Thermal processing of milk: Effects on nutrient content and physical properties

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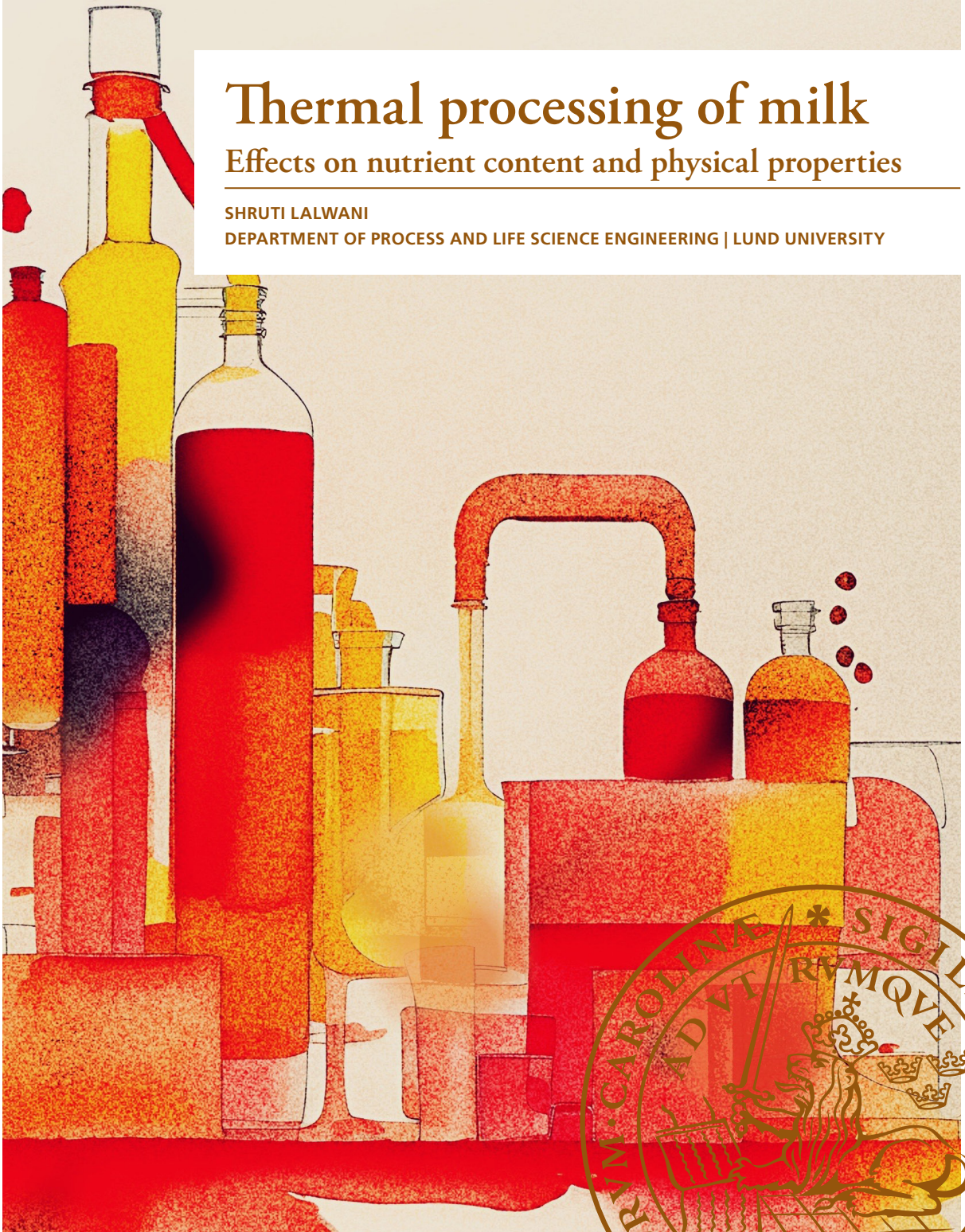

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Title and subtitle: Thermal Processing of Milk - Effects on nutrient content and physical properties

Abstract: Milk is a nutrient-dense fluid which is essential for a healthy balanced diet. Thermal processing is the dominating approach in the dairy industry to ensure food safety, however, heat treatment can alter both the composition and quality of the milk. The aim of this thesis has been to evaluate the effects of various processing technologies on the nutrient content and physical properties of milk and cream, mainly in the production of low- and high-pasteurised milk, milk with extended shelf-life (ESL) milk and ultra-high temperature (UHT) treated milk. The studies were performed in laboratory, pilot and dairy production scale to evaluate thermal processing effects on the milk system and to develop kinetic models for vitamin losses. The results showed that the concentration of macro components in milk did not change due to heat treatment. However, vitamin degradation was observed in milk heat-treated at laboratory scale as well as at pilot scale for ESL/UHT treatment using both direct and indirect heating systems. The degradation increased with higher heating temperatures and longer holding times, although no differences were observed between heating systems. At dairy production scale, high pasteurisation caused the most significant vitamin degradation, while limited losses were observed for high-temperature-short-time (HTST) pasteurisation, ESL, and UHT processing. During storage, vitamin losses varied between 1-22% for different heat treatments, and more pronounced effects of vitamin degradation were observed with higher heat treatment and longer storage times. The concentration of vitamin B_{12} tended to decrease more over time than vitamins B_1, B_2, and E, and more losses of vitamins occurred during storage than after heat treatment. The total mineral concentration in milk and cream showed limited losses. Additionally, a decrease in ionic calcium concentration was observed after heat treatment, while no significant changes were observed during shorter storage times.An increase in fat globule size and aggregate formation were observed in milk heat-treated using direct heating at pilot scale, with more pronounced effects at higher temperatures. However, the physical stability of milk remained unaffected on the day of production. Heat treatments performed at dairy production scale resulted in an increase in casein micelle size but had no effect on the physical stability of milk. However, during storage of milk after heat treatment at both pilot and dairy production scale, physical stability of milk was affected. Further, during storage a decrease in pH was observed with longer storage times. Minimal changes in milk colour were observed after heat treatment, whereas larger changes in colour were observed during prolonged storage. Laboratory scale experiments were used to fit kinetic models of vitamin degradation in milk (vitamins B_1, B_2 and E), which were validated with five different heat treatment processing lines at dairy production scale. Results showed that the predicted values obtained from laboratory scale experiments fit the validation data well. This means that simplified laboratory scale experiments can give valid predictions of thermal degradation of vitamins in milk during processing at dairies. The thesis has highlighted that heating systems for ESL/UHT treatment may influence milk quality, although no clear distinction between direct and indirect heating systems was observed at pilot scale for the investigated quality parameters. Furthermore, the thesis has shown that process techniques for heat treatment used in dairy production scale today are mild and have low effects on the nutrient content and physical properties of milk and cream, and that more pronounced changes primarily occur during longer storage times.

Key words: Bovine milk, heat treatment, storage, nutrient, vitamin degradation, physical property, UHT milk, ESL milk, dairy production, kinetic modelling

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Milky Ways: A doctoral quest into processing marvels

Table of Contents

Abstract

Milk is a nutrient-dense fluid which is essential for a healthy balanced diet. Thermal processing is the dominating approach in the dairy industry to ensure food safety, however, heat treatment can alter both the composition and quality of the milk. The aim of this thesis has been to evaluate the effects of various processing technologies on the nutrient content and physical properties of milk and cream, mainly in the production of low- and high-pasteurised milk, milk with extended shelf-life (ESL) milk and ultra-high temperature (UHT) treated milk. The studies were performed in laboratory, pilot and dairy production scale to evaluate thermal processing effects on the milk system and to develop kinetic models for vitamin losses.

The results showed that the concentration of macro components in milk did not change due to heat treatment. However, vitamin degradation was observed in milk heat-treated at laboratory scale as well as at pilot scale for ESL/UHT treatment using both direct and indirect heating systems. The degradation increased with higher heating temperatures and longer holding times, although no differences were observed between heating systems. At dairy production scale, high pasteurisation caused the most significant vitamin degradation, while limited losses were observed for high-temperature-short-time (HTST) pasteurisation, ESL, and UHT processing. During storage, vitamin losses varied between 1-22% for different heat treatments, and more pronounced effects of vitamin degradation were observed with higher heat treatment and longer storage times. The concentration of vitamin B_{12} tended to decrease more over time than vitamins B_1, B_2, and E, and more losses of vitamins occurred during storage than after heat treatment. The total mineral concentration in milk and cream showed limited losses. Additionally, a decrease in ionic calcium concentration was observed after heat treatment, while no significant changes were observed during shorter storage times.

An increase in fat globule size and aggregate formation were observed in milk heat-treated using direct heating at pilot scale, with more pronounced effects at higher temperatures. However, the physical stability of milk remained unaffected on the day of production. Heat treatments performed at dairy production scale resulted in an increase in casein micelle size but had no effect on the physical stability of milk. However, during storage of milk after heat treatment at both pilot and dairy production scale, physical stability of milk was affected. Further, during storage a decrease in pH was observed with longer storage times. Minimal changes in milk

colour were observed after heat treatment, whereas larger changes in colour were observed during prolonged storage.

Laboratory scale experiments were used to fit kinetic models of vitamin degradation in milk (vitamins B_1, B_2 and E), which were validated with five different heat treatment processing lines at dairy production scale. Results showed that the predicted values obtained from laboratory scale experiments fit the validation data well. This means that simplified laboratory scale experiments can give valid predictions of thermal degradation of vitamins in milk during processing at dairies.

The thesis has highlighted that heating systems for ESL/UHT treatment may influence milk quality, although no clear distinction between direct and indirect heating systems was observed at pilot scale for the investigated quality parameters. Furthermore, the thesis has shown that process techniques for heat treatment used in dairy production scale today are mild and have low effects on the nutrient content and physical properties of milk and cream, and that more pronounced changes primarily occur during longer storage times.

Popular science summary

Milk is a nutritious food that is crucial for a healthy and balanced diet. Heat treatment is the dominating approach in the dairy industry to ensure food safety since it facilitates the destruction of pathogens and most spoilage bacteria and enzymes and thereby extends the shelf-life of dairy products. Heat treatment of milk can alter both the composition and quality of the milk. Process techniques for heat treatment used in the dairy industry today probably have smaller effects on milk quality than older technologies that form the basis for nutritional values declared to consumers. The aim of this study was to evaluate the effects of various processing technologies on the nutritional quality and physical properties of milk, mainly in the production of low- and high-pasteurised milk, milk with extended shelf-life (ESL) milk and ultra-high temperature (UHT) treated milk. The studies were performed in laboratory, pilot and dairy industrial scale to evaluate thermal processing effects on the milk system.

The thesis examined raw and processed milk and/or cream that were heat-treated with low- and high-pasteurisation as well as ESL and UHT treatment. Samples were stored at different temperatures and times. Additionally, a pilot-scale study on ESL/UHT treatment with direct and indirect heating systems was conducted. The products were analysed for macro- and micro components as well as physical properties. The data and results were evaluated with statistical models and used to develop models that predict the rate of vitamin degradation (kinetic models).

In this thesis, minimal losses were demonstrated for macro components and total mineral content for milk and cream after low- and high-pasteurisation, as well as ESL and UHT treatment in dairy production. A decrease in the concentration of vitamin B_1, B_2, and B_{12}, and free calcium in milk was observed after high pasteurisation. The size of the casein micelles, the main protein group in milk, increased but no effect on physical stability was found between heat treatments. During storage, a decrease in pH was observed with longer storage times. Vitamin losses varied between 1-22% for different heat treatments, and more pronounced effects of vitamin degradation were observed with higher heat treatment and longer storage times. The concentration of vitamin B_{12} tended to decrease more over time than vitamins B_1, B_2, and E, and more losses of vitamins occurred during storage than after heat treatment. Effects on physical stability and colour changes in milk and cream were observed during prolonged storage. This suggests that heat

treatment techniques used at dairies today are mild with low effects on milk quality and that major changes primarily occur during longer storage periods.

A detailed study in pilot scale for ESL/UHT treatment with direct and indirect heating systems showed no impact on the fat, protein, and lactose contents in milk, while pH and ionic calcium were affected by the different heating systems. Vitamin degradation was observed for both direct and indirect heating and the degradation increased with increasing temperature and time, however no difference could be seen between heating systems. No effect of heating system was observed on milk colour. Large aggregates were formed during direct heat treatment, influenced by temperature, due to the formation of fat aggregates. This suggests that heating system may influence milk quality, however, no clear differences between direct and indirect heating were observed for the quality parameters investigated.

Kinetic models for vitamin degradation during heat treatment were developed, which can be used to estimate vitamin losses at dairy level. Although kinetic models for the degradation of milk components have been presented previously, this is the first study to validate and compare such models at the dairy production level using contemporary heat treatment techniques.

In summary, the thesis has shown that process techniques for heat treatment used in dairy production today are mild and have low effects on the nutritional and physical properties of milk and dairy products, and that more pronounced changes primarily occur during longer storage times. This demonstrates that the nutritional quality and physical properties of milk and dairy products are maintained after processing at dairy plants and that these can be declared as products with high nutrient density. The development of kinetic models for vitamin degradation during heat treatment have great potential for further optimization of dairy processes. The thesis has contributed with knowledge to ensure the quality of milk claimed to consumers, which may lead to increased added value for milk as a raw material in dairy products and milk-based foods.

Populärvetenskaplig sammanfattning

Mjölk är ett näringsrikt livsmedel som är avgörande för en hälsosam och balanserad kost. Värmebehandling är det dominerande tillvägagångssättet inom mejeriindustrin för att säkerställa livsmedelssäkerhet, eftersom det avdödar patogena och de flesta produktförstörande bakterier och enzymer och därmed förlänger hållbarheten av mejeriprodukter. Värmebehandling av mjölk kan förändra både mjölkens sammansättning och kvalitet. Tekniker för värmebehandling som idag används inom mejeriindustrin har sannolikt mindre effekt på mjölkens kvalitet än äldre processtekniker som ligger till grund för näringsvärden som deklareras till konsumenterna. Syftet med denna avhandling var att utvärdera påverkan av olika processtekniker på näringskvalitet och funktionalitet hos mejeriprodukter, främst vid produktion av låg- och högpastöriserad mjölk samt mjölk med förlängd hållbarhet (ESL-mjölk) och ultrahögpastöriserad (UHT) mjölk. Studierna utfördes i laboratorie-, pilot- och mejeriindustriskala för att utvärdera effekter av värmebehandling på mjölksystemet.

I doktorandprojektet undersöktes obehandlad och processad mjölk och/eller grädde som värmebehandlats med låg- och högpastörisering samt ESL- och UHT-behandling. Proverna lagrades vid olika temperatur och tid. Dessutom gjordes en pilotskalestudie på ESL/UHT-behandling med direkt och indirekt värmesystem. Produkterna analyserades för makro- och mikrokomponenter samt funktionella egenskaper. Datan och resultaten har utvärderats med statistiska modeller samt använts för att utveckla modeller för hastigheten av vitaminnedbrytning (kinetikmodeller).

Avhandlingen har visat minimala förluster i makrokomponenter och totalt mineralinnehåll för mjölk och grädde efter låg- och högpastörisering samt ESL- och UHT-behandling. En minskning i koncentration av vitamin B_1, B_2 och B_{12} och fritt kalcium i mjölk observerades efter högpastörisering. Storleken på mjölkens kaseinmiceller, den största gruppen av proteiner i mjölk, ökade men ingen effekt på stabilitet visades mellan värmebehandlingarna. Under lagring observerades en minskning av pH vid längre lagringstider. Vitaminförlusterna varierade mellan 1-22% för olika värmebehandlingar och mer tydliga effekter av vitaminnedbrytning observerades vid kraftigare värmebehandling och längre lagringstid. Vitamin B_{12}-koncentrationen tenderade att minska mer över tid än vitamin B_1, B_2 och E och mer förluster av vitaminer skedde under lagring än efter värmebehandling. Effekter på stabilitet och färgförändringar i mjölk och grädde observerades under långvarig

lagring. Detta tyder på att värmebehandlingstekniker som används i industriell skala idag är milda med låga effekter på mjölkkvaliteten och att större förändringar främst sker under längre lagringstider.

En detaljerad delstudie i pilotskala för ESL/UHT-behandling med direkt och indirekt värmesystem visade ingen påverkan på fett-, protein- och laktoshalten i mjölk, medan pH och fritt kalcium påverkades av de olika värmesystemen. Nedbrytning av vitaminer observerades för både direkt och indirekt värmesystem och nedbrytningen ökade med ökande temperatur och hålltid, dock kunde ingen skillnad ses mellan de studerade värmesystemen. Ingen effekt av värmesystem observerades gällande mjölkens färg. Vid direkt värmebehandling bildades stora aggregat som påverkades av temperaturen, vilket berodde på bildning av fettaggregat. Detta tyder på att värmesystemet kan påverka mjölkkvaliteten, men inga tydliga skillnader mellan direkt och indirekt värmesystem observerades för de undersökta kvalitetsparametrarna.

Kinetikmodeller för nedbrytningshastighet av vitaminer under värmebehandling utvecklades, som kan användas för att uppskatta vitaminförluster på mejerinivå. Även om kinetikmodeller för nedbrytning av mjölkens beståndsdelar har presenterats tidigare är detta den första studien som validerar sådana modeller på mejerinivå med hjälp av nutida värmebehandlingstekniker.

Sammanfattningsvis har avhandlingen visat att värmebehandlingstekniker som används i mejeriproduktionsskala idag är milda och har låga effekter på näringsmässiga och funktionella egenskaper hos mjölk och mejeriprodukter och att större förändringar främst sker under längre lagringstider. Detta visar att den näringsmässiga kvaliteten och funktionaliteten hos mjölk och mejeriprodukter bibehålls efter processing på mejerierna och att dessa kan deklareras som produkter med hög näringstäthet. Utvecklingen av kinetikmodeller för nedbrytning av vitaminer under värmebehandling har stor potential för ytterligare optimering av mejeriprocesser. Avhandlingen har bidragit med kunskap för att säkerställa kvaliteten som levereras till konsumenterna, vilket kan leda till ökat mervärde på mjölk som råvara i mejeriprodukter och mjölkbaserade livsmedel.

List of papers

Paper I

Lalwani S., Glantz M., Paulsson M. and Håkansson A. (2021). **The effect of free convection on apparent vitamin degradation kinetics.** *Food and Bioproducts Processing*, 130, 182-194.

Paper II

Lalwani S., Lewerentz F., Håkansson A., Babic M., Madina P., Edén J., Paulsson M. and Glantz M. **A pilot scale study on the effects of various temperature and time conditions on milk quality using direct and indirect heating systems.** *Accepted in International Dairy Journal*

Paper III

Lalwani S., Lewerentz F., Håkansson A., Löfgren R., Eriksson J., Paulsson M. and Glantz M. (2024). **Impact of thermal processing on micronutrients and physical stability of milk and cream at dairy production scale.** *International Dairy Journal*, 153, 105901.

Paper IV

Lalwani S., Lewerentz F., Håkansson A., Löfgren R., Eriksson J., Paulsson M. and Glantz M. **Changes in nutritional and technological properties of heat-treated milk and cream at dairy production scale during storage.** *International Dairy Journal*, 154, 105927.

Paper V

Lalwani S., Lewerentz F., Löfgren R., Paulsson M., Glantz M. and Håkansson A. **Exploring predictive kinetic modelling of thermal degradation from laboratory to production scale – a case study on three vitamins in milk.** *Under review*.

Author's contribution to the papers

Paper I

The author designed the study together with the co-authors, performed the experimental work with one of the co-authors, evaluated the data with one of the co-authors, commented on the paper and revised the paper together with the co-authors.

Paper II

The author designed the study together with the co-authors, performed the pilot-scale trials and experimental work together with two master students, evaluated the data with the co-authors, wrote the first version of the paper and finalized the paper with some of the co-authors.

Paper III

The author designed the study together with the co-authors, performed the experimental work, evaluated the data, wrote the paper and revised the paper together with some of the co-authors.

Paper IV

The author designed the study together with the co-authors, performed the experimental work, evaluated the data, wrote the paper and revised the paper together with some of the co-authors.

Paper V

The author designed the study together with the co-authors, performed the experiments and developed the kinetic fitting method, analysed and interpreted the findings and wrote the paper together with one of the co-authors.

Abbreviations

HTST	High-temperature-short-time
Past.	Pasteurisation
UHT	Ultra-high temperature
ESL	Extended shelf-life
HPLC	High-performance liquid chromatography
TSI	Turbiscan stability index
EDTA	Ethylenediaminetetraacetic
JND	Just noticeable difference
MFGM	Milk fat globule membrane
ICP-OES	Inductively coupled plasma optical emission spectroscopy
N	Number of replicates

Symbols

L	Lightness (dark to white)
a^*	colour chroma (green-red)
b^*	colour chroma (blue-yellow)
A, B	Reparameterization constant
c_0	Initial (vitamin) concentration, µg mL^{-1}
c_{exp}	Experimentally measured concentration, µg mL^{-1}
c_{mod}	Modelled concentration, µg mL^{-1}
E_A	Activation energy, J mol^{-1}
$k(T)$	Degradation rate at temperature T, μg^{1-n} mL^{n-1} s^{-1}
k_{ref}	Degradation rate at the reference temperature, s mL^{n-1} μg^{n-1}
n	Reaction order, -

R	Gas constant, J mol^{-1} K^{-1}
t	Time, s.
T	Temperature, K
T_{ref}	Reference temperature, K.

Introduction

Milk is a highly consumed product globally and milk production has increased worldwide during the last three decades by over 59% from 530 million tonnes in 1988 (FAO, 2021) to 936 million tonnes in 2022 (IDF, 2023). In 2022, farms in the European Union produced an estimate of 149.9 million tonnes of raw milk, out of which 15% accounted for drinking milk (EuroStat, 2022). In Sweden, all the milk that is sold to consumers need to undergo heat treatment to ensure the microbiological quality and, in addition, all prepacked food must have a nutrition declaration according to given rules in order to provide consumers with information about nutritional content (LIVSFS 2016:5, Livsmedelsverket; EU Regulation 1169/2011). Moreover, the declared nutritional value of the product should be applicable throughout the entire shelf-life stated on the package (Swedish Food Agency, 2024). Therefore, providing nutritional content declaration to consumer becomes a crucial parameter when consumers are choosing a nutritious food product to consume.

Milk is a nutrient-dense fluid which is essential for a healthy balanced diet. It consists of macro components like fat, lactose, and proteins, but also micro components like vitamins and minerals (Walstra et al., 2005). Milk has high nutritional value and high-water activity, making it an excellent growth medium for various microorganisms and is therefore prone to spoilage (Fatih et al., 2021). Thermal processing is the most widely used processing technique in the dairy sector, and it not only increases product safety by facilitating the deactivation of pathogenic microorganisms and enzymes, but it also improves milk quality and extends the shelf-life of milk and dairy products (Deeth, 2020; Walstra, 2005). Although heat treatment has many advantages, it also causes chemical changes in the milk which alters the nutritional and technological properties of milk, e.g. vitamin degradation, protein denaturation, physical stability and colour change (Kilic-Akyilmaz et al., 2022; Walstra et al., 2005). These alterations in milk also occur during storage and thus the shelf-life of milk and dairy products is not only determined by the severity of the heat treatment but also due to other factors including raw milk quality, type of packaging, distribution and storage conditions affecting the longevity of a product (Rauh and Xiao, 2022; Sunds et al., 2018). Consequently, it is crucial to understand the effects of processing and storage on milk quality for minimising nutritive losses, changes in chemical and physical properties as well as improving storage stability.

In order to keep the deleterious alteration of the nutritional quality of milk to a minimum, a good process design for heat treatment should be selected. Since it is challenging to monitor the chemical changes in milk in real-time, empirical data is used to comprehend the reaction kinetics of the heat treatment (Kessler, 2002). In understanding how heat treatments affect the nutritional degradation in the milk system, kinetic studies and mathematical models are required. Therefore, kinetics can aid in understanding chemical changes in milk, minimise losses as well as make heat treatment efficient. This study is novel as it provides a comprehensive analysis of thermal processing of milk across laboratory, pilot and dairy production scale. The inclusion of different heating temperatures, holding times as well as heating systems adds more detailed understanding of the factors affecting the nutrient content and physical properties of milk. Moreover, the incorporation of data on commercially produced products and the changes occurring in milk and cream after processing as well as storage makes this study unique.

Hypothesis

The hypothesis of this thesis is that the process technologies for heat treatment, which are used in the dairy industry today, probably have smaller effects on the nutritional content and physical properties of milk than older process technologies which are the basis for the calculations of nutritional values declared to consumers. By understanding the effects of thermal processing on the nutrient content and physical properties of milk, it will be possible to create kinetic models and improve the processing of milk and dairy products.

Aim

The overall aim of this thesis was to evaluate the effects of thermal processing on the nutrient content and physical properties of milk and cream. The overall aim was further divided into specific objectives:

- investigating the effect of heat treatment on vitamin degradation in a laboratory-scale setup (**Papers I and V**)
- examining how thermal processing of milk affects the nutrient content of milk and cream at laboratory, pilot and dairy production scale (**Papers II, III and V**)
- examining how thermal processing of milk affects the physical properties of milk and cream at pilot and dairy production scale (**Papers II and III**)

- evaluating the effects of storage on the nutritional content and physical properties of heat-treated milk and cream (**Papers II and IV**)
- developing kinetic calculation model to understand and predict the effects of heat treatment on vitamin degradation (**Papers I and V**)

The results obtained in this thesis can be used to understand the effects of thermal processing on milk which could increase the added value of milk as raw material and in the long-term increase profitability for the Swedish dairy and agricultural industry.

Background

Thermal processing of milk

Thermal processing of milk is the most commonly used processing technique in the dairy industry (IDF, 2022; Tetra Pak, 2015). Thermal processing plays a vital role as it helps transform raw material by destroying spoilage and pathogenic microorganisms into safe-to-consume food products with an extended shelf-life (Ifie & Marshall, 2018). Heat treatment of milk can be performed at different temperature-time conditions depending on achieving specific goals of safety, quality, product characteristics and shelf-life (Lewis & Deeth, 2008; Walstra et al., 2005). During the processing of milk using heat exchangers, milk undergoes three heating stages including a pre-heating stage, high heating stage/final heating followed by a subsequent cooling stage (Datta & Deeth, 2002; IDF, 2022; Tetra Pak, 2015). The heat treatments used in the industry can be further categorised based on direct consumption of milk, like HTST past., ESL, UHT and in-container sterilisation, or as a raw material for the production of yogurt, cheese or milk powders (IDF, 2022). This thesis mainly focuses on some of the heat treatments applied in the industry, i.e. HTST past., high past., ESL indirect as well as UHT direct and indirect. Table 1 lists the most commonly used heat treatments in the dairy industry, with temperature-time combinations used for heat treatment and the shelf-life conditions as well as both temperature-time combinations and shelf-life conditions used in this thesis.

Table 1. Heat treatments performed at dairy production scale with heating temperature/holding time and shelf-life conditions for the products (IDF, 2022; Tetra Pak, 2015) as well as temperture-time combinations and shelf-life conditions used in this thesis.

		General		Thesis	
Product	**Heat treatment**	**Heating temperature and holding time**	**Shelf-life condition**	**Heating temperature and holding time**	**Shelf-life condition**
Milk	Pasteurisation (HTST)	72-76 ºC for 15 s	Refrigerated; 5-10 days	77 ºC for 15 s	Refrigerated (8 ºC); 10 days
	High pasteurisation	Pre-heating; 75 ºC for 15 s Final heating: 90-95 ºC for 5-10 mins	Yogurt: 30 days	Pre-heating; 77 ºC for 15 s Final heating: 94 ºC for 420 s	
	Extended shelf-life (ESL)	120-140 ºC for 10-1 s	Refrigerated; 15-60 days	129 ºC for 2 s	Refrigerated (8 ºC); 30 days
	Ultra-high temperature (UHT)	Pre-heating: 75 ºC for 15s Final Heating: 135-150 ºC for 10-1 s	Ambient; Up to 1 year	Pre-heating: 75 ºC for 15 s Final Heating: 140 ºC for 4 s	UHT direct: Refrigerated (8 ºC); 40 days UHT indirect: Ambient (20 ºC); 4 months
Cream	Pasteurisation (HTST)	>80 ºC for 1-5 s	Refrigerated; 5-10 days	Pre-heating; 60 ºC for 1800 s Final heating: 93 ºC for 15 s	Refrigerated (8 ºC); 10 days
	Ultra-high temperature (UHT)	Pre-heating; 75 ºC for 15 s Final heating: 142-143 ºC for 4s	Refrigerated; upto 90 days	Pre-heating; 77 ºC for 15 s (skim milk fraction) 90 ºC for 1 s (cream fraction) Final heating: 137 ºC for 4s	Refrigerated (8 ºC); 75 days

High-temperature-short-time (HTST) pasteurisation

Pasteurisation is one of the oldest heat treatment practices used for heat treatment of milk. The term 'pasteurisation' comes from Louis Pasteur who in the middle of the 19th century studied the detrimental effects of heat on microorganisms by employing heat treatment as a technique for preservation (Tetra Pak, 2015). Pasteurisation can be carried out either in batches with Low-Temperature-Long-Time (LTLT) at 63 ºC for 30 min or continuously with High-Temperature-Short-Time (HTST) usually performed at 72-76 ºC for 15 s (IDF, 2022). Pasteurization of milk deactivates the enzyme alkaline phosphatase, and kills pathogens, yeasts and molds while keeping the flavour of milk unaltered as well as keeping the nutritional profile of milk (Walstra et al., 2005). HTST pasteurisation of milk is commercially used in the manufacturing of milk as well as cream production and cheese manufacturing (IDF, 2022). Packaging of HTST pasteurised milk is carried out in a clean environment but non-aseptically with a shelf-life of about 10 days stored under refrigerated conditions (IDF, 2022).

High pasteurisation

High pasteurisation is a method which is performed at higher temperatures for longer times compared to HTST pasteurisation. When milk is heat treated with high pasteurisation, it first goes through a pre-heating step at 75 ºC for 15 s followed by a final heat treatment at 90-95 ºC for 5-10 min (IDF, 2022; Tetra Pak, 2015). High pasteurisation of milk destroys most of the non-spore-forming bacteria, deactivates most enzymes and causes a complete denaturation of whey proteins in milk (IDF, 2022; Walstra et al., 2005). High pasteurisation of milk is performed prior to the fermentation of milk during yogurt production which helps in increasing the viscosity of yogurt by the formation of whey protein-κ-casein complexes as well as enhancing the water binding capacity (IDF, 2022).

Extended shelf-life (ESL) treatment

The short shelf-life of pasteurised milk led to the development of UHT milk which could be stored at ambient temperatures for up to 1 year, however, UHT milk may be perceived to have a 'cooked' taste by the consumer causing the development of extended shelf-life (ESL) milk. ESL milk has a longer shelf-life at refrigerated temperatures than HTST past. milk while keeping a similar taste profile as that of HTST past. milk (Rysstad & Kolstad, 2006). There is no standard definition for temperature and time conditions for ESL milk, however, the treatment is performed at temperature ranging from 120-140 ºC for 10-1 s, most commonly it is carried out at 125-130 ºC for 2-6 s (IDF, 2022). ESL heat treatment destroys all non-spore-

forming bacteria as well as psychotropic and mesophilic spores while causing a slight variation in the flavour of milk (IDF, 2022). ESL treatment is used for long shelf-life drinking milk as well as for the production of cream. Packaging of ESL milk is often performed under very clean conditions in a chamber with sterile air providing a shelf-life of up to 60 days in refrigerated conditions (IDF, 2022, Rysstad & Kolstad, 2006, Tetra Pak, 2015).

Ultra-high temperature (UHT) treatment

UHT processing of milk results in a product that is 'commercially sterile', implying that the heat treatment may not eliminate or destroy all bacteria but destroys the bacteria which is prone to grow under ambient storage conditions (IDF, 2022). Although high-temperature heat treatment is desirable since it destroys the pathogenic microbes, high heat treatment may cause unwanted changes in milk like the production of sulphurous compounds giving a cooked flavour to milk as well as affecting the appearance and altering the nutritional value of milk (Tetra Pak, 2015; Walstra et al., 2005). The temperature-time conditions for UHT milk are 135-150 ºC with a holding time of 10-1 s (IDF, 2022, Tetra Pak, 2015). UHT milk is packaged aseptically ensuring that no bacterial growth occurs after the heat treatment is performed, with a shelf-life of 9-12 months at ambient temperature (IDF, 2022, Tetra Pak, 2015).

Heating system

In the dairy industry, heat treatment of milk is performed with the help of heat transfer either in the form of convection or through conduction (Tetra Pak, 2015). The heating system used for ESL or UHT milk can be either direct (steam infusion or injection) or indirect (using plate or tubular heat exchangers) heating (Deeth & Lewis, 2017). This thesis investigates the effect of direct and indirect heating systems on milk quality in both pilot scale as well as dairy production scale (Papers **II, III and IV**).

Direct heating

Direct heating of milk involves bringing steam in direct contact with the product, eliminating the requirement of heat transfer through a wall of heat exchanger resulting in a higher heat transfer coefficient between milk and steam (Eisner, 2021). Direct contact of the product with steam helps in a faster heating of the product because of the condensation of steam which releases latent heat (Eisner, 2021). Direct heat treatment of milk is performed either using steam infusion or steam injection (Deeth & Datta, 2011; Eisner, 2021). Steam infusion of milk is performed by dispersing the milk droplets by spaying into the steam-filled chamber while steam injection is performed by injecting the steam into the milk through an

injection nozzle (Eisner, 2021). In direct heating systems, the rate of heating is extremely fast causing minimal chemical changes in milk as well as it is less prone to fouling (IDF, 2022, Eisner, 2021).

Indirect heating

Indirect heating of milk involves heat transfer between the heating medium (steam or hot water) and milk using a heat exchanger (Datta et al., 2002). Indirect heat treatment of milk is performed either using a plate or tubular heat exchanger (Eisner, 2021). One of the biggest advantages of using indirect heating of milk is that it is energy efficient and cost-effective since up to 90% of the energy used can be recovered (Eisner, 2021; Deeth, 2010). The indirect heating system is more prone to fouling in heat exchangers and thus to avoid it a pre-heating step is usually performed (Eisner, 2021).

Milk composition

Bovine milk is a nutrient-rich food composed of both macro and micro components. Milk is a good source of many vital nutrients including a substantial quantity of vitamins and minerals. The average composition of milk consists of 4.7% lactose, 4.2% fat, 3.5% proteins and 1% vitamins and minerals (Lindmark-Månsson et al., 2012; Walstra et al., 2005). There are many factors causing variation in milk composition, like season, breed of the cow, feed, and lactation period (Fox et al., 2015). The chemical composition of milk exerts a substantial impact on its nutritional value, flavour, microbial growth and chemical reactions occurring in milk (Walstra et al., 2005).

Macro components

Fat

Milk fat consists of fat droplets known as fat globules with size ranging from 0.1 to 10 μm in diameter (Walstra et al., 2005). The fat globules present in milk vary in size with numerous small globules and few larger globules. Each fat globule is naturally enveloped by the milk fat globule membrane (MFGM) which is composed of phospholipids, proteins, and enzymes (Michalski et al., 2002). Fat globules in milk are mainly composed of tri-, di- and monoglycerides, free fatty acids, sterols, fat-soluble vitamins, and water. The fatty acids present in milk contribute to milk flavour, however, these fatty acids are sensitive to heat and when exposed to higher temperatures, changes in double bond structure in the fatty acid occur, causing oxidation and off-flavours in milk (Walstra et al., 2005). Apart from heat treatment, homogenisation also affects the milk fat globules which is performed in milk to

reduce creaming and coalescence of fat droplets and increase the stability of milk (Walstra et al., 2005). During the processing of milk, homogenisation is used to reduce the milk fat globule size to less than 1 μm, depending on homogenisation pressure (Rauh and Xiao, 2022; Tetra Pak, 2015). Figure 1 illustrates the fat globules present in milk before and after homogenisation. Homogenisation of milk is usually performed at temperatures between 50 to 60 ºC (where the milk fat is in a melted state) in two stages, where in the first stage, fat is disrupted at high pressure followed by the second stage where the aggregates of fat globules and proteins formed during the first stage are disrupted at a lower pressure (Rauh and Xiao, 2022; Tetra Pak, 2015). During storage of milk, fat globules can aggregate causing fat separation, which is highly dependent on factors like the fat content in milk, storage temperature and the initial fat globule size (Ramsey et al., 1984; Lu et al., 2013). Homogenisation efficiency affects the rate of fat separation where higher homogenisation efficiency causes a larger reduction in the size of fat globules and in turn, causes less fat separation (Lu et al., 2013).

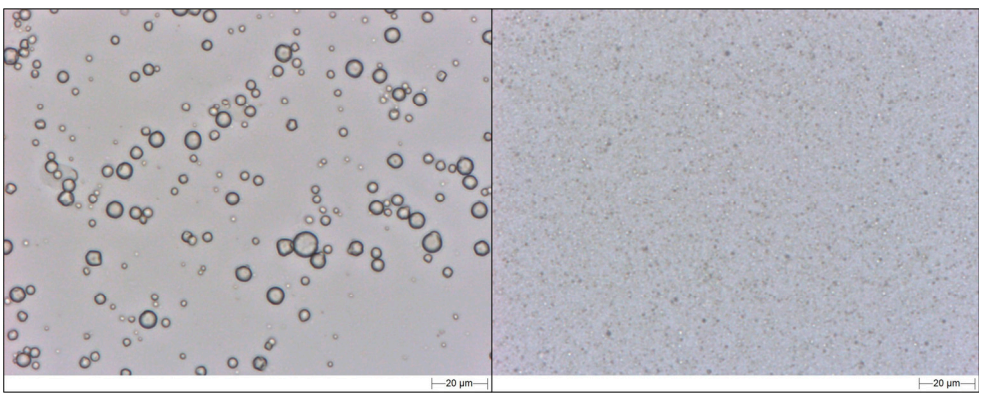

Figure 1. Micrographs of raw unhomognised milk (*left*) and homogenised milk (*right*) with 3% fat.

Lactose

Lactose is the primary sugar present in milk. Lactose is a disaccharide consisting of glucose and galactose linked with β-1,4-glycosidic bond (Walstra et al., 2005). When milk is heat treated, lactose undergoes different reactions. Isomerisation of lactose to lactulose may occur, which is used as an indicator of heat treatment intensity in heated milk products (Walstra et al., 2005) Other reactions including caramelisation and Maillard reactions also take place during heat treatment of milk which can result in the generation of flavour compounds as well as browning of the milk (Walstra et al., 2005). The extent of the Maillard reactions is influenced by the heat treatment and the chemical composition of the product (Rauh and Xiao, 2022). The more intense the heat treatment of milk is, more precursors are formed during the various stages of the Maillard reactions, and the most notable change can be observed during the shelf-life of milk (Rauh and Xiao, 2022). Hydrolysed lactose

products, i.e. glucose and galactose, exhibit a noticeable enhancement of Maillard reaction as the reducing sugar concentration increases due to hydrolysis of lactose into glucose and galactose, with a higher reactivity of galactose to Maillard reaction than lactose (Jansson et al., 2012; Naranjo et al. 2013). Furthermore, the Maillard reaction can also trigger a colour change in milk both after heating and during storage of the dairy products (Walstra et al., 2005), which is explained in the later in the thesis (see section *Colour*).

Proteins

Bovine milk contains about 3.5% proteins and the milk proteins are divided into the main protein groups whey proteins and caseins. The ratio of casein to whey protein in milk is about 80:20 (Fox et al., 2015). In milk, caseins are present in a micellar form which are large colloidal aggregates. Despite the vast research performed on casein micelles, its structure is still not fully known and many models have been proposed (Fox et al., 2015). It has thus been concluded that casein micelles are colloidal, polydisperse, fairly spherical particles ranging from 50 to 600 nm in diameter, with an average diameter of about 200 nm (Fox & Brodkorb, 2008; Holt et al., 2003). Casein present in milk includes α_{S1}-casein, α_{S2}-casein, β-casein and κ-casein while whey proteins present in milk are mainly α-lactalbumin and β-lactoglobulin. On acidification to a pH of 4.6, caseins precipitate whereas the whey proteins remain soluble (Fox et al., 2015). Whey proteins are heat labile and starts denaturing at about 70 ºC and can be completely denatured due to heating at 90 ºC for 10 min (Fox et al., 2015). However, compared to whey proteins, caseins are disordered proteins causing them to be highly resistant to heat denaturation (Walstra et al., 2005). During heat treatment of milk, the formation of complexes between β-lactoglobulin and κ-casein occurs due to an interaction with thiol disulfide exchange (Walstra et al., 2005). This takes place when free thiol groups present in whey proteins are subjected to high temperatures and reacts with the -S-S- group in the casein micelles. Sulphur bridges are formed between β-lactoglobulin and κ-casein (Walstra et al., 2005). The association of whey proteins and casein micelles is dependent on heating conditions where a prolonged heating time causes an increase in the association of β-lactoglobulin with the casein micelles (Anema et al, 2003). During storage, physical changes can occur in milk which causes aggregation of fat or protein particles resulting in creaming, sedimentation, separation or gelation (Rauh and Xiao, 2022). Sedimentation occurs due to the formation of aggregates of protein, fat and minerals during heat treatment of milk which can be formed either in the milk or because of fouling in the heat exchanger (Anema, 2019). Casein also plays a role in creaming of stored milk, as during storage fat globule flocculation or coalescence occur which is caused due to the proteolysis of the caseins that covers the fat globule surface (Anema, 2019).

Micro components

Vitamins

Vitamins are organic compounds that are integral to human health and should be present in dietary intake since humans cannot naturally synthesise them (Fox et al., 2015). Vitamin concentration is dependent on various factors including breed, feed composition as well as breeding practices (Godoy, 2021). Vitamins are divided into two categories: water-soluble vitamins (thiamine, riboflavin, pyridoxine, cyanocobalamin, and ascorbic acid, niacin, biotin, folate, and pantothenic acid) and fat-soluble vitamins (retinol, calciferol, tocopherol and menaquinones). Preserving these micronutrients is crucial since vitamins are sensitive and could degrade during handling, processing as well as storage (Godoy et al., 2021). During heat treatment, the structure of vitamins can be altered due to the breakage in chemical bonds within the molecule leading to loss of nutritional potency (Fox et al., 2015; Kilic-Akyilmaz et al., 2022; Walstra et al., 2005). Milk is a good source of most of the vitamins, both water-soluble vitamins including vitamins B_1, B_2 and B_{12} as well as fat-soluble vitamins like vitamins A, D and E (Walstra et al., 2005; Fox et al., 2015). Milk is a good source of vitamins, however, in this study a few of the vitamins were investigated due to research focus as well as resource limitations. Table 2 depicts the vitamins that were examined in this study including the concentration of the vitamins in raw milk (Lindmark-Månsson, 2003) as well as the importance of the vitamins (Fox et al., 2015; Ostan et al., 2013). Vitamins present in milk can be measured using high-performance liquid chromatography (HPLC), as was performed in this thesis (Papers **II, III, IV and V**).

Table 2: Concentration and importance of vitamins present in raw milk investigated in this thesis (Fox et al., 2015; Lindmark-Månsson, 2003, Ostan et al., 2013).

Vitamin	Concentration	Importance / function
B_1: Thiamine	0.40 mg/100g	Plays a critical role in energy metabolism and cell growth
B_2: Riboflavin	1.41 mg/100g	Growth and red blood cell production
B_{12}: Cyanocobalamin	4.12 µg/100g	Formation of red blood cells and functioning of nerve tissue
A: Retinol	0.36 µg/100g	Vision process, reproduction and growth in the immune system
E: α-Tocopherol	1.01 mg/100g	Protective effect on red and white blood cells and functioning of lungs

Thiamine, also known as vitamin B_1 (Figure 2), consists of two heterocyclic rings (substituted pyrimidine and thiazole) connected by a methylene bridge (Fox, 1998). Vitamin B_1 is a heat-sensitive vitamin and susceptible to oxidising and reducing agents (Godoy et al., 2021; Voelker et al., 2018). Studies have shown that pasteurisation and UHT milk heat treatment can cause a loss of about 10% in

vitamin B_1 concentration, however, sterilisation of milk leads to a reduction of about 20 to 50% (Fox et al., 1998; Vidal-Valverde, 1990). Heat treatment system including direct or indirect heating also have a minimal influence on vitamin B_1 levels in milk (Burton et al., 1970). During storage, no significant losses were observed for short-term storage either of pasteurised or UHT milk, however during prolonged storage of UHT milk the loss was in the range of 10 to 40% (Fox et al., 1998; Görner et al., 1980).

Vitamin B_2 (riboflavin; Figure 2) comprises of an isoalloxazine ring linked to an alcohol derived from ribose (Fox et al., 2015). Heat treatment has little to no effect on vitamin B_2 since it is a heat-stable vitamin, although vitamin B_2 is a light-sensitive vitamin and its stability decreases when exposed to light (Cardoso et al., 2017; Hadad et al., 1983; Ottaway, 2010). Vitamin B_2 present in milk is stable during heat treatment as well as storage and could be affected by light intensity, storage temperature and packaging material (Fox et al., 1995; Ottaway, 2002).

Cyanocobalamin or vitamin B_{12} (Figure 2) consists of a corrin ring which is a porphyrin-like structure attached to four reduced pyrrole rings with a central cobalt atom chelated at its core which is linked to a nucleotide base, ribose and phosphoric acid (Fox et al., 2015). Vitamin B_{12} is a fairly heat-stable vitamin where the losses due to heat treatment can range from negligible to 30% depending on the heat treatment employed (Andersson & Öste, 1992, Gregory et al., 1965; Kilic-Akyilmaz et al., 2022). Notably, vitamin B_{12} is not only affected by thermal treatment, destabilisation of vitamin B_{12} can also occur due to the presence of oxygen-reactive compounds (Kilic-Akyilmaz et al., 2022). Furthermore, the impact of storage on vitamin B_{12} concentration varies across studies, where a complete loss in vitamin B_{12} concentration in UHT-treated milk after 20 weeks of storage was observed (Godoy et al.,2021; Oamen et al., 1989) while Walstra et al. (2005) reported a loss of about 20 to 50% of vitamin B_{12} in UHT milk after 3 months of storage. The drop in vitamin B_{12} concentration noticed during storage is caused not only by the storage time but also by the presence of dissolved oxygen in the package (Oamen et al., 1989; Walstra et al., 2005).

This thesis mainly focused on the water-soluble vitamins B_1, B_2 and B_{12}, however there are other vitamins present in milk like niacin (B_3) pantothenic acid (B_5) and biotin (B_7) which are heat-stable vitamins and are stable during subsequent storage (Friend et al., 1983; Zhu et al., 2020). Furthermore, the stability of vitamin C is affected by multiple factors including the presence of antioxidants, exposure to oxygen as well as heat treatment and storage duration (Kilic-Akyilmaz et al., 2022).

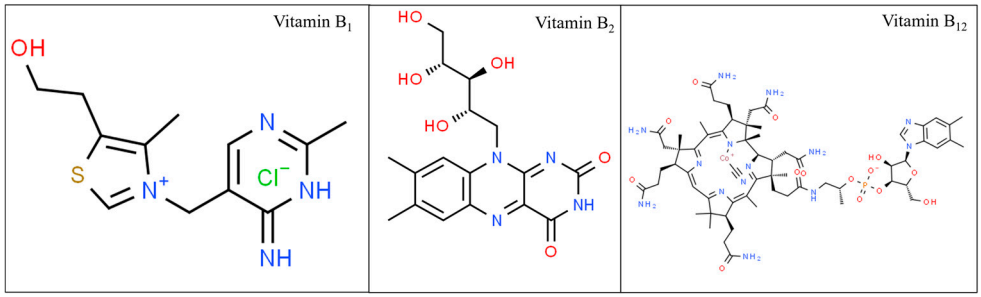

Figure 2. Chemical structures of vitamin B_1, B_2 and B_{12} (The Royal Society of Chemistry, 2024).

Fat-soluble vitamins present in milk includes vitamin A, D, E and K. Retinol or Vitamin A (Figure 3) is a precursor to a group of chemicals called retinoids, possessing the biological activity of vitamin A (Fox et al., 2015). Heat treatment of milk showed no significant difference in vitamin A concentration (Bilic et al., 1988), however, the presence of light and air leads to vitamin A loss (Ottaway, 2010). The high stability of vitamin A is also aided due to the presence of milk fat (Godoy et al., 2021), where loss of vitamin A due to exposure to light in full-fat milk is negligible when compared to skim milk (Whited, 2000). Vitamin E (Figure 3) is an antioxidant comprised of two groups of compounds. namely tocopherols (α-, β-, γ-, and δ-tocopherol) and tocotrienols (α-, β-, γ-, and δ-tocotrienol). Tocopherols consist of a chromanol ring and a phytyl chain (Fox et al., 2015). Vitamin E is a heat-stable vitamin (Macrae et al., 1993) while previous storage studies suggest no loss of vitamin E (Fox, 1995; Lorenzen et al., 2011) to a complete loss of vitamin E during storage (Ajmal et al., 2019). Vitamin D is also a heat-stable vitamin however, most of the milk in Sweden is fortified with vitamin D (Swedish Food Agency, 2024).

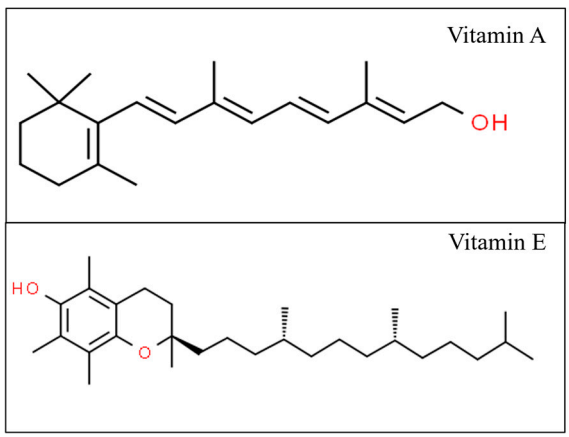

Figure 3. Chemical structures of vitamin A and E (The Royal Society of Chemistry, 2024).

Minerals

The minerals in milk are an essential group of micronutrients constituting about 1% of the milk content (Walstra et al., 2005). From a nutritional point of view, minerals are vital components that promote functioning of immune system as well as inflammation regulation (Weyh et al., 2022). The minerals present in milk are divided into macro elements including calcium, magnesium, sodium, potassium, phosphorus, and chloride as well as trace minerals including iron, copper, zinc, manganese, selenium, iodine, chromium, cobalt, molybdenum, fluorine, arsenic, nickel, silicon, and boron (Cashman, 2011). The mineral concentration of milk is affected by a variety of factors including seasonal variations, lactation stage, environmental and genetic factors (Cashman, 2011; Fox et al., 2015). While the minerals present in milk make up a relatively small percentage, they play a crucial role in both technological properties of milk like stability of proteins and buffering capacity along with nutritional functions like growth and maintenance of teeth and bones (Cashman, 2011; Gaucheron, 2005). Inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed to determine the mineral concentration in milk in this thesis (**Paper III**). Calcium in milk is present partly in serum form as well as partly in the casein micelles as calcium phosphate. Calcium in milk exists as a stable complex with citrate and about 10% of the total calcium in milk exists in ionic form, termed ionic calcium (Gaucheron, 2005; Lewis, 2011). Ionic calcium in milk is also affected by change in pH, with a decrease in pH an increase in ionic calcium is observed (Lewis, 2011). Heat-induced changes in the mineral concentration of milk are largely impacted by processing conditions, e.g. holding time, heating temperature and flow conditions which cause fouling in the heat exchangers resulting in a drop in mineral concentration in milk (Huppertz et al., 2022). Heat treatment of milk also results in a decrease in the concentration of ionic calcium which is caused due to the changes in mineral distribution between colloidal and serum phases of milk and the extent of these changes in mineral balance is dependent on the intensity of heat treatment (Deeth et al., 2017; Fox et al., 2015). Thermal treatment of milk could cause precipitation of calcium phosphate leading to a decrease in ionic calcium concentration (de la Fuente, 1998). Previous studies have suggested that a higher reduction in ionic calcium concentration in milk is observed with more intense heat treatments, for instance, UHT heat treatment has a larger effect on ionic calcium than pasteurisation (Holt, 1995; Deeth et al., 2017). Storage of milk leads to a slight decrease or no change in ionic calcium concentrations (Anema, 2017; Lewis, 2011).

Physical properties of milk

The physical and chemical properties of milk are dependent on intrinsic factors like composition and structure as well as extrinsic factors such as milk treatment and temperature (McCarthy et al., 2009). Thus, understanding the physical properties of milk is of importance concerning designing processing equipment, analysis of milk quality as well as comprehending complex reactions taking place and the microstructure of milk. In this thesis, several physical properties of milk were analysed and their effects on milk both before and after heat treatment as well as during storage (Papers **II, III and IV**). Various physical properties of milk like pH, colour, particle size distributions of fat globules and casein micelles, physical stability of milk as well as structural changes in milk were investigated.

pH

pH of raw milk is in the range of 6.6 to 6.8 (Walstra et al., 2005; Fox et al. 2015). pH of milk affects the conformation of proteins, enzyme activity as well as the dissociation of acids in milk which contributes to an acidic taste and can inhibit microbial activity (Walstra et al., 2005) making it an important parameter to examine. pH is strongly associated with temperature as after thermal processing of milk, a decrease in the pH of milk can be observed because of lactose breakdown at higher temperatures which causes the production of organic acids like formic acid (Nieuwenhuijse et al., 2003; Tsioulpas et al., 2010). Moreover, the association of calcium and phosphate into complexes in milk is affected by thermal processing causing a decrease in pH (Fox et al., 1998). Previous studies have suggested minor changes in the pH of milk after heat treatment using commercial heat treatments like UHT or HTST pasteurisation (Deeth et al., 2017; Karlsson et al., 2019). The type of heating system used for heat treatment also influences the pH of milk as a small decrease in pH is expected after indirect heating of milk (Walstra et al., 2005) while direct heating increases the pH of milk (Lee et al., 2017; Hammershøj et al., 2010). The increase in pH of milk after direct heating could possibly be due to degassing or pressure of steam which causes a removal of CO_2 during flash cooling (Dickow et al., 2012; Hammershøj et al., 2010). Decrease in the pH of milk is expected during storage due to many factors including the breakdown of lactose-producing organic acids (Limacher et al., 2008), proteolysis of casein micelles, temperature-dependent dephosphorylation (Al-Saadi et al., 2008; Rauh et al., 2014) and protons release caused due to association of calcium and phosphate (Gaucheron, 2005).

Colour

The colour of milk and dairy products could indicate physio-chemical changes. The white colour of milk is caused by the dispersion of light by fat globules and casein

micelles, and when the milk is homogenised, the colour of milk becomes even whiter due to the scattering of light by smaller homogenised fat globules (Fox et al., 2015). Moreover, the carotenoids (β-carotene) present in milk give a yellow colour to milk fat (Walstra et al., 2005). Colour changes in milk could occur both after being heat treated as well as overtime during storage, making it an important parameter for identifying the quality of dairy products. For measuring colour, different methods could be used, however, in this thesis, CIELab 1976 was used for colour measurements (Papers **II and IV**). CIELab uses three values to detect the colour which are L, a* and b* where L represents lightness with intensity ranging between dark to white (0 to 100). The a* and b* values indicate the colour chroma with values ranging from positive (+) and negative (-), where the positive a* value indicates redness and negative a* value indicates greenness and positive b* value indicates yellowness and negative b* value indicates blueness (McGuire, 1992). In addition, L, a* and b* values can be used to calculate the colour changes between samples with a total colour difference (△E) as follows (Baldevbhai et al., 2012):

$$\Delta E = \sqrt{(L_2 - L_1)^2 + ({b^*}_2 - {b^*}_1)^2 + ({a^*}_2 - {a^*}_1)^2} \quad (1)$$

If the value of the detected colour difference (△E) between samples is below 2.3, which corresponds to just noticeable difference (JND), this suggests that the change between the samples is undetected by the human eye (Baldevbhai et al., 2012). Maillard reactions are chain reactions that occur in the milk system and are divided into three stages. In the initial stage, lactose, which is the reducing sugar in milk, undergoes lactosylation which is a reaction with amino group resulting in the formation of Amadori compounds (van Boekel, 1998). This is followed by an intermediate stage where volatile compounds are formed (Sunds et al., 2018). Lastly, in the final stage of the Maillard reactions, a non-enzymatic browning reaction occurs involving condensation of amino compounds and sugar fragments, leading to the formation of brown melanoidins and intra-micellar protein cross-links (Al-Saadi & Deeth, 2008; van Boekel, 1998). Browning and colour changes induced by the Maillard reactions are often considered a defect in quality (Rauh and Xiao, 2022). Maillard reactions in milk and dairy products occur faster for temperatures above 100 °C while the rate of reaction is slower at lower temperatures or during storage at ambient temperatures and is close to zero in refrigerated products (Rauh and Xiao, 2022). The offset of the Maillard reactions is significantly influenced by the heat treatment of the products. If the products undergo a higher initial heat treatment more precursors from intermediate and final stage Maillard products are formed compared to products that undergo lower initial heat treatment (Rauh and Xiao, 2022). During storage of milk, Maillard reactions are ongoing however, for products that are stored above 20-25 °C the changes occurring in milk are significant (Karlsson, 2019; Rauh and Xiao, 2022).

Particle size distribution

Fat globule sizes in raw milk range from 1 to 10 µm and usually in industry for processed milk when homogenisation is applied to milk the resulting mean fat globule size is less than 1 µm (Rauh and Xiao, 2022). The average casein micelle size in milk ranges from 0.17 to 0.20 µm. (Bijl et al., 2014; Walstra et al., 2005). Particle size distributions of casein and fat globules in milk can be measured using laser diffraction, as was performed in this thesis (Papers **II, III and IV**). One of the drawbacks of this method is that particles larger in size, e.g., fat globules, will overlap particles of smaller size such as casein micelles due to static measurements and thus blocking the signal. This shortcoming can be overcome for raw milk where the fat is not homogenised by de-fatting the milk with the help of centrifugation. However, for processed heat-treated milk, which is homogenised during processing, defatting of milk is not plausible and therefore a reactive solution is added to dissolve the casein micelles giving an accurate measurement for fat globules. The protein dissolving solution, sol A is a mixture of Tween 20, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-Na_2) and MilliQ water where EDTA acts as a casein dissociating substance while Tween 20 is an emulsifier which hinders fat globule aggregation (Ransmark et al., 2019). Figure 4 depicts the particle size distribution curve for HTST past. milk. When the milk is analysed in the Mastersizer without any addition of sol A, two peaks are seen in the PSD curve where one shows the casein micelle peak while the other shows the fat globule peak (Figure 4, blue curve). However, with the addition of sol A, casein micelles are dissolved and only one peak for fat globules is detected (Figure 4, red curve)

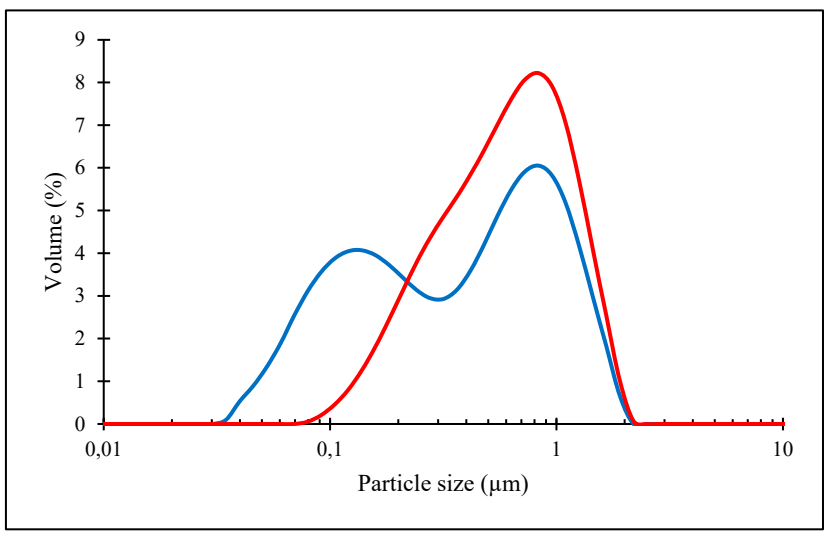

Figure 4. Particle size distribution of HTST past. milk, with (red) and without (blue) the addition of sol A (adapted from Paper II).

An increase in the size of casein micelles is expected with an increase in heating temperature and holding time. This increase in casein micelle size is linked with the level of denatured whey proteins associating with the casein micelles leading to the formation of whey protein and κ-casein complexes (Anema & Li, 2003; Ono et al., 1999; Singh et al., 1993). During storage of milk, a slight decrease in casein micelle size can be observed, which is caused by the dissociation of β-lactoglobulin-κ-casein complexes from casein micelles (Akkerman et al., 2021). The heating system also has an influence on the particle size distribution since in an indirect heating system, the denatured whey protein associates at the micellar surface with κ-casein in the colloidal phase, however, direct heating of milk causes the κ-casein and whey protein complexes to split between the serum and colloidal phase (Anema, 2021). Direct heat treatment of milk also causes disruption of fat globules due to shear and elongation forces during steam injection (Dickow et al., 2012; Ye et al., 2005). This disruption in turn causes the casein micelles and dissociated κ-casein to attach to the surface of fat globules (Corredig & Dalgleish, 1996) which causes a shift in particle size distribution of milk heat treated with direct steam injection compared to indirect heat treatment. These changes occurring in milk due to direct steam injections are usually minimised by homogenisation which is commonly placed downstream at dairy production scale (Eisner, 2021; Malmgren, 2007). These aggregates formed in the milk due to different heating systems can also be visualised with automated static imaging using Raman spectroscopy (**Paper II)**.

Physical stability

The stability of a food product undergoes gradual changes over time which impact the shelf-life of the product. Milk is a colloid system consisting of casein micelles and fat globules which can be subjected to various physical changes including creaming, aggregation, and coalescence (Walstra et al., 2005). Creaming of milk is a result of fat globules rising due to the difference in density between fat globules and milk plasma (Walstra et al., 2005). Creaming is an undesirable quality in milk, however, it is not considered as a shelf-life limiting factor if the fat can be easily dispersed back by shaking (Rauh and Xiao, 2022). Aggregation occurs for casein micelles and fat globules where particles form clusters, while coalescence is a phenomenon forming one large particle (Walstra et al., 2005). The milk system can also experience partial coalescence and Ostwald ripening while particle size also influences the physical stability of milk dispersion (Walstra et al., 2005).

Multiple static light scattering is the technique used for stability analyses which works on the principle of Mie theory. The backscattering value of a system increases with an increase in particle size ranging between 0.1 to 0.6 μm while the backscattering reduces as the particle size becomes higher than 0.6 μm (Carrentero et al., 2005). The physical stability of milk was evaluated using the Turbiscan stability index (TSI; Papers **II, III and IV**) which illustrates the destabilisation

kinetics (Carrentero et al., 2005; Meng et al., 2020). A low TSI value (on a scale of 0-10) indicates a stable emulsion and as the TSI value increases the stability reduces. During long storage of milk, a destabilisation mechanism could occur due to the coalescence of fat globules and/or aggregation caused by the proteolysis of casein present on the surface of these fat globules causing the formation of fat-protein aggregates which can potentially cause fat separation or creaming in milk (Anema, 2019; Stoeckel et al., 2016).

Reaction kinetics

As previously discussed, heat treatment of milk and dairy products is necessary for ensuring product safety and increasing shelf-life, however, the heating temperature and holding time could also have a detrimental effect on milk quality in terms of nutritional profile and physical properties. Therefore, to minimize the undesirable alterations in milk while deactivating spoilage bacteria as well as pathogenic microorganisms, it is essential to have a good process design for heat treatment (Kessler, 2002). To understand the impact of heat treatment on nutrient degradation, kinetic studies and mathematical models can be used (van Boekel, 2008). Chemical kinetics is the study of the rates at which chemical reactions progress (van Boekel, 2008). Chemical changes, e.g. vitamin degradation taking place in milk during heat treatment, can be theoretically quantified with the help of reaction kinetics. Additionally, a good understanding of reaction kinetics can enhance the formulation and fortification of food products to both preserve the nutrients present as well as minimizing the undesirable breakdown of the product (Heldman & Lund, 2007). Thermal changes occurring during heating can be described with the help of rate law (van Boekel, 2008).

$$Rate = -\frac{dC}{dt} = kC^n \qquad (2)$$

where C is the vitamin concentration, n is the reaction order, t is time, and k is a temperature-dependent reaction rate.

The temperature dependence of the rate constant (k) can be described by Arrhenius equation:

$$k\,(T) = k_o * \exp\left(-\frac{E_a}{RT}\right) \qquad (3)$$

Where k is the reaction rate constant, R is the gas constant, E_a is the activation energy and T is temperature (absolute).

Determination of the kinetic parameters (k_o and E_a) is essential for each nutrient for the specific food or formulation as the food matrix has an influence on the reaction

kinetics (Lešková et al., 2006; Villota and Hawkes, 2006). To determine the reaction order kinetics, typically the food (milk) is subjected to constant temperature for different durations and quantifying the nutrient concentration, followed by estimation of kinetic parameters. Kinetic parameters in food can be determined by a two-step method which is the traditional way, where at each temperature, the rate constant is determined followed by determining the activation energy. While another, more contemporary approach is the one-step approach, where both parameters are computed simultaneously using non-linear regression (Claeys et al., 2001, 2001b; Greiby et al., 2017; Jewell, 2012).

Despite the large importance and widespread use of milk worldwide, there are relatively few studies on kinetics on milk or dairy products. Kessler & Fink (1986) intensively investigated the changes occurring in milk including loss of vitamin B_1 due to heat treatment over a wide temperature-time range and interpreting the change by reaction kinetics. Another study on vitamin B_2 in skimmed milk was conducted Alvarado et al. (2022) to obtain a degradation kinetic model. Other relevant studies on vitamin B_1 and B_2 have been performed on different food products including soy milk (Kwok et al., 1998) as well as rosehip nectar (Kadakal et al., 2017). It is worth noting that, the studies mentioned above often use traditional kinetic fitting methods, manually testing reaction orders against data to find the best fit.

Methodological consideration

In this thesis a global parameter fitting model was applied where the temperature dependence of k is modelled with Arrhenius equation (Villota and Hawkes, 2015; van Boekel 2008; van Boekel, 2021, 2022):

$$k(T) = k_{ref} \cdot \exp\left(\frac{E_A}{R \cdot T_{ref}}\left(1 - \frac{T_{ref}}{T}\right)\right) \quad (4)$$

where, k_{ref} is the reaction rate at a reference temperature T_{ref}, E_A is the activation energy, T is the absolute temperature and R is the gas constant.

To attain the model in the best practice way, modifications were done to obtain a good quality fitting of the parameters obtained. Firstly, to minimize the standard error of the activation energy estimation, a reference temperature was chosen according to the suggestion from Schwab and Pinto (2007).

$$T_{ref} = \frac{\sum_{i=1}^{n}[c_i \cdot \ln c_i]^2}{\sum_{i=1}^{n}\frac{[c_i \cdot \ln c_i]^2}{T_i}} \quad (5)$$

where c_i and T_i refers to the experimentally observed concentration and temperature combinations. Secondly, reparameterization of parameters were performed to improve convergence behaviour and decrease modelling errors as described by van Boekel (2022).

$$A = \frac{E_A}{R \cdot T_{ref}} \quad (6)$$

$$B = \ln\left(k_{ref}\right) \quad (7)$$

Finally, it is important to mention that the initial concentration was used as an additional parameter for fitting in each experiment. It is essential to clarify that these parameters were only used as a tool to determine the kinetic parameters and are not actually part of the kinetic model.

Ideally, the best approach would be to fit the kinetic models using data obtained from actual production-scale experiments, using the real processing equipment and thermal treatment processes that are commonly used in industry. However, this is rarely feasible due to the high costs involved and the limited availability of production-scale facilities. In addition, the range of operating parameters is often too narrow to achieve the necessary separation required for parameter fitting. Nevertheless, despite these limitations, it is still crucial to validate and evaluate the performance of the kinetic parameters obtained from typical laboratory conditions (such as heating and holding vials) when applied to production-scale experiments. Therefore, in this thesis, kinetic studies on vitamin degradation were performed in a standard test-tube-in-water-bath experiment in laboratory-scale. The experimental setup was adopted from Paper **I**, where the thermocouple was placed carefully at the centre of the test tube. The kinetic model for thermal degradation of vitamins was obtained and was further used for validation by comparing the vitamin concentration of milk after the thermal process in dairy production scale as well as compared to the pilot scale heat treatment (Papers **I, II, III and V).**

Effects of processing and storage on nutrient content of milk

Processing of milk and dairy products can cause an effect on the nutrient content which is dependent on various factors during processing including heating temperature, holding time as well as heating system e.g. direct or indirect heating. As mentioned previously, these changes in milk also occur continuously during storage. In order to understand the effects of processing and storage on milk and cream, heat treatment trials followed by storage were conducted in laboratory, pilot and dairy production scale (Papers **II, III, IV and V).** Laboratory scale experiments performed in thesis aimed to investigate vitamin degradation using small quantity of milk to understand the feasibility of the methods when heating and holding samples in vial. Followed by pilot scale studies aiming to replicate the real-world condition of heat treatment and to understand the effects on milk quality. The heat treatment was performed with 5 different temperatures ranging from 124-143 °C for 1 and 4s in both direct and indirect heating systems. Industrial scale studies were then performed to confirm the effects of heat treatment on nutrients and physical properties occurring in commercially produced milk and cream. The investigated heat treatments included HTST past., high past., ESL indirect, UHT direct and UHT indirect heat treatment. Each set-up had its own limitation like, laboratory scale may lack real-world relevance, pilot scale studies might overlook full production complexities, and industrial scale studies can be resource intensive. Despite their differences, these studies shared a common goal of understanding of how thermal processing and storage affects the milk quality and where in the process significant changes occur.

Macro components

The average composition of raw milk samples collected from the silo tanks at dairy plants used in this thesis had an average fat content of 4.13% (SD 0.06%), average protein content of 3.32% (SD 0.07%) and average lactose content of 4.60% (SD 0.17%) (Papers **III and V**). Macro component values of raw milk obtained were in range with previously reported values of Swedish raw milk obtained from silo tanks at dairy plants by Lindmark-Månsson et al. (2003) and Karlsson et al. (2019).

To understand the effect of heating system in pilot scale on the macro component concentration of milk, HTST past. milk (starting material) with an average fat content of 2.93%, protein content of 3.51% and lactose content of 4.83% was heat-treated using direct and indirect heating systems. The average macro component composition of milk after heat treatment using the direct and indirect heating systems is shown in Figure 5. No changes in milk composition were observed due to heat treatment. Overall, the macro component composition of milk was not affected by heating temperature, holding time as well as heating system (Paper **II**).

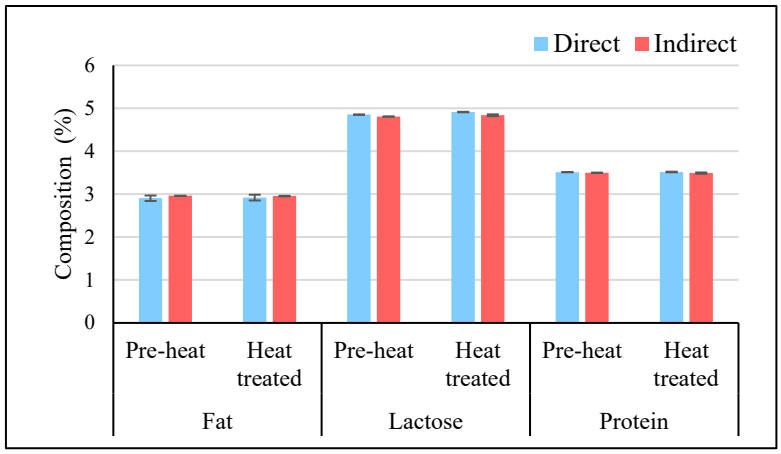

Figure 5. Macro component (fat, lactose, protein) composition in raw milk and heat-treated milk when subjected to direct and indirect heating at pilot scale (adapted from Paper II).

The effects of processing on macro components (fat, lactose and protein contents) for raw milk and heat-treated milk when subjected to different heat treatments at dairy production scale is presented in Figure 6 (Paper **III**). The results showed that there were only small changes in lactose and protein concentration observed in milk after heat treatment. During processing at dairy production scale, milk undergoes homogenisation to reduce the fat globule size in order to avoid creaming and milk was standardised to obtain the desired fat content in milk. In Figure 6, it can be observed that the fat content for heat-treated milk was standardised to be in line with the values declared for macro components on the package of the respective product. Overall, minimal changes in the macro components of milk were observed after heat treatment in both pilot scale as well as dairy production scale studies.

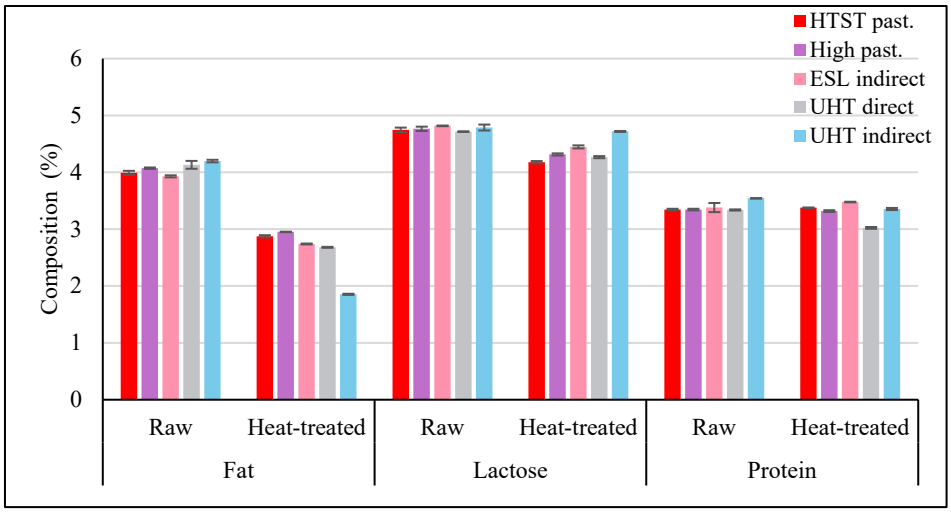

Figure 6. Macro component (fat, lactose, protein) composition in raw milk and heat-treated milk when subjected to HTST past., high past., ESL indirect, UHT direct and UHT indirect heat treatment (adapted from Paper III).

Mineral composition

Mineral analyses were performed on milk heat treated in dairy scale. Figure 7 depicts the mineral concentration in raw milk and heat-treated milk when subjected to different heat treatments (Paper **III**). The observed mineral concentration in the raw milk was within the range observed in an earlier study by Lindmark-Månsson et al. (2003) performed on Swedish raw milk, except for potassium and sodium, which were slightly lower in concentration, and iron, which was higher in concentration. Since the mineral concentration in cow milk is dependent on various factors such as season and breed (Walstra et al., 2005), the differences obtained in the mineral concentration could therefore be due to such factors. From Figure 7, it can be observed that the heat treatment of milk had little to no effect on the mineral concentration of milk. A similar trend was observed for cream samples heat treated with HTST past. and UHT indirect heat treatment (Paper III). Mineral concentration is dependent on processing conditions such as heating temperature, holding time, and flow conditions, which could potentially cause a build-up of fouling in the heat exchangers, leading to a decrease in mineral concentrations. However, in this thesis, the thermal processing investigated for milk and cream at dairy production scale had minimal impact on the mineral concentration.

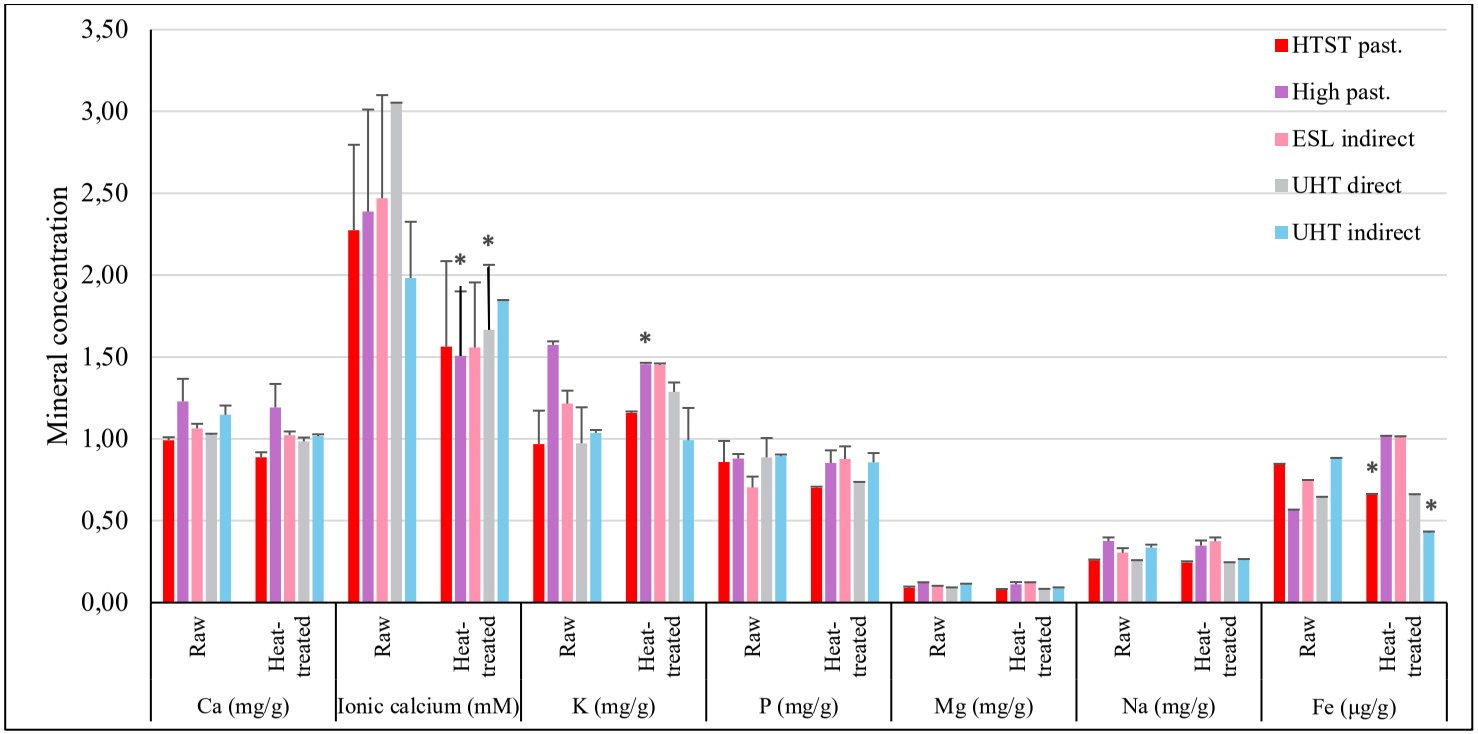

Figure 7. Mineral concentration of total calcium, ionic calcium, potassium, phosphorus, magnesium, sodium and iron in raw milk and heat-treated milk when subjected to HTST past., High past., ESL indirect, UHT direct and UHT indirect heat treatment. *P*-values indicate significant differences between raw milk and day of production (day 0) for each heat treatment (* $P < 0.05$) (adapted from Paper III).

Ionic calcium

The concentration of ionic calcium in milk plays a critical role in factors such as thermal stability, protein conformation and stability, as well as aggregation behaviours during heating (Walstra et al., 2005). Therefore, understanding the changes occurring in the ionic calcium concentration in milk due to heating is important. The ionic calcium concentration normalized to the initial concentration (C/Co) in milk after thermal processing by direct and indirect heating at different temperatures and holding times at pilot scale is shown in Figure 8 (Paper **II**). It can be observed that the ionic calcium concentration in milk tended to decrease as the heating temperature increased from 124 to 140 ºC for both direct and indirect heating, as well as the different holding times. However, an increase in the concentration of ionic calcium was observed at 143 ºC, which was unexpected. In Figure 7, the ionic calcium concentration in milk before and after heat treatment at dairy production scale for different heat treatments showed a similar trend, with a decrease in ionic calcium concentration observed after heat treatment (Paper **III**). This decrease in the concentration of ionic calcium was expected after heat treatment of milk due to the changes occurring in the distribution of minerals between the colloidal phase and serum phase of milk (Akkerman et al., 2021; Fox et al., 2015; On-Nom et al., 2010). Furthermore, the intensity of heat treatment determines the extent of changes in the mineral balance of milk (Fox et al., 2015).

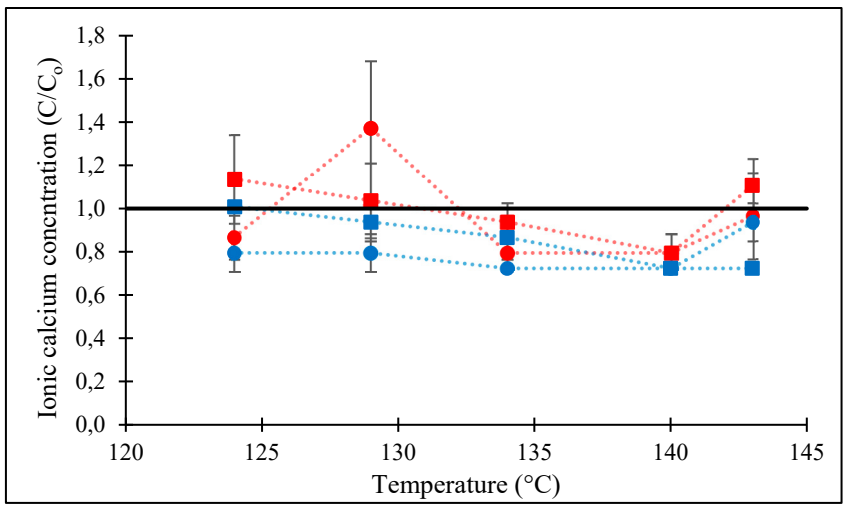

Figure 8. Ionic calcium concentration normalised to initial concentration (C/C_0) in milk after heat treatment with direct (blue) and indirect (red) heating system at different temperatures with a holding time of 1 s (O) and 4 s (□) (N=4). The straight line denotes the value for HTST past. milk (Paper II).

Ionic calcium concentrations for milk and cream were also monitored during storage after heat treatment at dairy production scale, as shown in Figure 9 (Paper **IV**). It

can be seen that, as the storage time progressed, no statistically significant changes in the ionic calcium concentrations for milk and cream were observed, except for UHT indirect heat-treated milk, where a significant decrease was observed after 8 months of storage. Previous studies suggest that heat treatment of milk does induce a decrease in ionic calcium concentration, but ionic calcium concentration recovers during cold storage of milk (Lewis, 2011). This is in line with our findings, as a decrease in ionic calcium concentration was observed when milk was heat treated (Figure 7), while during storage, the ionic calcium concentration remained unchanged (Figure 9). On the other hand, a significant decrease that is observed in ionic calcium concentration during long storage of UHT indirect milk is somewhat unexpected as a decrease in pH (Figure 13) would result in dissociation of calcium from casein micelles, leading to an increase in ionic calcium concentration (Broyard & Gaucheron, 2015). However, the pH change was rather small to cause a change and other factors could possibly cause this decrease in ionic calcium concentration. Furthermore, studies exploring the change in ionic calcium concentration for longer storage of milk heat treated with UHT showed inconsistent results, with some suggesting an increase while others suggested no change in ionic calcium concentration (Grewal et al., 2017; Li et al., 2017). In summary, ionic calcium concentration in milk decreased notably due to heat treatment. The findings also suggest that the ionic calcium concentration in milk and cream subjected to different heat treatments remains constant for shorter storage times, however, for longer storage time there may be a decrease in the concentration that may result in change in mineral equilibrium and therefore in the stability of casein micelles.

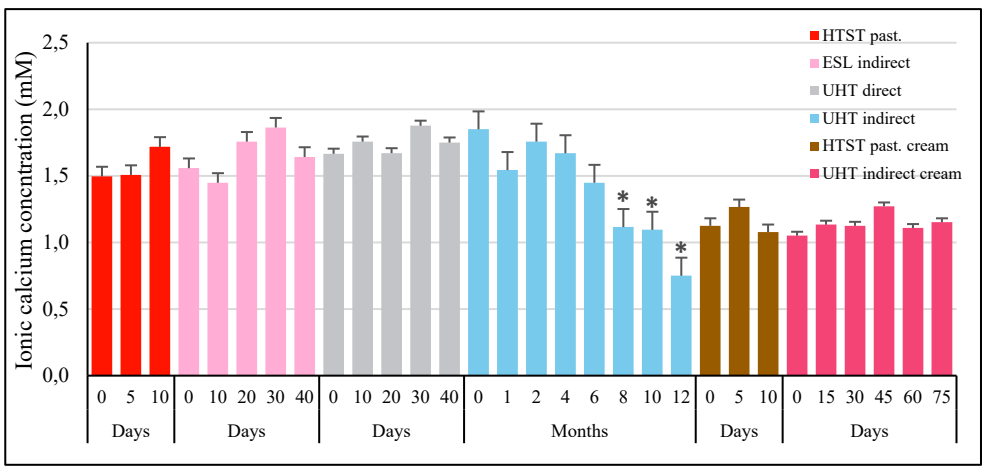

Figure 9. Ionic calcium concentration as a function of storage time for milk subjected to HTST past., ESL indirect, UHT direct and UHT indirect as well as cream subjected to HTST past. and UHT indirect (N=4). All samples were stored at 8 ºC except for UHT indirect milk which was stored at 20 ºC. *P*-values indicate significant differences for milk and cream, respectively, between day of production (day 0) and storage for each heat treatment (* $P < 0.05$) (adapted from Paper IV).

Vitamin composition

Milk is a good source of vitamins, particularly vitamin A and most B-vitamins, in terms of its nutritional value. However, the concentration of vitamins is affected by thermal processing and storage. In this thesis, the changes in vitamin concentration in milk due to heat treatment and storage were investigated (Papers **II, III, IV and V**). The vitamin concentration present in Swedish raw milk, as reported by Lindmark-Månsson (2003), ranges as follows: vitamin B_1 concentration of 0.40-0.44 µg/mL, vitamin B_2 concentration of 1.41-1.50 µg/mL, vitamin B_{12} concentration of 0.41-0.44 ng/mL, and vitamin E concentration of 1.01-1.10 µg/mL. The vitamin concentration in raw milk obtained in this study ranged from 0.43-0.58 µg/mL for vitamin B_1, 1.20-1.43 µg/mL for vitamin B_2, 0.33-0.44 ng/mL for vitamin B_{12} and 0.94-1.37 µg/mL for vitamin E (Papers **III and V**). The results are in line with the concentration of vitamins in raw milk obtained in this thesis.

Figure 10 shows the concentrations of vitamin B_1, vitamin B_2, and vitamin E in milk subjected to heat treatment at pilot scale, using both direct and indirect heating systems at different temperatures and holding times (Paper **II**). The concentration is normalized to the initial concentration of HTST past. milk (C/C_0). It was observed that vitamin degradation occurred in both direct and indirect heating systems, with the extent of degradation increasing as the heating temperature and holding time increased. Notably, vitamin B_1 exhibited the most significant decline, ranging from 1% to 11% across different temperature and time combinations, while vitamin B_2 showed a decrease between 2% and 8%, and vitamin E showed a reduction ranging from 1% to 8%. When comparing the direct and indirect heating systems, no significant difference in vitamin loss was observed between the two systems ($P > 0.05$). Previous studies that compared direct and indirect heating systems yielded similar results, indicating that there were no notable differences in terms of vitamin loss in milk (Burton et al., 1970; Lorenzen et al., 2011). Overall, the results of the pilot -scale study indicate that the degradation of vitamins is highly dependent on the temperature/time conditions applied during heat treatment, irrespective of whether a direct or indirect heating system is utilized.

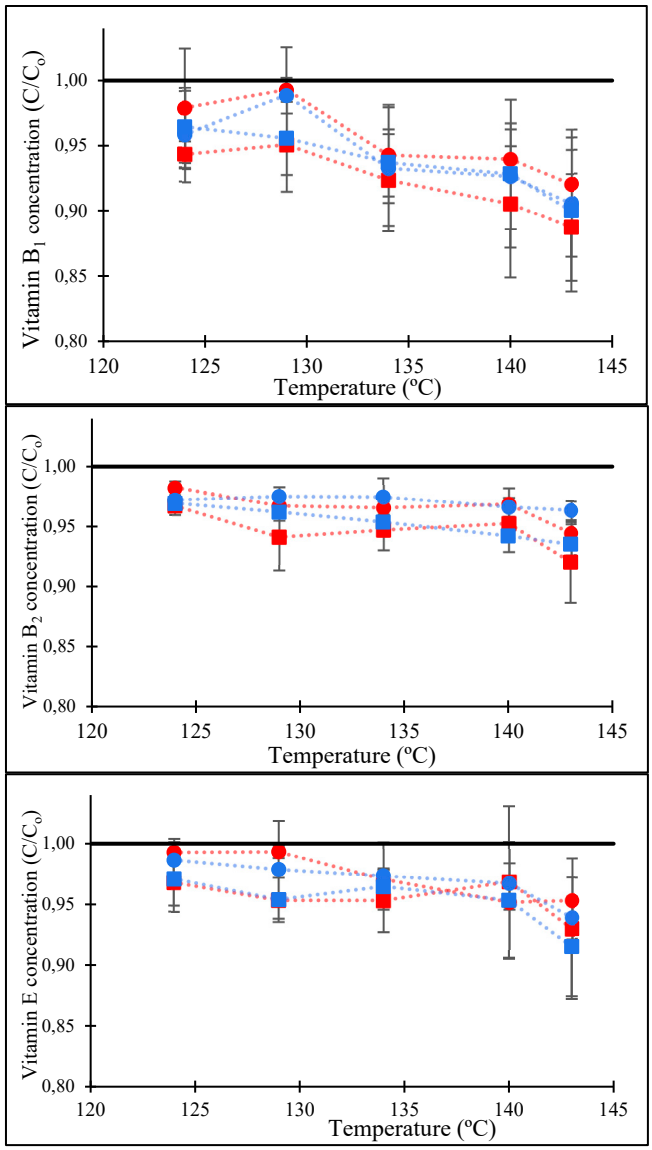

Figure 10. Concentrations of vitamin B_1, vitamin B_2 and vitamin E normalised to initial concentration (C/C_o) in milk after heat treatment with direct (blue) and indirect (red) heating system at different temperatures with a holding time of 1 s (○) and 4 s (□) (N=4). The straight line denotes the value for HTST past. milk (Paper II).

Figure 11 depicts the vitamin B_1, B_2, B_{12} and E concentrations in raw milk and heat-treated milk when subjected to different heat treatments at dairy production scale (Paper **III**). In Figure 11, it can be observed that the investigated vitamins showed a slight degradation after heat treatment. HTST past. showed no significant loss for

each of the vitamins studied. Furthermore, High past. of milk resulted in significant losses of vitamins B_1, B_2 and B_{12} ($P < 0.05$), whereas no significant loss for vitamin E was observed ($P > 0.05$). ESL indirect and UHT direct showed a significant loss of vitamin B_2, whereas the other vitamins remained unaffected. Finally, UHT indirect heat treatment on milk resulted in no significant loss of any of the investigated vitamins. Vitamin A was also investigated for pre-heated cream as well as after final heating with HTST past. and UHT indirect heat treatment resulting in no significant losses in vitamin A concentration after heat treatment (Paper III). Previous studies have contradictory findings where a few suggest that after heat treatment, no loss in the investigated vitamins occur (Andersson & Öste, 1994; Bendicho et al., 2002; Haddad & Loewenstein, 1983; Zhu et al., 2020) while others suggest losses of up to 30% after HTST past. (Andersson & Öste, 1992; Burton et al., 1967; Renner et al., 1989; Walstra et al., 2005). High past. heat treatment had the highest impact on the vitamin losses which was expected as the heat treatment conditions applied are intense. High past. heat treatment is usually used during yoghurt production, which helps improving the texture and consistency of yoghurt through heat-induced aggregation of whey proteins with the casein micelles.

To compare the degradation of vitamins during heat treatment at pilot scale study and dairy scale study (Papers **II** and **III**), it was observed that heat treatments conducted at pilot scale for ESL temperature of 129 °C with holding times of 1s and 4s resulted in a 2% and 4% loss of vitamin B_1, 3% and 3% loss of vitamin B_2, and 1% and 4% loss of vitamin E, respectively. In dairy production, following ESL indirect treatment (129 °C/2s), there was a 5% loss of vitamin B_1, 1% loss of vitamin B_2, and 1% loss of vitamin E. Heat treatments in the pilot scale at a UHT temperature of 140°C with holding times of 1s and 4s resulted in a 6% and 8% loss of vitamin B_1, 3% and 5% loss of vitamin B_2, and 4% and 3% loss of vitamin E, respectively. In dairy production, after ESL indirect treatment (129°C/2s), there was a 6% loss of vitamin B_1, 1% loss of vitamin B_2, and 8% loss of vitamin E. Losses of vitamin B_1 in both pilot scale and dairy production scale were observed are similar, whereas smaller losses in vitamin B_2 were observed in dairy production scale compared to pilot scale and more losses of vitamin E in dairy production scale for UHT processing compared to pilot scale. From these findings, varying degrees of vitamin degradation for pilot scale and dairy production scale experiments were observed. No clear conclusions could be drawn and therefore these insights highlight that there is a need for more research.

Overall, the concentration of vitamin B_{12} showed the most significant change among the studied vitamins, whereas other vitamins displayed minor losses. High past. heat treatment showed the most severe thermal degradation of the investigated vitamins due to the high heat load. Nevertheless, no distinct trends regarding the impact of heat treatment on the studied micronutrients could be definitively concluded, suggesting that multiple factors influence the changes during production.

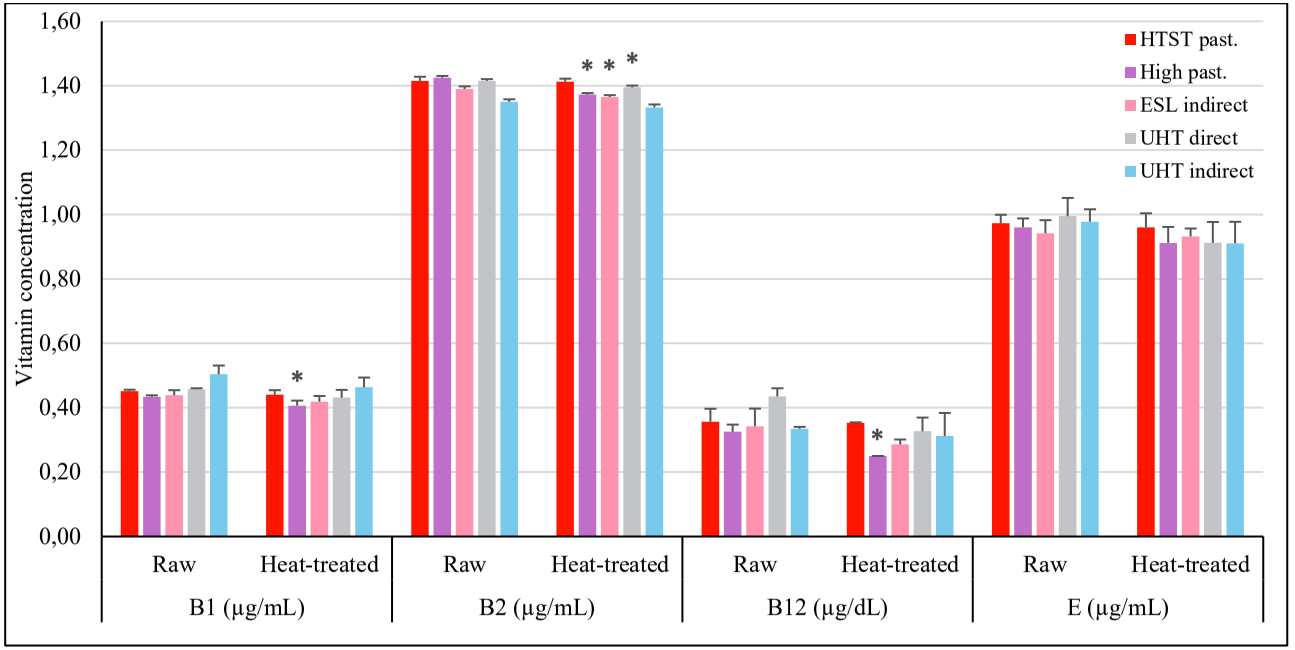

Figure 11. Vitamin concentrations of B_1, B_2, B_{12} and E in raw milk and heat-treated milk when subjected to HTST past., High past., ESL indirect, UHT direct and UHT indirect heat treatment. Values for raw milk and processed milk have been re-calculated based on a fat content of 3%. *P*-values indicate significant differences between raw milk and day of production (day 0) for each heat treatment (* $P < 0.05$; adapted from Paper III).

Table 3. Retention (%) of vitamins B_1, B_2, B_{12} and E on best before date for milk subjected to HTST past., ESL indirect and UHT direct stored at 8 ºC and UHT indirect stored at 20 ºC. Retention was quantified and presented as percentage of vitamin concentration compared to day 0, corresponding to 100% (adapted from Paper IV).

	Retention (%)				
Heat treatment	**Best-before date**	**Vitamin B1**	**Vitamin B2**	**Vitamin B12**	**Vitamin E**
HTST past.	10 days	96.47±2.50	100.00±0.58	97.74±0.00	96.54±2.04
ESL indirect	30 days	96.65±4.62	99.45±0.61	97.55±0.70	91.66±2.63
UHT direct	30 days	93.69±5.05	97.49±1.47	77.98±1.22	91.30±4.10
UHT indirect	4 months	90.50±4.61	94.93±1.13	81.73±1.60	95.42±2.78

Retention of vitamins B_1, B_2, B_{12} and E on the best before date for milk subjected to HTST past., ESL indirect, UHT direct and UHT indirect is shown in Table 3 (Paper **IV**). For vitamin B_1, a loss of about 3.5% was observed for HTST past. after 10 days of storage, while the maximum loss of about 10% was observed for UHT indirect after 4 months of storage. Previous studies investigating vitamin B_1 loss in milk suggest a loss of about 10-20% in UHT milk after 3 months of storage (Walstra et al., 2005), while Eberhard et al. (2003) reported a loss of 15% in UHT direct milk after 4 weeks of storage. Lorenzen et al. (2011) found no loss in ESL indirect milk during cold storage, but El-Sayed et al. (2014) noted a 100% loss after 4 months at 25 ºC for UHT milk. These variations in vitamin B_1 concentration may depend on the light exposure during storage which could influence the losses observed in the studies (Fox et al., 2015; Walstra et al., 2005). For vitamin B_2 rather small changes in concentration were observed during storage with the maximum loss of about 5% observed after 4 months of storage of UHT indirect milk. Vitamin B_2 is a stable vitamin both to heat treatment as well as to storage, however, the stability of vitamin B_2 is reduced when exposed to light (Cardoso et al., 2012; Kilic-Akyilmaz et al., 2022). Other studies have only shown a small reduction of about 3 to 4% in vitamin B_2 concentration for UHT treated milk stored for 5 months (El-Sayed et al., 2014; Oamen et al., 1989).

In Table 3, small losses of vitamin B_{12} less than 3% were observed for HTST past. and ESL indirect on the best before date which is in line with previous studies on HTST past. and ESL indirect showing no significant loss after storage (Andersson & Öste, 1994; Lorenzen et al., 2011). However, losses of about 20% were observed for UHT direct after only 30 days of storage and for UHT indirect after 4 months. As vitamin B_{12} is sensitive to the presence of oxygen, these higher losses in vitamin B_{12} concentration in UHT direct milk could possibly be due to exposure to oxygen during heat treatment with steam injection which might have stimulated the destabilisation of vitamin B_{12} or due to the presence of dissolved oxygen in the package (Kilic-Akyilmaz et al., 2022; Walstra et al., 2005). Previous studies have shown contradictory results of vitamin B_{12} losses during storage in UHT milk with

no effect to complete loss of the vitamin (Fox, 1995). For vitamin E losses of up to 10% were observed during storage. In conclusion, it is clear that vitamin losses can occur not only during heat treatment but also during storage. The intensity and duration of heat treatment are directly linked to the amount of these losses. As milk undergoes more intense heat treatments and longer storage times, the degree of vitamin degradation increases. These results emphasize the importance of precise control over processing and storage conditions to protect the nutritional quality of milk products.

Effects of processing and storage on physical properties of milk

Processing of milk and dairy products can cause an effect on the physical properties of milk which is dependent on different factors during processing including heating temperature, holding time as well as heating system e.g., direct or indirect heating. As mentioned previously, these changes in milk also occur continuously during storage. To understand the effects of processing and storage on milk and cream, heat treatment trials followed by storage were conducted in pilot and dairy production scale (Papers **II, III and IV)**.

pH

In Figure 12, the pH of HTST past. milk is shown, with an average pH of 6.69±0.03. Additionally, the pH of milk after heat treatment using both direct and indirect heating systems at pilot scale can be seen (Paper **II)**. It was observed that heat treatment resulted in an overall increase in the pH of the milk. After direct heat treatment, a slight increase in pH was observed for each temperature/time combination. On the other hand, the pH of milk after indirect heating either slightly increased or remained similar across the various heating temperatures and holding times. Previous studies (Dickow et al., 2012; Hammershøj et al., 2010; Lee et al., 2017) have also shown an increase in pH of milk after direct heat treatment. These studies suggested that this increase in pH could possibly be attributed to degassing or the steam pressure causing the removal of CO_2 during flash cooling.

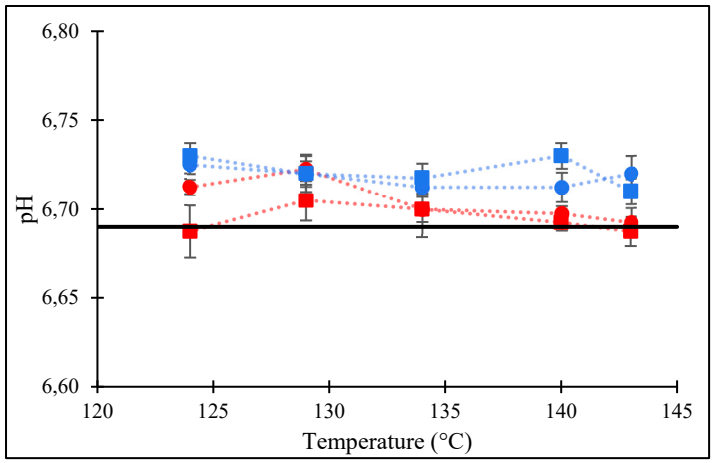

Figure 12. pH of milk after heat treatment with direct (blue) and indirect (red) heating system at different temperatures with a holding time of 1s (○) and 4 s (□) at pilot scale Nn=4). The straight line denotes the value for HTST past. milk (Paper II).

Figure 13 depicts the pH of raw milk, heat-treated milk as well as on the best-before date for milk subjected to various heat treatments at dairy production scale (Papers III and IV). It can be observed that the pH of raw milk is in the range of 6.68 to 6.75, which is comparable to previous studies by Karlsson et al. (2019) and Lindmark-Månsson et al. (2003). After heat treatment of milk, there is a decrease in pH, which could possibly be due to the formation of organic acids like formic acid which at high temperatures causes the breakdown of lactose (Nieuwenhuijse & van Boekel, 2003; Tsioulpas et al., 2010). Additionally, heat treatment causes a decrease in pH due to the association of calcium and phosphate in milk (Fox & McSweeney, 1998). The pH changes observed in Figure 13 suggest a minor effect on the milk pH after heat treatment at the dairy production scale which is consistent with previous studies by Deeth and Lewis (2017) and Karlsson et al. (2019) who reported only minor changes in milk pH after HTST past. and UHT heat treatment. Furthermore, a decrease in pH was observed for stored milk samples. This decrease in pH during storage occurs due to various factors such as temperature-dependent dephosphorylation and proteolysis of casein micelles, breakdown of lactose, which in turn produces organic acids, as well as the association of calcium and phosphate ions, which release protons (Limacher et al., 2008; Gaucheron, 2005; Rauh et al., 2014). To conclude, the pH changes in milk take place both during processing and storage due to biochemical changes in the milk system. The data obtained in the present thesis offers valuable insights into the changes occurring in the pH of milk due to heating temperature, holding time as well as heating systems, and complexities during dairy scale processing, assisting producers in enhancing product quality. The subtle pH changes indicate the essential balance between the formation of organic acids and lactose breakdown, which are further crucial in

determining texture, flavour, and safety (Nieuwenhuijse et al., 2003; Tsioulpas et al., 2010; Fox et al., 2015). Tracking these pH variations acts as a guide to extending the shelf life of milk, preserving its nutritional content, and ultimately satisfying consumer's needs.

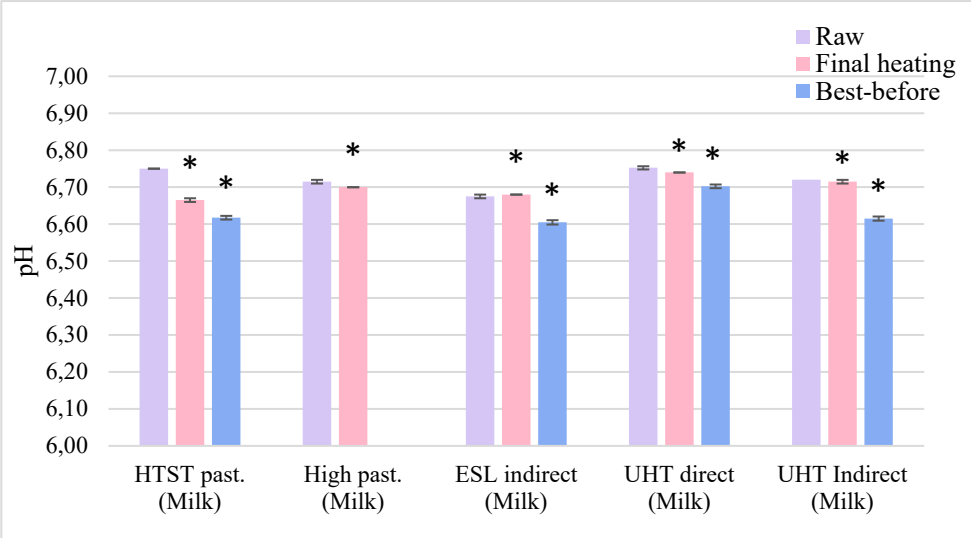

Figure 13. pH of milk (raw, heat-treated as well as on the best-before day) subjected to HTST past., High past., ESL indirect, UHT direct and UHT indirect at dairy production scale. All samples were stored at 8 ºC except for UHT indirect milk which was stored at 20 ºC (adapted from Papers III and IV).

Colour

Figure 14 (a, b, c) depicts L (dark-light), a* (green-red) and b* (blue-yellow) values of milk after heat treatment at pilot scale, using direct and indirect heating systems at different temperatures and holding times on the day of production (Paper **II)**. When compared to the HTST past. milk, heat-treated milk samples showed slightly higher L and b* values, and a lower a* value indicating that the heat-treated milk is whiter, greener and more yellow. However, the observed colour change(△E) is small and will not be detectable by consumers. This is because the colour change (△E) value is lower than 2.3, which is the just noticeable difference (JND) value. The JND value represents the threshold below which changes in colour are undetected. Additionally, there is no clear trend suggesting a change in colour of milk at different heating temperatures and times caused by direct or indirect heating systems.

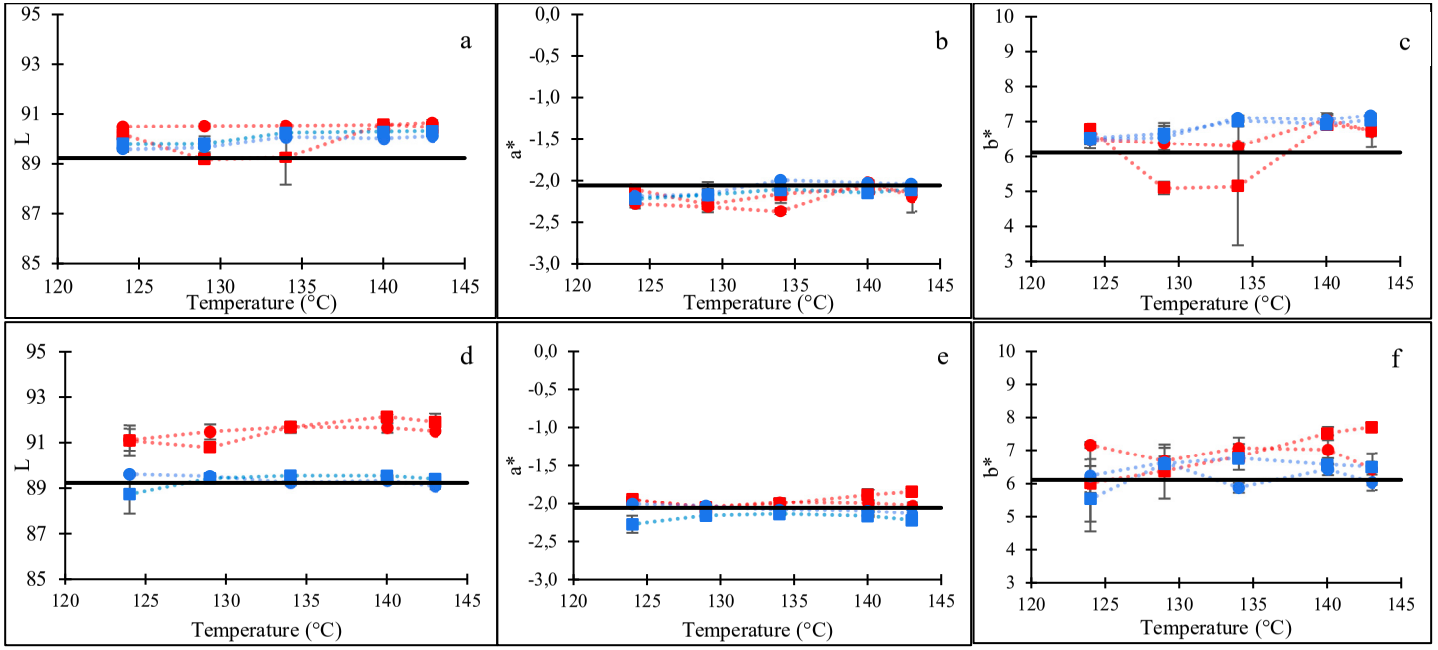

Figure 14. L (dark-light), a* (green-red) and b* (blue-yellow) values of milk after heat treatment with direct (blue) and indirect (red) heating system at different temperatures with a holding time of 1s (○) and 4 s (□) at day of processing (a, b, c) at pilot scale and stored for 28 days at 4 ºC (d, e, f) (N=4) at pilot scale. The straight line denotes the value for HTST past. milk (Paper II).

Figure 14 (d, e, f) shows the L, a* and b* values of milk heat-treated with direct and indirect heating systems after 28 days of storage (Paper **II**). The lightness of milk after 28 days of storage was lower than the day of production for direct heat-treated milk, while the lightness was higher for indirect heat-treated milk. Additionally, a larger change in lightness during storage in milk was observed for the heat treatment at higher temperatures (134 to 143 °C). When compared to the day of production, no effect of heating system was seen in the lightness (L value) of the milk. Therefore, the difference observed in the milk must have occurred during storage of the milk. One plausible reason for the change in lightness could be that the lightness is correlated to particle size (McClements et al., 2019), and thus a change in particle size of milk samples during storage could affect the colour of the milk. This is also supported by the fact that the physical stability of the milk was lower after 28 days of storage (Figure 20 b), with a more significant impact of storage observed in indirect heat-treated milk (Paper **IV)**.

The colour change ($\triangle$E) as a function of storage time for milk and cream subjected to different heat treatment in dairy production scale when compared with the day of production (day 0) is shown in Figure 15 (Paper **IV**). It can be seen that during storage, a change in colour is observed for both milk and cream samples. However, the $\triangle$E values were lower or about the just noticeable difference (JND), which has been previously suggested to be 2.3 (Baldevbhai & Anand, 2012). Thus, the minor changes would be unnoticeable by the consumers. However, during prolonged storage of UHT indirect milk, after 6 months the $\triangle$E value is above 2.3 suggesting a noticeable change in the colour of the milk. The colour change observed in UHT indirect milk could possibly be due to the Maillard reaction occurring in milk during storage (Al-Saadi & Deeth, 2008) or due to the change in particle size of milk during storage (McClements et al., 2019). Exploring the colour changes in milk during heating and storage revealed that while the initial impact of heat treatment and heating system on milk colour may not be apparent, more significant changes emerge during storage. Storage time is crucial, as a gradual change in the colour of milk is observed as the storage period progresses while over prolonged storage, these colour changes become more significant. This understanding of colour change in milk enables a thorough investigation and control of these changes, offering valuable insights into the development and quality of dairy products.

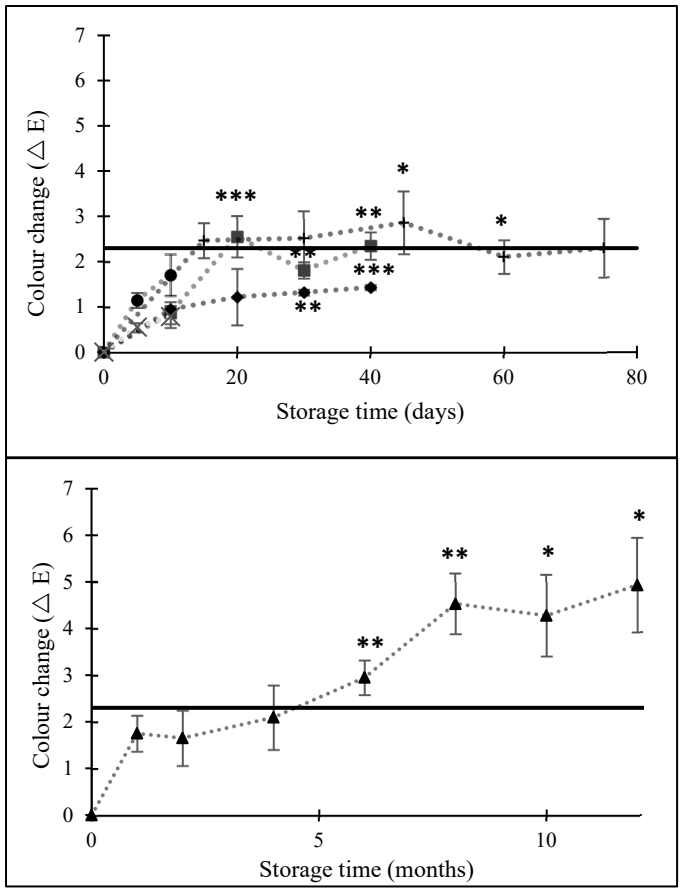

Figure 15. Colour change (△E) as a function of storage time for milk at dairy production scale subjected to HTST past. (●), ESL indirect (■), UHT direct (♦), UHT indirect (▲) and cream subjected to HTST past. (×) and UHT indirect (+) (n=4). The just noticeable difference (JND) is 2.3, which is denoted by a straight line. *P*-values indicate significant differences for milk and cream, respectively, between colour change (△E between day of production (day 0) and first storage point) and storage time within each heat treatment (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) (Paper IV).

Particle size distribution

The particle size distribution of milk was analysed during both pilot scale and dairy production scale experiments (Papers **II, III and IV**). In the pilot scale trials, the impact of heating temperature and holding time were studied using both direct and indirect heating systems. The starting material for the experiments was homogenized HTST pasteurized milk, and no homogenisation was conducted upstream. Homogenized HTST pasteurized milk was used as a starting material to

solely investigate the effects of heat treatment on milk without homogenization. In the dairy scale experiments, the particle size distribution of milk was assessed in skimmed raw milk, heat-treated milk samples, and storage samples to observe changes in the particle size distribution.

Figure 16 shows the effects of the heating system on the size of fat globules and casein micelles (Paper **II**). The effect of different temperatures on the particle size distribution of milk treated with indirect heating systems was minimal, while the effect was more pronounced for milk heat-treated using direct heating system. In the case of direct heating, there was a noticeable shift towards larger particles in the particle size distribution, and this effect was more significant at higher temperatures. To analyse the size of fat globules (Figure 16 c, d), a protein dissolving solution (sol A) was added to the milk. In Figure 16 (c), no effect of indirect heating on fat globule size distribution was observed compared to HTST past. milk, suggesting that the small differences seen in Figure 16 (a) could possibly be attributed to changes in casein micelle size. However, in Figure 16 (d), which shows the particle size distribution for milk treated with direct heating, it can be seen that not only were casein micelles dissolved but some of the larger particles also disappeared after the addition of the sol A solution, suggesting that the aggregates present in heat-treated milk were formed due to the interaction of proteins on the surface of fat globules (Sharma & Dalgleish, 1993; Walstra et al., 2005).

Moreover, to further understand the increase in fat globule size, Dv (90) values were investigated with and without the addition of the sol A for HTST past. milk, as well as for direct and indirect heat-treated milk (Figure 17). It is evident that the Dv (90) value for indirect heat-treated milk was similar to that of the starting material (HTST past.). While a higher Dv (90) value was observed for milk treated with direct heating, and the effect on particle size was more pronounced at higher temperatures. Finally, microscopic analysis of the milk sample was performed to further investigate this increase in fat globule size, as shown in Figure 18. It is apparent that direct heat-treated milk exhibited different particles of larger size compared to HTST past. milk and indirectly heat-treated milk. The micrographs (Figure 18) confirm that there was both full coalescence and aggregation of fat globules caused by the heating system.

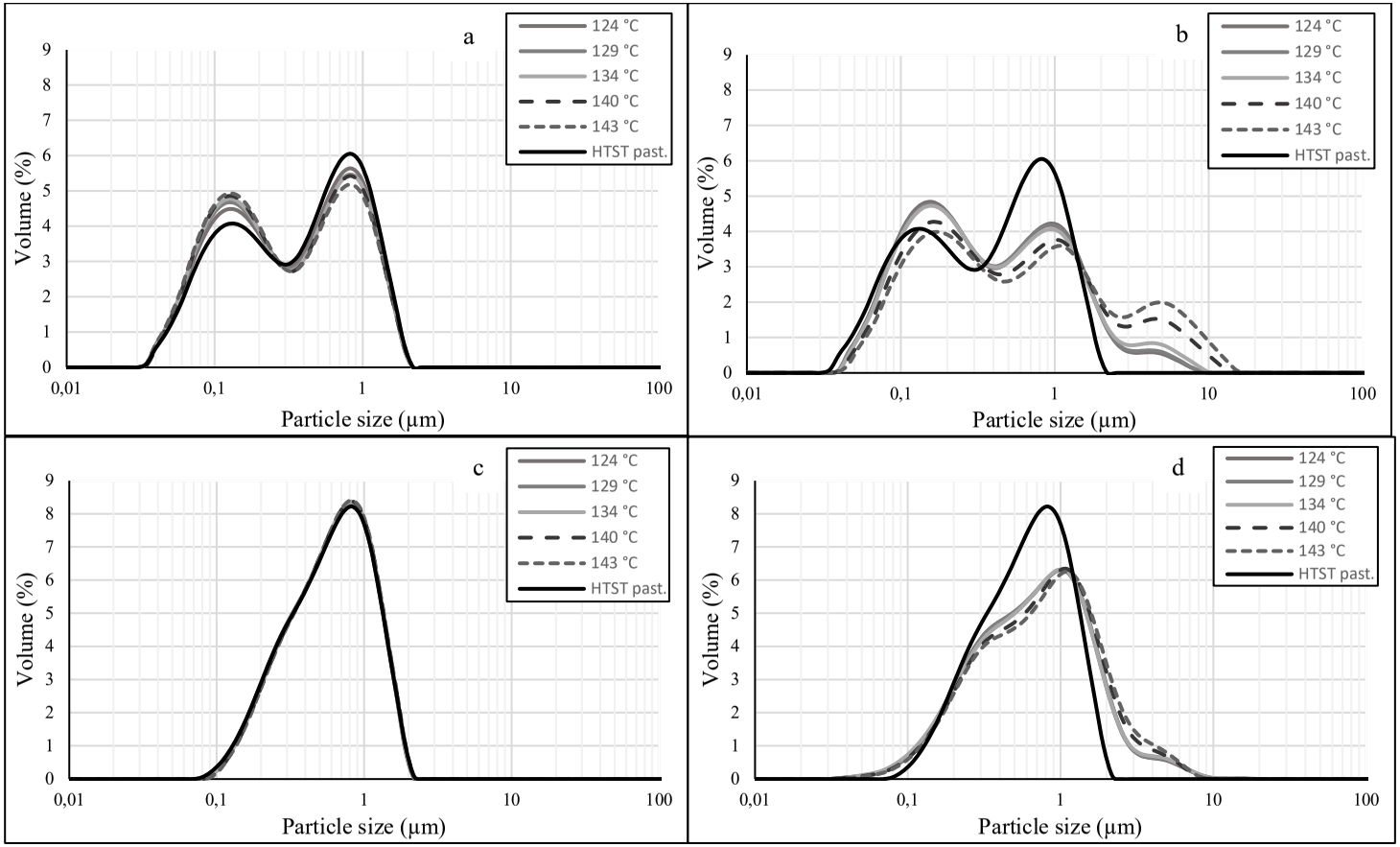

Figure 16: Particle size distributions of milk heated at different temperatures for 4 s with indirect heating system (a), direct heating system (b), indirect heating system with addition of solA to the milk (c) and direct heating system with addition of solA to the milk (d) at pilot scale (Paper II).

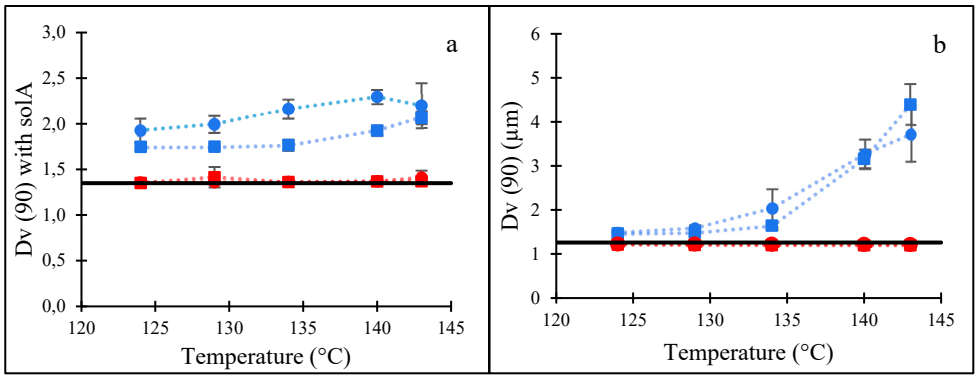

Figure 17. Dv (90) of milk particle size distribution with (a) and without (b) addition of sol A after heat treatment with direct (blue) and indirect (red) heating systems at different temperatures with a holding tme of 1 s (○) and 4 s (□) (N=4) at pilot scale. The straight line denotes the value for HTST past. milk (Paper II).

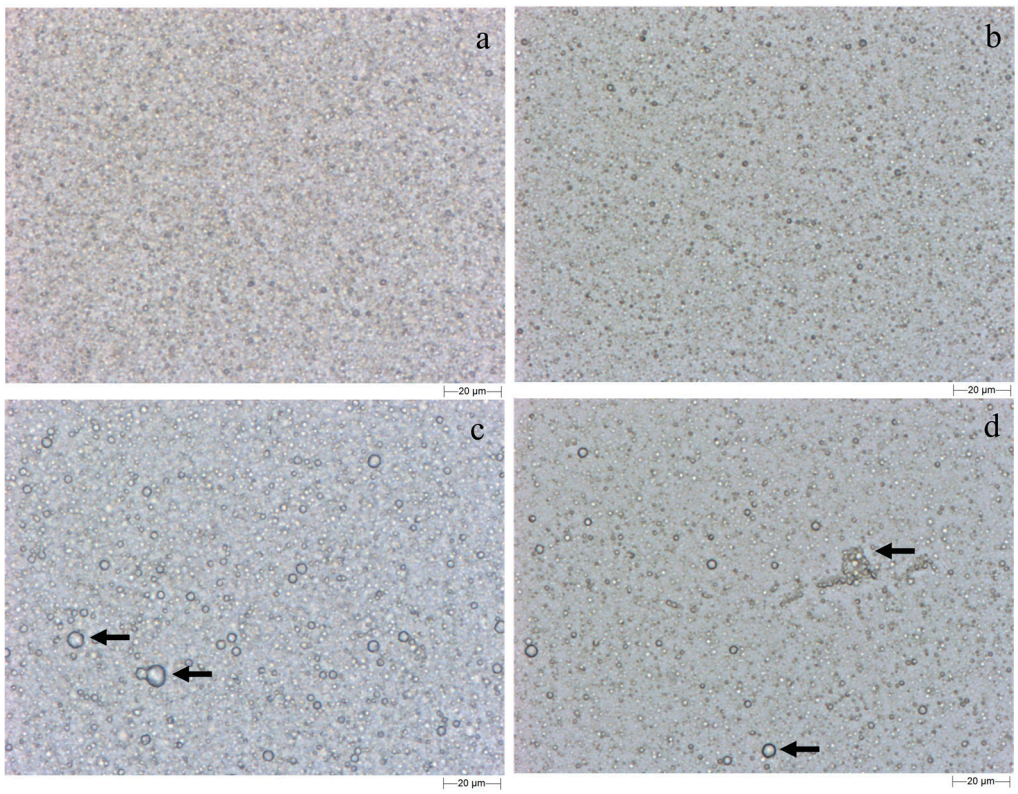

Figure 18. Micrographs for a) HTST past. milk, b) indirect heat treated milk 143 ºC/ 4 s, c) direct heat-treated milk 134 ºC/ 1 s, and d) direct heat treated milk 140 ºC/ 4 s. The arrows indicate large fat globules indicating full coalescence and aggregates of small fat globules (Paper II).

Previous studies investigating the particle size distribution of fat globules have been conducted using unhomogenized milk as the starting material (Corredig & Dalgleish, 1996; van Boekel & Folkerts, 1991; Zadow, 1969). A significant distinction in this study is that the milk was homogenized before undergoing direct heating. Homogenisation enhances the stability of fat globules against hydrodynamic breakup by reducing their size and creating a more stable membrane. The replacement of a portion of the native milk fat globule membrane with casein micelles and whey proteins increases the interfacial tension from approximately 1.5 to 10 mN·m-1 (Walstra et al., 2005). This is because whey proteins and casein micelles partially replace the native milk fat globule membrane, resulting in increased interfacial tension (Walstra et al., 2005). The rate of both particle breakup and coalescence is influenced by turbulence, and the dominant process depends on the interfacial stability of the droplet and the intensity of turbulence (Håkansson, 2019; McClements, 2015). As a result, a decrease or increase in fat globule size is not unexpected in a turbulent zone, whether in unhomogenized or homogenized milk. Therefore, the coalescence of fat globules observed in direct heat-treated milk could have occurred either in the holding cell or during flash cooling in the steam injection step. Even though fat globule coalescence is observed, this is usually mitigated with the help of homogenization which is usually performed upstream at the dairies.

The particle size distribution for milk heat-treated at dairy production scale was performed, and the results are shown in Table 4 (Paper **III**). The fat globule size in heat-treated milk ranged from 0.28 to 0.85 µm due to the different homogenization pressure applied to the milk. Furthermore, the volume-weighted average diameter D[4,3] of casein micelles in skimmed raw milk was 0.13 µm for each of the heat treatments. After the milk samples were heat-treated, the apparent casein micelle size increased, ranging from 0.17 µm to 0.23 µm. This increase in casein micelle size due to increasing temperature and holding time was expected and is also correlated to the casein micelles associated with denatured whey protein (Anema & Li, 2003; Ono et al., 1999). It is thus expected that high past. and UHT direct and indirect heat-treated milk would have the highest effect on the apparent casein micelle size however this was not observed in our data. One possible reason for the small increase in apparent casein micelle size in high past. milk compared to the other heat treatments could be that the whey protein aggregates can also be formed in the serum phase during heating and therefore they do not associate with casein micelles. This, in turn, results in a lower level of denatured whey proteins associated with casein micelles.

Table 4. Mean ± standard deviation (N=4) of physical stability expressed as Turbiscan stability index (TSI), volume-weighted average diameter (D[4,3]) of casein micelles and fat globules as well as after final heating for processed milk subjected to different heat treatments, as well as comparison in physical properties between heat treatments (Paper III).[1]

Property	Heat treatment				
	HTST past.	High past.	ESL indirect	UHT direct	UHT indirect
TSI	0.90±0.25^{a}	1.21±0.23^{a}	0.97±0.12^{a}	1.22±0.20^{a}	0.88±0.10^{a}
Apparent casein micelle size, D[4,3] (µm)	0.19±0.00^{a}	0.17±0.01^{b}	0.23±0.00^{c}	0.17±0.01^{b}	0.21±0.01^{d}
Fat globule size, D[4,3] (µm)	0.85±0.04^{a}	0.37±0.01^{b}	0.63±0.00^{c}	0.28±0.00^{d}	0.32±0.00^{e}

[1]Different superscript letters indicate significant differences (P ≤ 0.05) within a row, comparing physical properties between heat treatments. The total homogenisation pressures applied during processing were for HTST past. 140 bar, High past. 170 bar, ESL indirect 160 bar, UHT direct 160 bar and UHT indirect 180 bar.

The volume-weighted diameter, D[4,3], for fat globules and casein micelles in milk during storage for dairy production scale study is depicted in Figure 19 (Paper **IV**). It can be seen in Figure 19 (a), that a slight increase in fat globule size after 30 days of storage for ESL indirect milk was observed whereas fat globule size for HTST past. and UHT direct remained unchanged during their respective storage times. In Figure 19 (b), it is apparent that the fat globule size of milk in UHT indirect milk changed significantly with the largest effect observed after 10 months of storage. The apparent casein micelle size was in the range of 0.16 to 0.21 µm on the day of production, no effect of storage was observed on apparent casein micelle size for HTST past and UHT direct treated milk while a slight decrease in UHT indirect milk after eight months of storage was observed (Figure 19). Overall, the particle size distribution of milk during storage remained stable apart from prolonged stored milk samples.

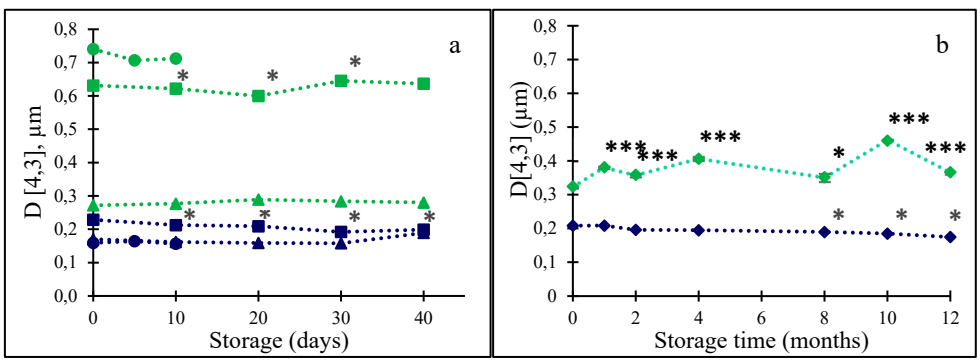

Figure 19. Fat globule size, D[4,3] (green) and apparent casein micelle size, D[4,3] for milk (blue) as a function of storage time for milk subjected to HTST past. (●), ESL indirect (■), UHT direct (▲) stored at 8 °C (a) and UHT indirect (♦) stored at 20 °C (b). *P*-values indicate significant differences for milk between day of production (day 0) and storage time within each heat treatment (* *P* < 0.05; ** *P* < 0.01; * *P* < 0.001; Paper IV).**

Physical stability

The particle size distribution of milk in direct and indirect heat-treated milk at pilot scale was discussed in the previous section, and these particle size distributions can have an effect on the stability of the milk system. However, Figure 20 (a) which compares the TSI value of direct and indirect heat-treated milk compared to the HTST past. milk, shows no clear effect on the stability of heat-treated milk (Paper **II**). On day 0 (day of production), there was no difference in the TSI value between direct and indirect heat-treated milk, even though direct heat-treated milk had larger fat globules and aggregates. When the same samples were stored in cold storage for 28 days it was observed that the TSI value increased for both direct and indirect heat-treated milk (Figure 20, b), with a more pronounced effect on the instability in indirect heat-treated milk. The differences observed in size distribution did not have a significant effect on the physical stability of the milk samples both on the day of production and during storage, indicating that the milk was fairly stable in terms of physical stability.

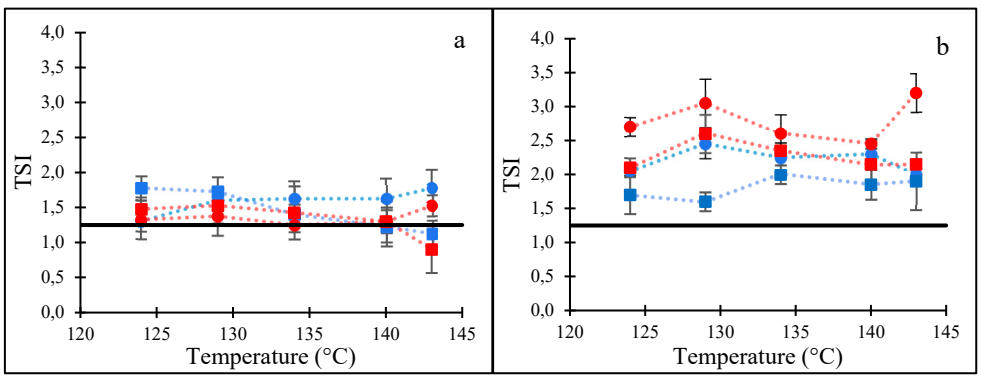

Figure 20. Physical stability expressed as Turbiscan stability index (TSI) for day 0 (a) and day 28 (b) of milk after heat treatment with direct (blue) and indirect (red) heating system at different temperatures with a holding tim of 1 s (O) and 4 s (□) (N=4) in pilot scale. The straight line denotes the value for HTST past. milk (Paper II).

The physical stability, TSI, of milk subjected to different heat treatments in dairy production scale is presented in Table 4 (Paper **III**). UHT indirect treatment had the lowest TSI value, with a fat content of milk of 1.86%, compared to about 3% for the other processed milk samples. An increase in fat content could result in higher instabilities in milk due to flocculation creaming and aggregation of fat globules present in milk (Walstra et al., 2005). However, the TSI values for processed milk ranged from 0.9 to 1.2 with no significant differences indicating a stable system.

Figure 21 depicts the physical stability (TSI) for milk and cream during storage (Paper **IV**). The TSI value for milk on the day of production ranged from 0.9 to

1.22, while the TSI value for cream was in the range of 2.6 to 2.8, showing that cream is more unstable than milk. However, the TSI value is still within the acceptable range of product quality (TSI < 3; Carrentero et al., 2005). In Figure 21, the physical stability of UHT direct milk remained stable throughout the storage, while for HTST past and ESL indirect instability was observed from day 10. For UHT indirect instability was observed at month 10 and 12. Even though the TSI value for cream was higher on the day of production compared to milk, the instability of the cream during storage did not increase. Previous studies have suggested that during the storage of UHT milk, creaming occurs due to fat globule flocculation or coalescence caused by proteolysis of casein that covers the surface of fat globules (Anema, 2019). Proteolysis caused by plasmin is inhibited when milk is stored at lower temperatures. However, in this present study, the milk was stored at 8°C. More research is needed to understand the risk of proteolysis in cold-stored milk, which was not studied in the present study. In summary, the changes in the physical stability of milk and cream during storage were significant. However, the observed TSI value was still not very high, suggesting that the product was of an acceptable quality even after the best-before date.

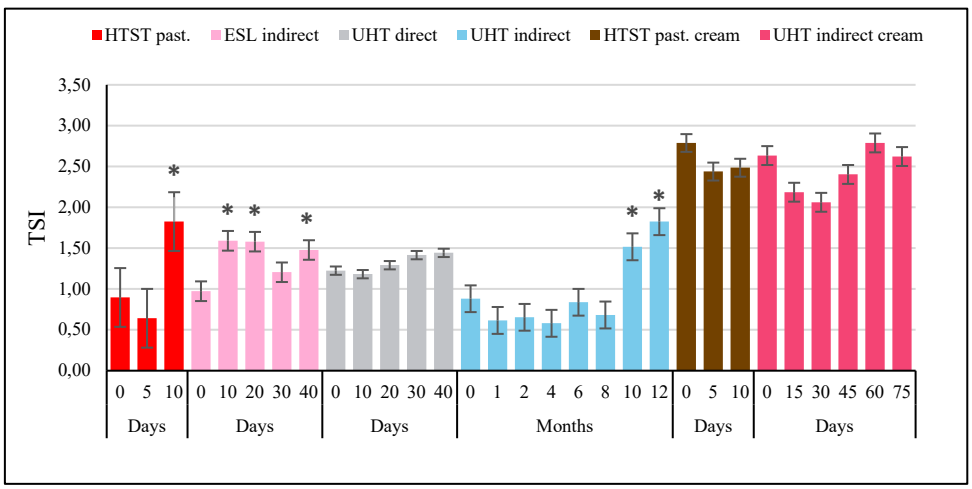

Figure 21. Physical stability expressed as Turbiscan stability index (TSI) for milk as a function of storage time for milk subjected to HTST past., ESL indirect, UHT direct s and UHT indirect as well as cream subjected to HTST past. and UHT indirect in dairy production scale. *P*-values indicate significant differences for milk between day of production (day 0) and storage time within each heat treatment (* $P < 0.05$; adapted from Paper IV).

Kinetic modelling

Kinetic thermal degradation models play a crucial role in optimising food processing methods. Ideally, these models should be fitted to data obtained from real production scale experiments using the actual large-scale processing equipment and the thermal treatment processes used in the industry. However, this is often not possible due to the high cost and limited accessibility of production-scale facilities. Additionally, the range of operating parameters in these facilities may not provide enough variation to accurately fit the model parameters. As a result, it is necessary to validate and test the performance of kinetic parameters fitted to typical laboratory conditions (heating and holding of vials) when applied to production scale conditions. To understand the thermal degradation of vitamins in milk, a laboratory scale vial heat and hold experiment was performed at five different temperatures ranging from 70 °C to 110 °C and were held for up to 35 mins. The kinetic model fitting for three vitamins (B_1, B_2 and E) were performed in laboratory scale and the obtained models were further used for validation of dairy scale experiment results obtained as discussed in the previous sections (Paper **V**).

Vitamin degradation in laboratory scale experiments

Degradation of vitamins in laboratory scale experiments is depicted in Figure 22. The markers show the concentration of vitamin B_1, B_2 and E as a function of processing time at different heating temperatures (70, 80, 90, 100 and 110 °C). The vitamin concentration in raw milk ranged from 0.48-0.58 µg/mL for vitamin B_1, 1.20-1.33 µg/mL for vitamin B_2 and 1.30-1.37 µg/mL for vitamin E. It can be observed that each of the vitamin is degrading due to thermal processing and the rate of degradation increases with increasing temperature (Paper **V**).

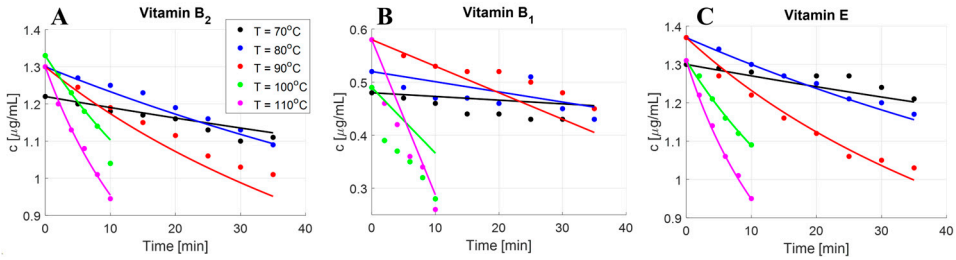

Figure 22. Vitamin concentration, *c*, as a function of processing time, at different processing temperatures (T = 70 °C, 80 °C, 90 °C, 100 °C, 110 °C) for the laboratory scale experiments (markers). Lines display the predicted vitamin concentrations according to the kinetic models in Table 5. A) Vitamin B_2, B) Vitamin B_1 and C) Vitamin E (Paper V).

Fitting kinetic models

To the experimentally obtained vitamin values in laboratory scale, global parameter fitting (see section *Reaction kinetics*) was performed to derive kinetic model for each of the studied vitamin. Table 5 summarises the kinetic parameters obtained by model fitting. The overall fit appears to be quite good when comparing the modelled vitamin concentration to the experimentally measured concentrations in Figure 23. This is further supported by the coefficients-of-determination (R^2) values, which are 98% for vitamin B_2, 93% for vitamin B_1, and 99% for vitamin E. Although the adjusted-R^2 values, which consider the number of parameters used in the estimations, are slightly lower, however, all the values are above 90% (see Table 5). It can be observed that the overall fit is lower for vitamin B_1 in comparison to the other two vitamins (Paper **V**).

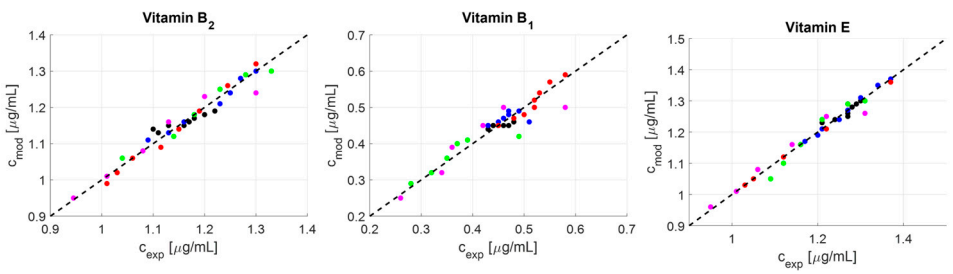

Figure 23. Vitamin concentration predicted by the kinetic model, c_{mod}, compared to the experimentally measured concentration, c_{exp} for the three vitamins (A: vitamin B_2, B: vitamin B_1 and C: vitamin E) in the laboratory-scale heat treatments. Marker colours refer to the heat treatment temperature (see legend in Figure 22; Paper V).

Table 5. Vitamin degradation kinetic parameters (n, k_{ref}, E_A) obtained from using the global model on the laboratory scale data (standard deviations in parenthesis). Also showing the model fitted (experiment-specific) initial concentrations and the coefficient of determination (R^2) and the adjusted version (R^2_{adj}) for the fit to the laboratory scale data (Paper V).

	Parameter	Vitamin B_2 (Riboflavin)	Vitamin B_1 (Thiamine)	Vitamin E (α-Tocopherol)
Kinetic model	*n* [-]	2.23 (0.65)	0 (0.0)	2.44 (0.62)
	k_{ref} [$\mu g^{1-n}\, s^{-1}\, mL^{n-1}$]	0.0070 (0.00073)	0.004194 (0.0005)	0.0045 (0.00058)
	E_A *[kJ/mol]*	71.74 (2.81)	102.2 (8.0)	77.57 (3.18)
	T_{ref} [K]	361.30	361.28	356.48
Experiment-specific	$c_{0,70°C}$ [µg/mL]	1.21 (0.01)	0.46 (0.01)	1.32 (0.01)
	$c_{0,80°C}$ [µg/mL]	1.31 (0.01)	0.51 (0.01)	1.38 (0.01)
	$c_{0,90°C}$ [µg/mL]	1.31 (0.01)	0.58 (0.01)	1.33 (0.01)
	$c_{0,100°C}$ [µg/mL]	1.32 (0.01)	0.43 (0.01)	1.32 (0.01)
	$c_{0,110°C}$ [µg/mL]	1.30 (0.01)	0.55 (0.02)	1.32 (0.01)
Fit	R^2 [%]	98	93	99
	R^2_{adj} [%]	97	91	98

Furthermore, in Figure 22, along with the experimentally measured vitamin concentration (markers), the model predictions (solid lines) for vitamin degradation obtained from Table 5 can be observed. Figure 22 shows that the remaining lack of fit arises from the fact that the kinetic model predictions deviate more under certain conditions. While the fit is typically of the same quality regardless of processing time and temperature for vitamin E, the lack of fit for vitamin B_2 is largely influenced by a single point (T = 100 °C, t = 10 min), which not only deviates from the kinetic model but also from the trend of the other data points at that processing temperature. Therefore, part of the lack of fit here could be attributed to some unidentified experimental uncertainty. The fit for vitamin B_1 is somewhat lower compared to the other investigated vitamins. Figure 22 shows that this is mainly due to difficulties in estimating the degradation rates during prolonged processing at high temperatures (Paper **V**).

Validation and comparison of kinetic models

The validation of the kinetic model obtained was performed by predicting the vitamin degradation due to processing at dairy scale. Since, the industrial dairy processing of milk differs considerably from the laboratory scale experiments which was used to develop the kinetic model, both in the heating temperature-time

combinations as well as heat treatment method. Therefore, a proper validation of the model needs to be performed with the dairy scale production condition. Figure 24 depicts the comparison between the predicted concentration using the model obtained in Table 5 and the experimental concentration of vitamin obtained after five different dairy scale heat treatment processes (Paper **III**), where the error bar depicts the standard deviation of the experimentally measured concentration. It can be observed that the fit of the model is relatively good with the obtained experimental value of vitamins as the error bar overlaps the model prediction. However, some discrepancies can also be observed between predicted and measured vitamin concentration, for example high past. heat treatment predictions for vitamin B_1 and B_2 are deviating the most from the experimental value leading to overprediction of loss. While vitamin E has larger error bar causing difficulty in concluding the different heat treatments.

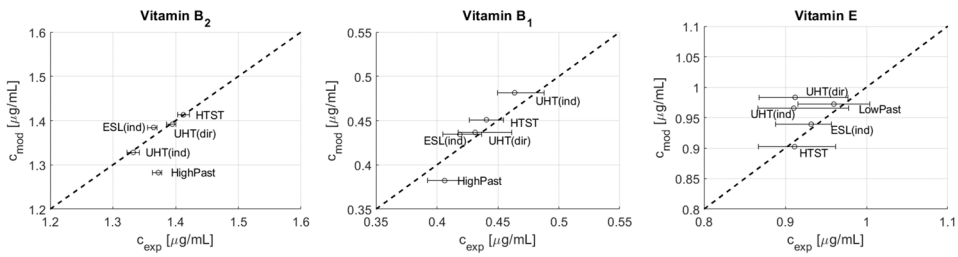

Figure 24. Vitamin concentrations predicted by the kinetic model, c_{mod}, compared to the experimentally measured concentration, c_{exp} for the three vitamins (A: vitamin B_2, B: vitamin B_1 and C: vitamin E) in the dairy scale heat treatments. Error bars display estimate plus/minus one standard deviation of the experimentally measured concentrations (Paper V).

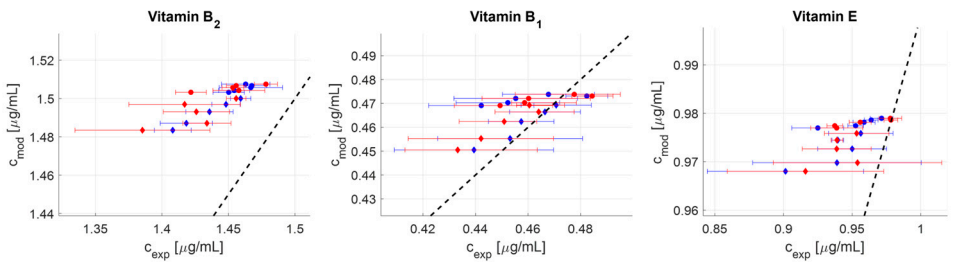

Figure 25. Vitamin concentrations predicted by the kinetic model, c_{mod}, compared to the experimentally measured concentrations, c_{exp} for the three vitamins (A: vitamin B_2, B: vitamin B_1 and C: vitamin E) in the pilot-scale heat treatments. Error bars display estimate plus/minus one standard deviation of the experimentally measured concentrations. Blue/red markers: direct/indirect heating. Disks/diamonds: 1/4 s holding time (Paper V).

Furthermore, the obtained kinetic model for vitamin degradation was further compared to the pilot scale experiments investigating the vitamin degradation (Paper **II**). Figure 25 depicts the comparison between the predicted concentration using the model obtained in Table 5 and the experimental concentration of vitamin

obtained after heat treatment at pilot scale. The fit of the model is poor compared to dairy scale dataset and the model systematically underpredicts the vitamin concentration under majority conditions. This is less obvious for vitamins B_1 and E, as the model predictions still fall within the range of experimental uncertainty (as indicated by the error bars). However, this discrepancy is more apparent for vitamin B_2. It is important to note that there are differences between pilot scale data and laboratory and dairy scale data which could influence the comparison with the model obtained. Differences include the type of raw material used (raw milk versus pre-pasteurised consumption milk) and the treatment of samples (such as assessing vitamin content in fresh samples versus frozen and thawed samples). Therefore, it is difficult to determine whether the observed deviation is due to limitations in the kinetic modelling approach or the systematic impact of freezing and thawing on vitamin determination.

Overall, the laboratory fitted kinetic model obtained showed a relatively good fit between the predicted and measured vitamin concentrations for dairy production scale heat treatments, despite the differences between the laboratory scale experiments and the production scale. However, when comparing the model with pilot scale experiments, the results were less conclusive. It is important to note that there are inherent differences in how milk is treated in these different types of experiments. Nonetheless, the approach of fitting kinetic parameters using laboratory experiments to predict thermal degradation in production scale processes still holds value.

Conclusions

In this thesis, the effects of thermal processing and storage on the nutrient content and physical properties of milk and cream were evaluated at laboratory, pilot and dairy production scale. The thesis has shown that:

- Thermal processing showed minimal losses of macro components and total mineral content in milk and cream, whereas degradation of vitamins B_1, B_2, and B_{12} in milk increased with increasing heating temperature and holding time. At dairy production scale, high pasteurisation caused the most significant vitamin degradation, while limited losses were observed for HTST pasteurisation, ESL, and UHT processing. At pilot scale, no differences were observed between direct and indirect heating systems for ESL/UHT treatment (**Papers II, III and V**).
- Thermal processing showed no changes in physical stability of milk, whereas an increase in casein micelle size was observed at dairy production scale. An increase in fat globule size and aggregate formation were observed for direct heating for ESL/UHT treatment at pilot scale with more pronounced effects with increasing temperature. Minimal colour changes occurred (**Papers II, III and IV**).
- During storage, vitamin losses ranged from 1-22% for different heat treatments and more pronounced effects of vitamin degradation was observed with more intense heat treatment and longer storage times. Vitamin B_{12} concentration tended to decrease more over time than vitamins B_1, B_2 and E and more losses in vitamin concentration were found after storage than after heat treatment. Significant effects on physical stability and colour changes in milk and cream were observed during prolonged storage (**Papers II and IV**).
- Kinetic models for vitamin degradation during heat treatment were developed, which can be used to estimate vitamin losses at dairy level. This is the first study to validate and compare such a model at the dairy production level using contemporary heat treatment techniques (**Papers I and V**).

The thesis has highlighted that heating systems for ESL/UHT treatment may influence milk quality, although no clear distinction between direct and indirect

heating systems was observed at pilot scale for the investigated quality parameters. Furthermore, the thesis has shown that process techniques for heat treatment used in dairy production scale today are mild and have low effects on the nutrient content and physical properties of milk and cream, and that more pronounced changes primarily occur during longer storage times.

Future outlook

This thesis has demonstrated the effects of thermal processing and storage on nutrient content and physical properties of milk. However, there are many aspects of milk quality that need to be investigated that are affected by processing. For instance, lactulose and furosine content in milk, which indicate changes occurring in milk due to heat treatment could be investigated to further understand the effects of processing. Investigating protein denaturation in milk caused by heat treatment could be of interest by examining how heat changes the three-dimensional structure of proteins. This alteration affects properties such as gel formation, functional characteristics, and nutritional quality. It is essential to comprehend these transformations to improve dairy processing techniques for achieving specific product textures and qualities while maintaining nutritional value. Additionally, this thesis only investigated a few vitamins and milk is a good source of many vitamins, and therefore the effect of heat treatment on different vitamins could be of interest.

Since processing of milk does not only include heat treatment but also other steps such as homogenisation, which has a great effect on the stability of milk, incorporating various processing steps could be an interesting approach to understand changes occurring in milk. In this study, 5 different processing lines in a dairy were investigated, while more of such processing lines could be investigated like thermisation, low temperature long time (LTLT) pasteurisation and in container sterilisation. Moreover, during the pilot scale study, only the tubular heat exchanger for indirect heating and steam injection for direct heating were compared, while it could be interesting to look at plate heat exchangers as well as steam infusion and their effects on milk quality.

The kinetic model developed in this study could further help in understanding the degradation of vitamins at different temperature and time conditions. Such an approach of calculating the degradation of nutrients with the help of modelling could therefore be used instead of performing experiments in a large production scale.

References

Ajmal, M., Nadeem, M., Imran, M., Mushtaq, Z., Ahmad, M. H., Tayyab, M. & Gulzar, N. (2019). Changes in fatty acids composition, antioxidant potential and induction period of UHT-treated tea whitener, milk and dairy drink. *Lipids in Health and Disease, 18*, 1-9.

Akkerman, M., Johansen, L. B., Rauh, V., Poulsen, N. A., & Larsen, L. B. (2021). Contribution of casein micelle size and proteolysis on protein distribution and sediment formation in UHT milk during storage. *International Dairy Journal, 117*, 104980.

Akkerman, M., Johansen, L. B., Rauh, V., Sørensen, J., Larsen, L. B., & Poulsen, N. A. (2021). Relationship between casein micelle size, protein composition and stability of UHT milk. *International Dairy Journal, 112*, 104856.

Al-Saadi, J. M., & Deeth, H. C. (2008). Cross-linking of proteins and other changes in UHT milk during storage at different temperatures. *Australian Journal of Dairy Technology, 63*(3), 93.

Alvarado, U., Zamora, A., Arango, O., Saldo, J., & Castillo, M. (2022). Prediction of riboflavin and ascorbic acid concentrations in skimmed heat-treated milk using front-face fluorescence spectroscopy. *Journal of Food Engineering, 318*, 110869.

Andersson, I., & Öste, R. (1992). Loss of ascorbic acid, folacin and vitamin B12, and changes in oxygen content of UHT milk. II: Results and discussion. *Milchwissenschaft, 47*(5), 299-302.

Andersson, I., & Öste, R. (1994). Nutritional quality of pasteurized milk. Vitamin B12, folate and ascorbic acid content during storage. *International Dairy Journal, 4*(2), 161-172.

Anema, S. G. (2017). Storage stability and age gelation of reconstituted ultra-high temperature skim milk. *International Dairy Journal, 75*, 56-67.

Anema, S. G. (2019). Age gelation, sedimentation, and creaming in UHT milk: A review. *Comprehensive Reviews in Food Science and Food Safety, 18*(1), 140-166.

Anema, S. G. (2021). Heat-induced changes in caseins and casein micelles, including interactions with denatured whey proteins. *International Dairy Journal, 122*, 105136.

Anema, S. G., & Li, Y. (2003). Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *Journal of Dairy Research, 70*(1), 73-83.

Baldevbhai, P. J., & Anand, R. (2012). Color image segmentation for medical images using L* a* b* color space. *IOSR Journal of Electronics and Communication Engineering, 1*(2), 24-45.

Bendicho, S., Espachs, A., Arantegui, J., & Martín, O. (2002). Effect of high intensity pulsed electric fields and heat treatments on vitamins of milk. *Journal of Dairy Research, 69*(1), 113-123.

Bijl, E., de Vries, R., van Valenberg, H., Huppertz, T., & Van Hooijdonk, T. (2014). Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. *International Dairy Journal, 34*(1), 135-141.

Bilic, N., & Sieber, R. (1988). Determination of retinol and alpha-tocopherol in milk and milk products using high-pressure liquid chromatography after saponification in serum vials. *Zeitschrift fur Lebensmittel-untersuchung und-forschung, 186*(6), 514-518.

Burton, H., Ford, J., Perkin, A., Porter, J., Scott, K., Thompson, S., & Edwards-Webb, J. (1970). Comparison of milks processed by the direct and indirect methods of ultra-high-temperature sterilization. IV. The vitamin composition of milks sterilized by different processes. *Journal of Dairy Research, 37*(3), 539-543.

Cardoso, D. R., Libardi, S. H., & Skibsted, L. H. (2012). Riboflavin as a photosensitizer. Effects on human health and food quality. *Food & function, 3*(5), 487-502.

Carrentero, A., Villepin, R., Brunel, L., & Carries, J. (2005). Turbiscan LAb User Guide. *Formulaction, France*.

Cashman, K. (2011). Milk salts: Macroelements, nutritional significance. *Encyclopedia Dairy Science*, second ed., Elsevier Inc., 2011, pp. 925–932.

Claeys, W. L., Ludikhuyze, L. R., & Hendrickx, M. E. (2001). Formation kinetics of hydroxymethylfurfural, lactulose and furosine in milk heated under isothermal and non-isothermal conditions. *Journal of Dairy Research, 68*(2), 287-301.

Claeys, W. L., Ludikhuyze, L. R., Van Loey, A. M., & Hendrickx, M. E. (2001). Inactivation kinetics of alkaline phosphatase and lactoperoxidase, and denaturation kinetics of β-lactoglobulin in raw milk under isothermal and dynamic temperature conditions. *Journal of Dairy Research, 68*(1), 95-107.

Corredig, M., & Dalgleish, D. G. (1996). Effect of different heat treatments on the strong binding interactions between whey proteins and milk fat globules in whole milk. *Journal of Dairy Research, 63*(3), 441-449.

Datta, N., Elliott, A. J., Perkins, M. L., & Deeth, H. C. (2002). Ultra-high-temperature (UHT) treatment of milk: Comparison of direct and indirect modes of heating. *Australian Journal of Dairy Technology, 57*, 211-227.

de la Fuente, M. A. (1998). Changes in the mineral balance of milk submitted to technological treatments. *Trends in Food Science & Technology, 9*(7), 281-288.

Deeth, H. (2010). Improving UHT processing and UHT milk products. In M.W. Griffiths (Ed.), Improving the safety and quality of milk (Vol. 1, pp. 302-329). Cambridge, UK: Woodhead Publishing.

Deeth, H. (2020). Stability and spoilage of lipids in milk and dairy products. *Advanced Dairy Chemistry, Volume 2: Lipids*, 345-373.

Deeth, H. C., & Datta, N. (2011). Ultra-high temperature treatment (UHT): Heating systems. In J. W. Fuquay, P. L. H. McSweeney, & P. F. Fox (Eds.), *Encyclopedia of dairy sciences* (2nd ed., pp. 699-707). Amsterdam, The Netherlands: Elsevier.

Deeth, H. C., & Lewis, M. J. (2017). *High temperature processing of milk and milk products*: Chichester, UK: John Wiley & Sons.

Dickow, J. A., Nielsen, M. T., & Hammershøj, M. (2012). Effect of Lenient Steam Injection (LSI) heat treatment of bovine milk on the activities of some enzymes, the milk fat globule and pH. *International Journal of Dairy Technology, 65*(2), 191-200.

Eberhard, P., Bütikofer, U., & Sieber, R. (2003). *Vitamine in gelagerter hocherhitzter Milch*: Eidg. Forschungsanstalt für Milchwirtschaft Liebefeld.

Eisner, M. D. (2021). Direct and indirect heating of milk–A technological perspective beyond time–temperature profiles. *International Dairy Journal, 122*, 105145.

El-Sayed, S., Hagrass, A., Asker, A., Malhat, F., El-Sayed, M., & El-Salam, M. (2014). Storage stability of thiamin, riboflavin and pyridoxine in UHT milk. *Egyptian Journal of Dairy Science, 42*(1), 1-6.

EuroStat. (2022). Available at: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics#Milk_production. (Accessed: 22 December 2023).

Fao.org (2021) Dairy production and products: Production. Available at: https://www.fao.org/dairy-production-products/production/en/ (Accessed: 22 December 2023).

Fatih, M., Barnett, M. P., Gillies, N. A., & Milan, A. M. (2021). Heat treatment of milk: a rapid review of the impacts on postprandial protein and lipid kinetics in human adults. *Frontiers in Nutrition, 8*, 643350.

Fox, P., & Brodkorb, A. (2008). The casein micelle: Historical aspects, current concepts and significance. *International Dairy Journal*, 18(7), 677-684.

Fox, P., Uniacke-Lowe, T., McSweeney, P., & O'Mahony, J. (2015). *Dairy chemistry and biochemistry* (2nd ed.). Cham, Switzerland: Springer International Publishing.

Fox, P. F., & McSweeney, P. L. (1998). *Dairy chemistry and biochemistry*. London: Chapman & Hall.

Friend, B., Shahani, K., Long, C., & Agel, E. (1983). Evaluation of freeze-drying, pasteurization, high-temperature heating and storage on selected enzymes, B-vitamins and lipids of mature human milk. *Journal of Food Protection, 46*(4), 330-334.

Gaucheron, F. (2005). The minerals of milk. *Reproduction Nutrition Development, 45*(4), 473-483.

Godoy, H. T., Amaya-Farfan, J., & Rodriguez-Amaya, D. B. (2021). Degradation of vitamins. In *Chemical Changes During Processing and Storage of Foods*. Academic Press: Cambridge, MA, USA, 2021; pp. 329–383.

Gorner, F., & Uherova, R. (1980). Vitamin changes in UHT-treated milk during storage. *Nahrung, 24*(4/5), 373-379.

Gregory, M. E., & Burton, H. (1965). The effect of ultra-high-temperature heat treatment on the content of thiamine, vitamin B6 and vitamin B12 of milk. *Journal of Dairy Research, 32*(1), 13-17.

Greiby, I., Mishra, D. K., Dolan, K. D., & Siddiq, M. (2017). Inverse method to estimate anthocyanin degradation kinetic parameters in cherry pomace during non-isothermal heating. *Journal of Food Engineering, 198*, 54-62.

Grewal, M. K., Chandrapala, J., Donkor, O., Apostolopoulos, V., & Vasiljevic, T. (2017). Predicting sediment formation in ultra high temperature-treated whole and skim milk using attenuated total reflectance-Fourier transform infrared spectroscopy. *International Dairy Journal, 74*, 39-48.

Haddad, G. S., & Loewenstein, M. (1983). Effect of several heat treatments and frozen storage on thiamine, riboflavin, and ascorbic acid content of milk. *Journal of Dairy Science, 66*(8), 1601-1606.

Håkansson, A. (2019). Emulsion formation by homogenization: Current understanding and future perspectives. *Annual review of food science and technology, 10*, 239-258.

Hammershøj, M., Hougaard, A. B., Vestergaard, J. S., Poulsen, O., & Ipsen, R. H. (2010). Instant infusion pasteurisation of bovine milk. II. Effects on indigenous milk enzymes activity and whey protein denaturation. *International Journal of Dairy Technology, 63*(2), 197-208.

Heldman, D., & Lund, D. (2007). Handbook of food engineering. (Ed. 2) USA: CRC, Taylor & Francis.

Holt, C. (1995). Effect of heating and cooling on the milk salts and their interaction with casein. *Heat-induced changes in milk.* (Ed. 2), 105-133. Brussels, Belgium: International Dairy Federation.

Holt, C., De Kruif, C., Tuinier, R., & Timmins, P. (2003). Substructure of bovine casein micelles by small-angle X-ray and neutron scattering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 213(2-3), 275-284.

Huppertz, T., & Nieuwenhuijse, H. (2022). Constituent fouling during heat treatment of milk: A review. *International Dairy Journal, 126*, 105236.

IDF (2022). *Heat treatment of milk*. Bulletin No 516/2022. Brussels, Belgium: International Dairy Federation.

IDF (2023). *The World Dairy Situation Report 2023*.Bulletin No 527/2023. Brussels, Belgium: International Dairy Federation.

Ifie, I., & Marshall, L. J. (2018). Food processing and its impact on phenolic constituents in food. *Cogent Food & Agriculture, 4*(1), 1507782.

Jansson, T., Clausen, M. R., Sundekilde, U. K., Eggers, N., Nyegaard, S., Larsen, L. B., Colin, R., Sundgren, A., Andersen, H. J., Bertram, H. C. (2014). Lactose-hydrolyzed milk is more prone to chemical changes during storage than conventional ultra-high-temperature (UHT) milk. *Journal of Agricultural and Food Chemistry, 62*(31), 7886-7896.

Jewell, K. (2012). Comparison of 1-step and 2-step methods of fitting microbiological models. *International Journal of Food Microbiology, 160*(2), 145-161.

Karlsson, M. (2019). Stability of ultra-high temperature treated milk *(thesis)*. Swedish University of Agricultural Sciences, Uppsala

Karlsson, M. A., Langton, M., Innings, F., Malmgren, B., Höjer, A., Wikström, M., & Lundh, Å. (2019). Changes in stability and shelf-life of ultra-high temperature treated milk during long term storage at different temperatures. *Heliyon, 5*(9).

Kessler, H. G. (2002). Food and Bio Process Engineering: Dairy Technology. Verlag A. Kessler, Munich, Germany, pp. 130–216.

Kilic-Akyilmaz, M., Ozer, B., Bulat, T., & Topcu, A. (2022). Effect of heat treatment on micronutrients, fatty acids and some bioactive components of milk. *International Dairy Journal, 126*, 105231.

Kwok, K. C., Shiu, Y. W., Yeung, C. H., & Niranjan, K. (1998). Effect of thermal processing on available lysine, thiamine and riboflavin content in soymilk. *Journal of the Science of Food and Agriculture, 77*(4), 473-478.

Lee, A., Barbano, D., & Drake, M. (2017). The influence of ultra-pasteurization by indirect heating versus direct steam injection on skim and 2% fat milks. *Journal of Dairy Science, 100*(3), 1688-1701.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and analysis, 19*(4), 252-276.

Lewis, M., & Deeth, H. (2008). Heat treatment of milk. In A. Y. Tamime (Ed.), *Milk processing and quality management*, 168-204. London, UK: Blackwell.

Lewis, M. J. (2011). The measurement and significance of ionic calcium in milk–a review. *International Journal of Dairy Technology, 64*(1), 1-13.

Li, S., Ye, A., & Singh, H. (2021). Physicochemical changes and age gelation in stored UHT milk: Seasonal variations. *International Dairy Journal,* 118, 105028.

Limacher, A., Kerler, J., Davidek, T., Schmalzried, F., & Blank, I. (2008). Formation of furan and methylfuran by Maillard-type reactions in model systems and food. *Journal of Agricultural and Food Chemistry, 56*(10), 3639-3647.

Lindmark-Månsson, H., Fondén, R., & Pettersson, H.E. (2003). Composition of Swedish dairy milk. *International Dairy Journal, 13*(6), 409-425.

Lorenzen, P. C., Clawin-Rädecker, I., Einhoff, K., Hammer, P., Hartmann, R., Hoffmann, W., Martin, D., Molkentin, J., Walte, H. G. & Devrese, M. (2011). A survey of the quality of extended shelf life (ESL) milk in relation to HTST and UHT milk. *International Journal of Dairy Technology*, 64(2), 166-178.

Lu, C., Wang, G., Li, Y., & Zhang, L. (2013). Effects of homogenisation pressures on physicochemical changes in different layers of ultra-high temperature whole milk during storage. *International Journal of Dairy Technology, 66*(3), 325-332.

Macrae, R., Robinson, R. K., & Sadler, M. J. (1993). *Encyclopaedia of food science, food technology, and nutrition*. London: Academic Press.

Malmgren, B. (2007). Evaluation of UHT milk processed by direct steam injection and steam infusion technology *(thesis)*. Lund University, Lund

McCarthy, O., & Singh, H. (2009). Physico-chemical properties of milk. *Advanced Dairy Chemistry: Volume 3: Lactose, Water, Salts and Minor Constituents*, (3rd ed.), pp. 691-758, Boston, Springer.

McClement, D. (2015). *Food Emulsions: principles, practices, and techniques*. Boca Raton: CRC Press, UK, 357-380.

McClements, D. J., Newman, E., & McClements, I. F. (2019). Plant-based milks: A review of the science underpinning their design, fabrication, and performance. *Comprehensive Reviews in Food Science and Food Safety, 18*(6), 2047-2067.

McGuire, R. G. (1992). Reporting of objective color measurements. *HortScience, 27*(12), 1254-1255.

Meng, F., Uniacke-Lowe, T., Lanfranchi, E., Meehan, G., O'Shea, C., Fox, P. F., Huppertz, T., Ryan, C., Kelly, A. L. (2020). Factors affecting the creaming of human milk. *International Dairy Journal, 108*, 104726.

Michalski, M.-C., Cariou, R., Michel, F., & Garnier, C. (2002). Native vs. damaged milk fat globules: membrane properties affect the viscoelasticity of milk gels. *Journal of Dairy Science, 85*(10), 2451-2461.

Naranjo, G. B., Gonzales, A. S. P., Leiva, G. E., & Malec, L. S. (2013). The kinetics of Maillard reaction in lactose-hydrolysed milk powder and related systems containing carbohydrate mixtures. *Food Chemistry, 141*(4), 3790-3795.

Nieuwenhuijse, J. A., & van Boekel, M. A. (2003). Protein stability in sterilised milk and milk products. In *Advanced Dairy Chemistry—1 Proteins: Part A/Part B* pp. 947-974, Cham, Switzerland: Springer.

Oamen, E., Hansen, A., & Swartzel, K. (1989). Effect of ultra-high temperature steam injection processing and aseptic storage on labile water-soluble vitamins in milk. *Journal of Dairy Science, 72*(3), 614-619.

On-Nom, N., Grandison, A., & Lewis, M. (2010). Measurement of ionic calcium, pH, and soluble divalent cations in milk at high temperature. *Journal of Dairy Science, 93*(2), 515-523.

Ono, T., Yoshida, M., Tanaami, H., & Ohkosi, H. (1999). Changes in casein micelle size induced by heating. *International Dairy Journal, 9*(3-6), 405-406.

Ostan, R., Laza, L., & Popescu, I. (2013). Comparative study of B12 vitamin distribution and assimilation. *Research Journal of Agricultural Science*, 45(4).

Ottaway, P. B. (2002). The stability of vitamins during food processing. *The nutrition handbook for food processors*, pp 247-264, Boca Raton, FL, USA: CRC Press

Ottaway, P. B. (2010). Stability of vitamins during food processing and storage. In *Chemical deterioration and physical instability of food and beverages* pp. 539-560, Oxford, UK: Woodhead Publishing.

Ramsey, J., & Swartzel, K. (1984). Effect of ultra high temperature processing and storage conditions on rates of sedimentation and fat separation of aseptically packaged milk. *Journal of Food Science, 49*(1), 257-262.

Ransmark, E., Svensson, B., Svedberg, I., Göransson, A., & Skoglund, T. (2019). Measurement of homogenisation efficiency of milk by laser diffraction and centrifugation. *International Dairy Journal, 96*, 93-97.

Rauh, V., & Xiao, Y. (2022). The shelf life of heat-treated dairy products. *International Dairy Journal, 125*, 105235.

Rauh, V. M., Sundgren, A., Bakman, M., Ipsen, R., Paulsson, M., Larsen, L. B., & Hammershøj, M. (2014). Plasmin activity as a possible cause for age gelation in UHT milk produced by direct steam infusion. *International Dairy Journal, 38*(2), 199-207.

Renner, E., Schaafsma, G., & Scott, K. (1989). Micronutrients in milk. *Micronutrients in Milk and Milk-based food products.*, 1-70.

Rysstad, G., & Kolstad, J. (2006). Extended shelf-life milk—advances in technology. *International Journal of Dairy Technology, 59*(2), 85-96.

Schwaab, M., & Pinto, J. C. (2007). Optimum reference temperature for reparameterization of the Arrhenius equation. Part 1: Problems involving one kinetic constant. *Chemical Engineering Science, 62*(10), 2750-2764.

Singh, H., & Latham, J. M. (1993). Heat stability of milk: Aggregation and dissociation of protein at ultra-high temperatures. *International Dairy Journal, 3*(3), 225-237.

Stoeckel, M., Lidolt, M., Achberger, V., Glück, C., Krewinkel, M., Stressler, T., von Neubeck, M., Wenning, M., Scherer, S. & Fischer, L. (2016). Growth of Pseudomonas weihenstephanensis, Pseudomonas proteolytica and Pseudomonas sp. in raw milk: Impact of residual heat-stable enzyme activity on stability of UHT milk during shelf-life. International Dairy Journal, 59, 20-28.

Sunds, A. V., Rauh, V. M., Sørensen, J., & Larsen, L. B. (2018). Maillard reaction progress in UHT milk during storage at different temperature levels and cycles. *International Dairy Journal, 77*, 56-64.

Swedish Food Agency (2024). The Swedish Food Agency food database, version 2023-06-13. Available at: https://soknaringsinnehall.livsmedelsverket.se/ (Accessed: 20 January 2024).

TetraPak. (2015). *Dairy processing handbook*. Lund, Sweden: Tetra Pak Processing systems AB.

The Royal Society of Chemistry (2024) *Royal Society of Chemistry*. Available at: https://www.rsc.org/ (Accessed: 07 February 2024).

Tsioulpas, A., Koliandris, A., Grandison, A., & Lewis, M. (2010). Effects of stabiliser addition and in-container sterilisation on selected properties of milk related to casein micelle stability. *Food Chemistry, 122*(4), 1027-1034.

van Boekel, M. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry, 62*(4), 403-414.

van Boekel, M. (2021). Kinetics of heat-induced changes in foods: A workflow proposal. *Journal of Food Engineering, 306*, 110634.

van Boekel, M. (2022). Kinetics of heat-induced changes in dairy products: Developments in data analysis and modelling techniques. *International Dairy Journal, 126*, 105187.

van Boekel, M. A. (2008). *Kinetic modeling of reactions in foods*. Boca Raton, FL, USA: CRC press.

van Boekel, M. A. J. S., & Folkerts, T. (1991). Effect of heat treatment on the stability of milk fat globules. *Milchwissenschaft, 46*(12), 758-765.

Vidal-Valverde, C., Reche, A., & Medhano, A. (1990). Determination of thiamin in milk by HPLC. *23 Reunion Bienal RSEQ*.

Villota, R., & Hawkes, J. G. (2018). Reaction kinetics in food systems. In *Handbook of food engineering.* pp. 225-484, Boca Raton, FL, USA: CRC Press.

Voelker, A. L., Miller, J., Running, C. A., Taylor, L. S., & Mauer, L. J. (2018). Chemical stability and reaction kinetics of two thiamine salts (thiamine mononitrate and thiamine chloride hydrochloride) in solution. *Food Research International, 112*, 443-456.

Walstra, P., Wouters, J. T., & Geurts, T. J. (2005). *Dairy science and technology*: Boca Raton, FL, USA: CRC Press. Taylor & Francis Group.

Weyh, C., Krüger, K., Peeling, P., & Castell, L. (2022). The role of minerals in the optimal functioning of the immune system. *Nutrients, 14*(3), 644.

Whited, L., Hammond, B., Chapman, K., & Boor, K. (2002). Vitamin A degradation and light-oxidized flavor defects in milk. *Journal of Dairy Science, 85*(2), 351-354.

Wishard, A., & Gibb, B. C. (2019). Dynamic light scattering–an all-purpose guide for the supramolecular chemist. *Supramolecular Chemistry, 31*(9), 608-615.

Ye, A., Singh, H., Taylor, M., & Anema, S. (2005). Disruption of fat globules during concentration of whole milk in a pilot scale multiple-effect evaporator. *International Journal of Dairy Technology, 58*(3), 143-149.

Zadow, J. (1969). Studies on the ultra-heat treatment of milk. *Australian Journal of Dairy Technology, 24*(2), 44.

Zhu, D., Kebede, B., Chen, G., McComb, K., & Frew, R. (2020). Effects of the vat pasteurization process and refrigerated storage on the bovine milk metabolome. *Journal of Dairy Science, 103*(3), 2077-2088.

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