	9.855	4.646	671.363	1038.386	2.707824	2.771283	821.2659	135.3912	138.5642	41063.29	4.6		10.96669	11.2237	3326.127
11.084	4.535	9.032	4.301	685.806	1047.041	2.466736	2.554684	828.1121	123.3368	127.7342	41405.6	5.2		10.7303	11.11287	3602.288
6.026	2.475	4.903	2.309	560.409	700.805	1.284255	1.304056	554.2345	64.21273	65.20279	27711.73	5		5.458082	5.542237	2355.497
20.904	8.372	17.178	8.023	765.974	1445.368	4.66925	4.891449	1143.194	233.4625	244.5725	57159.72	5.2		20.31124	21.2778	4972.895
4.774	1.955	3.874	1.842	517.472	610.122	0.985764	1.010861	482.503	49.28822	50.54307	24125.15	5.2		4.288075	4.397247	2098.888
10.854	4.575	9.193	4.467	775.931	1179.293	2.489696	2.658903	932.7253	124.4848	132.9451	46636.26	5.4		11.07915	11.83212	4150.627
5.865	2.337	4.767	2.214	560.752	673.47	1.20504	1.244412	532.6122	60.25199	62.22062	26630.61	10.2		8.254523	8.524225	3648.393
5.102	2.089	4.155	1.977	540.441	636.847	1.062683	1.095618	503.6429	53.13415	54.78089	25182.14	10.3		7.332512	7.559763	3475.136
4.442	1.809	3.618	1.71	498.452	561.237	0.901957	0.927988	443.8343	45.09787	46.39942	22191.71	9.8		5.998017	6.171123	2951.498
5.976	2.434	4.824	2.287	562.975	708.734	1.26072	1.290244	560.5065	63.03599	64.51218	28025.32	10.3		8.698967	8.902681	3867.495
5.313	2.187	4.302	2.046	538.274	642.811	1.118937	1.138938	508.3605	55.94685	56.94689	25418.02	10.5		7.832558	7.972564	3558.523
5.537	2.293	4.507	2.162	541.578	663.084	1.179783	1.211765	524.3967	58.98915	60.58827	26219.83	10.5		8.258481	8.482358	3670.777
5.258	2.163	4.269	2.024	545.831	659.65	1.10516	1.125126	521.6804	55.25802	56.25628	26084.02	9.8		7.349317	7.482085	3469.174
5.777	2.309	4.708	2.19	557.374	662.557	1.188967	1.229345	523.9798	59.44837	61.46723	26198.99	10.2		8.144426	8.42101	3589.262
													MEAN	9.390876	9.725607	3676.889
UHT milk 20 degrees C													MEDIAN	8.256502	8.451684	3659.585
Galactose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
5.814	2.371	4.676	2.176	560.222	697.498	1.224557	1.220555	551.6187	61.22783	61.02775	27580.93	10.1		8.326985	8.299774	3751.007
6.299	2.672	5.325	2.583	654.648	850.562	1.397337	1.47608	672.6944	69.86683	73.80399	33634.72	10.7		9.921089	10.48017	4776.13
6.481	2.61	5.262	2.456	576.732	718.658	1.361747	1.396346	568.3565	68.08737	69.8173	28417.83	10.4		9.464144	9.704605	3950.078
5.036	2.081	4.074	1.946	536.641	637.888	1.058091	1.076155	504.4663	52.90454	53.80776	25223.32	10.1		7.195018	7.317855	3430.371
15.932	6.404	12.854	5.979	718.081	1213.364	3.539579	3.608174	959.6759	176.9789	180.4087	47983.8	4.4		13.98134	14.25229	3790.72
10.868	4.442	8.659	4.121	633.445	938.81	2.413352	2.441675	742.4998	120.6676	122.0838	37124.99	5.4		10.73942	10.86545	3304.124
9.384	3.718	7.518	3.467	624.998	838.269	1.997761	2.031077	662.9705	99.88807	101.5539	33148.52	5.1		8.590374	8.733633	2850.773
8.357	3.58	6.976	3.441	707.957	989.422	1.918547	2.014754	782.5346	95.92733	100.7377	39126.73	5.1		8.24975	8.663442	3364.899
14.798	5.868	11.849	5.489	711.253	1161.689	3.231904	3.30054	918.8003	161.5952	165.027	45940.01	5.2		14.05878	14.35735	3996.781
14.59	5.973	11.694	5.529	717.337	1205.715	3.292176	3.325653	953.6255	164.6088	166.2826	47681.27	4.7		13.49792	13.63518	3909.864
5.492	2.22	4.354	2.037	542.522	645.556	1.13788	1.133287	510.5318	56.89398	56.66436	25526.59	4.7		4.665306	4.646478	2093.18
22.25	9.022	18.343	8.603	895.007	1643.842	5.042363	5.255588	1300.19	252.1181	262.7794	65009.5	5.1		21.68216	22.59903	5590.817
5.679	2.333	4.475	2.131	551.405	681.239	1.202744	1.192303	538.7576	60.13719	59.61514	26937.88	10.3		8.298932	8.22689	3717.427
5.867	2.377	4.639	2.188	553.196	682.756	1.228001	1.228089	539.9575	61.40003	61.40445	26997.88	9.9		8.227605	8.228196	3617.715
6.423	2.59	5.074	2.391	585.489	723.569	1.350267	1.355537	572.2412	67.51335	67.77687	28612.06	10.5		9.451868	9.488762	4005.688
6.107	2.597	5.018	2.442	642.841	818.685	1.354285	1.387557	647.4793	67.71425	69.37783	32373.96	10.5		9.479995	9.712896	4532.355
5.919	2.402	4.606	2.166	547.159	659.066	1.242351	1.214277	521.2184	62.11756	60.71384	26060.92	10.4		8.634341	8.439223	3622.468
6.417	2.599	5.017	2.339	562.811	685.772	1.355433	1.322891	542.3432	67.77165	66.14453	27117.16	10.4		9.42026	9.194089	3769.285
4.449	1.809	3.446	1.621	493.974	545.066	0.901957	0.872112	431.0428	45.09787	43.6056	21552.14	10.6		6.3588	6.14839	3038.852
5.373	2.194	4.136	1.957	532.565	630.952	1.122955	1.083061	498.9798	56.14775	54.15306	24948.99	10.2		7.692242	7.41897	3418.012
													MEAN	9.896816	10.02063	3726.527
UHT milk 30 degrees C													MEDIAN	8.256502	8.451684	3659.585
Galactose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
5.814	2.371	4.676	2.176	560.222	697.498	1.224557	1.220555	551.6187	61.22783	61.02775	27580.93	10.1		8.326985	8.299774	3751.007
6.299	2.672	5.325	2.583	654.648	850.562	1.397337	1.47608	672.6944	69.86683	73.80399	33634.72	10.7		9.921089	10.48017	4776.13
5.587	2.318	4.477	2.157	544.049	677.622	1.194134	1.208626	535.8965	59.70668	60.43132	26794.82	10		8.060401	8.158228	3617.301
5.47	2.2	4.38	2.033	549.528	652.877	1.126399	1.130776	516.3228	56.31996	56.5388	25816.14	10.6		7.941114	7.971971	3640.076
16.453	6.676	13.147	6.137	784.061	1306.546	3.695712	3.707371	1033.384	184.7856	185.3685	51669.21	5.4		16.44592	16.4978	4598.56
17.828	7.126	14.155	6.577	756.037	1295.818	3.954021	3.983614	1024.898	197.7011	199.1807	51244.91	5.4		17.59539	17.72708	4560.797
16.162	6.585	12.439	5.858	729.976	1237.889	3.643476	3.532207	979.0755	182.1738	176.6104	48953.78	5.1		15.66695	15.18849	4210.025
17.505	7.178	13.503	6.445	722.377	1290.182	3.98387	3.900741	1020.44	199.1935	195.037	51022	5.1		17.13064	16.77319	4387.892
17.616	7.074	13.195	6.126	725.55	1242.891	3.924172	3.700465	983.0322	196.2086	185.0232	49151.61	5.2		17.07015	16.09702	4276.19
14.015	5.605	10.486	4.848	684.469	1051.359	3.080937	2.898104	831.5277	154.0468	144.9052	41576.38	5.2		13.40208	12.60675	3617.145
12.121	4.858	8.85	4.123	659.917	975.729	2.652144	2.442931	771.7033	132.6072	122.1465	38585.16	5.2		11.53683	10.62675	3356.909
17.986	7.342	13.06	6.168	709.384	1241.69	4.078009	3.726833	982.0822	203.9005	186.3417	49104.11	5.1		17.53544	16.02538	4222.953
6.275	2.589	4.538	2.158	540.43	647.434	1.349693	1.209254	512.0173	67.48464	60.46271	25600.87	10.3		9.312881	8.343854	3532.92
6.955	2.833	4.931	2.321	575.328	702.337	1.489754	1.31159	555.4464	74.48769	65.57948	27772.32	10.3		10.2793	9.049969	3832.58
6.578	2.71	4.466	2.133	536.684	662.645	1.419149	1.193559	524.0494	70.95747	59.67793	26202.47	10.5		9.934045	8.35491	3668.346
6.284	2.547	4.319	2.026	539.368	632.776	1.325584	1.126381	500.4226	66.2792	56.31906	25021.13	10.5		9.279088	7.884669	3502.958
7.854	3.179	5.24	2.469	567.892	708.387	1.688365	1.404508	560.232	84.41823	70.22539	28011.6	10.5		11.81855	9.831554	3921.624
7.311	2.979	4.837	2.298	560.89	689.763	1.573561	1.29715	545.5002	78.67803	64.85748	27275.01	9.8		10.46418	8.626045	3627.576
7.367	2.994	4.787	2.257	552.573	669.251	1.582171	1.271409	529.2749	79.10855	63.57044	26463.74	10.7		11.23341	9.027003	3757.852
7.497	3.197	5.08	2.459	620.116	770.102	1.698697	1.39823	609.0494	84.93485	69.91148	30452.47	9.9		11.38127	9.368138	4080.631
													MEAN	12.21679	11.34694	3946.974
													MEDIAN	11.30734	9.599846	3795.216

Milk																
Galactose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
2.115	0.843	1.89	0.864	354.356	349.765	0.460144	0.538402	280.23	46.01445	53.84017	28023	10.5		6.442023	7.537624	3923.22
2.416	0.968	2.169	1	385.97	393.188	0.53417	0.626547	315.0157	53.41703	62.65474	31501.57	11.1		7.798887	9.147592	4599.229
													MEAN	7.120455	8.342608	4261.225
Lactose free milk													MEDIAN	7.120455	8.342608	4261.225
Galactose		Glucose		Lactose		Fitted line			Dilution c	100						
nC	nC*min	nC	nC*min	nC	nC*min	Galactose	Glucose	Lactose	Galactose	Glucose	Lactose	Milk (g)		Galactose	Glucose	Lactose
25.63	10.249	20.448	9.341			7.727018	7.843322		7727.018	7843.322	0	10.3		1066.328	1082.378	0
37.576	15.392	32.426	15.4	0.38	0.273	11.33058	12.45163	0.940039	11330.58	12451.63	940.0392	9.8		1506.967	1656.066	125.0252
33.446	13.498	27.437	12.662	0.804	0.652	10.0035	10.36918	1.277708	10003.5	10369.18	1277.708					
38.298	15.489	33.351	15.466	0.376	0.246	11.39854	12.50183	0.915984	11398.54	12501.83	915.9836	10.4		1584.397	1737.754	127.3217
39.226	15.979	34.072	16.094	0.373	0.227	11.74187	12.97946	0.899056	11741.87	12979.46	899.0556	10.4		1632.12	1804.146	124.9687
39.82	16.331	34.384	16.299	0.405	0.293	11.98851	13.13538	0.957858	11988.51	13135.38	957.8582	10.6		1690.38	1852.089	135.058
40.694	16.518	35.449	16.455	0.382	0.244	12.11953	13.25403	0.914202	12119.53	13254.03	914.2017	10.6		1708.854	1868.818	128.9024
41.631	17.011	35.909	17.005	0.423	0.309	12.46497	13.67235	0.972113	12464.97	13672.35	972.1133	10.2		1707.7	1873.111	133.1795
43.927	17.844	38.386	18.008			13.04863	14.4352		13048.63	14435.2	0	10.3		1800.71	1992.057	0
45.797	18.662	39.519	18.422	0.465	0.332	13.62178	14.75008	0.992605	13621.78	14750.08	992.6051	10.6		1920.671	2079.761	139.9573
32.249	13.114	27.916	13.024			9.734445	10.64451		9734.445	10644.51	0	9.9		1304.416	1426.364	0
34.856	14	29.898	13.76	0.361	0.24	10.35524	11.20429	0.910638	10355.24	11204.29	910.6379	10.7		1470.444	1591.009	129.3106
													MEAN	1581.181	1723.959	94.88396
													MEDIAN	1632.12	1804.146	127.3217

Appendix 5

A popular scientific summary of the report.

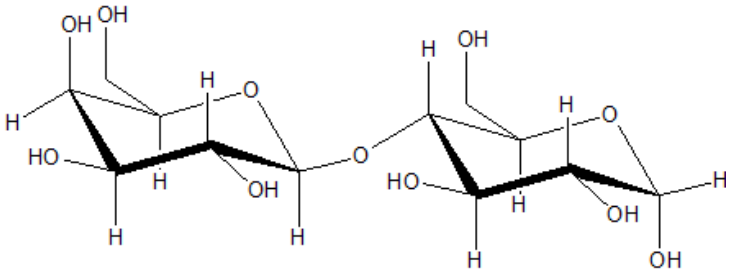
The holy dairy cow and the milk we drink

Recently, there has been a lot of debate on the subject of milk and milk consumption. The newspapers and social media have been flooded with opinions and thoughts about the holy dairy cow.

This fall, a study was published by Swedish scientist who claimed that excessive milk consumption lead to increased risk of fractures and risk of death. They had studied two large groups of Swedish men and women during 20 years, and could see that those who drank more than three glasses of milk a day were more ill, and had more traces of inflammation and ageing processes in their blood. The scientist, led by Karl Michaëlsson at Akademiska Sjukhuset in Uppsala, suggested the culprit could be the milk's content of carbohydrates.

Lactose is the main carbohydrate in milk, which is made up from the building blocks galactose and glucose. Galactose had been used in animal studies, where one is interested in studying ageing processes. Mice and rats that have been exposed to galactose show the same symptoms of ageing as "naturally" aged animals, but in much shorter time. The mechanism is not clear, but one suggests a higher level of blood glucose makes the cells age faster.

Therefore, the Uppsala scientists suggested the milks content of galactose to be the villain. One glass of milk, around two decilitres, contain 9.3 g of lactose. Divided by two, the total galactose content is then 4.7 g, which is the equal amount of the galactose used in the animal studies, per bodyweight. This leads to the question; by drinking milk, do we accelerate our own ageing?

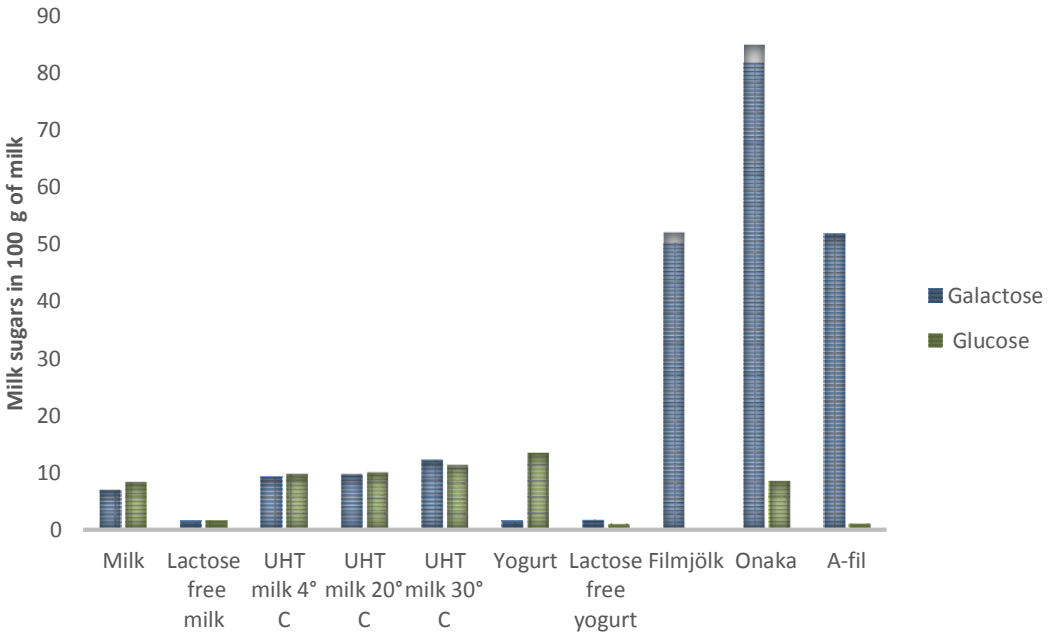


This is the lactose molecule, where the two building blocks galactose and glucose are connected via the oxygen (O) in the middle. The galactose is to the right.

In order to answer that question, we must first know how much galactose different dairy products contain. The dairy processes vary, for example some of the lactose is degraded when the milk is pasteurized. Also, the lactose is consumed by Lactic

acid bacteria in the production of yogurt. This is two example of dairy processes which affects the content of lactose, and also the content of galactose. By analysing the content of lactose, galactose and glucose in different dairy products found on the Swedish market, we can know what products we should avoid, provided that galactose is bad for us.

This study was performed at a project on the Swedish University of Agricultural Sciences. The result show that the amount of galactose in one glass of milk (low-pasteurized, 1.5% fat) gives the same dose as the one used in the animal studies, enough for a 93 kg human. In lactose free milk, the amounts are even higher, since the lactose split into galactose and glucose in the reduction process. In milk with extended shelf life (often called UHT milk) stored at warmer room temperature, the amounts of galactose has increased more than in milk stored at colder temperatures. The amount of free galactose in different dairy products is higher compared to milk, since the Lactic acid bacteria has broken down the lactose into separate parts. In the Uppsala study, no correlation between yogurt consumption, and risk of disease was seen, which contradicts the galactose theory.



The amount of galactose and glucose in different dairy products, found on the Swedish market. Since the lactose is broken down in the lactose free products, the amount of galactose and glucose is very much higher, the values are expressed as g/100 g. The other values are expressed as mg/100g.

But then again. This result needs to be carefully evaluated, before a conclusion can be drawn. Lactose, and galactose as well, are involved in the production of prebiotics found in yogurt, which have positive health effects. More research is needed, to know if the whole story of the milk we drink.